CHEMICAL CONTROL OF SOYBEAN RUST (*PHAKOPSORA PACHYRHIZI*) ON SOYBEANS

by

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submitted in fulfilment of the requirements for the degree of

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ABSTRACT

Soybean rust (SBR) caused by *Phakopsora pachyrhizi* Syd. is an aggressive wind-dispersed fungal disease which has spread around the world at an alarming rate in the last decade. The disease was first reported in South Africa (SA) in 2001. It has become well established in the province of KwaZulu-Natal. Reports are occasionally made from eastern Mpumalanga, late in the growing season, in years with good rainfall. Yield losses of 10 – 80% have been reported due to SBR infection. Literature was reviewed to better understand the pathogen in an attempt to find suitable disease management strategies. Many strategies involve delaying, rather than preventing, SBR infection. Of the two strategies to prevent infection, the use of fungicides was the only option for disease control in SA, as no resistant cultivars are available. Field trials were conducted to determine which fungicides are effective in controlling SBR. Further research was conducted to determine the timing, frequency and rate of fungicide applications for optimal control of SBR. Trials were evaluated for disease severity, seed yield and the effect of fungicides on seed quality.

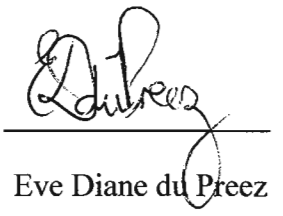
Fungicides from the triazole class of the sterol biosynthesis inhibiting group of fungicides were found to be the most effective in controlling SBR. A fungicide from the strobilurin group was found to be less effective than the triazoles at the suggested rate, but was found to be as effective when evaluated at higher dosage rates. Triazoles premixed with fungicides from the benzimidazole and strobilurin groups were also effective in controlling SBR. Timing of application was found to be critical for strobilurin fungicides, but not for triazole fungicides, which have a curative ability, unlike strobilurins. Strobilurin fungicides applied preventatively, before the appearance of disease symptoms were as effective as triazole fungicides applied after disease symptoms, but before infection levels had reached 10%. Across both wet and dry seasons two fungicide applications applied at 21d intervals at the R2 growth stage resulted in effective disease control. In wet seasons, a third fungicide application resulted in yields that were higher, albeit not statistically significant, than two fungicide applications. Assessments of individual fungicides for optimal dosage rate found that

registered rates were already optimal for some fungicides, but for others it appeared as if alterations were necessary to the rate suggested for registration.

This study was one of the first to extensively evaluate the efficacy of the new triazole and strobilurin fungicides on SBR control. The results have been shared globally, but particularly with newly affected countries in South and North America. Although this research has been groundbreaking, there are still many aspects of fungicide control which need to be studied in order to further optimise chemical control of SBR.

DECLARATION

I, Eve Diane du Preez, declare that the research reported in this thesis, except where otherwise indicated, is my own original research. This thesis has not been submitted for any degree or examination at any other university.



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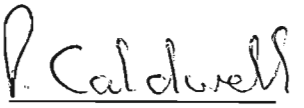

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GENERAL INTRODUCTION

When the first report of soybean rust (SBR), *Phakopsora pachyrhizi* (Syd.), on soybeans was made in Zimbabwe in 1998, South Africans knew it would only be a matter of time before its arrival in South Africa (SA). In October 1998 a workshop on SBR, sponsored by the Protein Research Foundation (PRF - formerly the Protein Research Trust) and the Agricultural Research Council (ARC), was convened at Potchefstroom, SA. SBR expert Dr Shanmugasundaram from the Asian Vegetable Research and Development Centre, Taiwan and researchers from Zimbabwe were invited to share their expertise with South Africans with the aim of preparing a strategic plan for the arrival of the disease in SA.

Although this plan included screening of SA soybean germplasm in Zimbabwe, the aspect of chemical control strategies was neglected. With the arrival of SBR in SA in 2001, researchers found themselves ill-prepared for managing the disease. The only information available was from Zimbabwe, where different cultivars, growing periods, environmental conditions and regulations on fungicide registrations meant that the information could only be used as a guideline. SA had to quickly develop a chemical control strategy to minimise the impact of the disease on soybean production.

In May 2001, a meeting held at the ARC Grain Crops Institute in Potchefstroom, saw the initiation of the National Soybean Rust Task Team (SBRTT) in SA. The SBRTT comprises researchers from various universities, seed and agrochemical companies, provincial Departments of Agriculture, the ARC, the PRF and Oil Crops Institute. National research needs were identified and assigned to various members of the SBRTT. A decision was taken not to make any artificial inoculations in areas where SBR had not occurred in the first season. Since the disease was only recorded from the province of KwaZulu-Natal (KZN), the research had to be conducted in KZN. The KZN Department of Agriculture and Environmental Affairs, which has an agricultural research farm based at Cedara, where SBR occurred in the first season, was assigned to research chemical control of SBR.

The lack of resistant cultivars makes fungicide control the best option for SBR management. Soybeans are very strongly inbreeding, making population breeding difficult because there is only a small proportion of outcropping. In the last four decades interspecies crosses have led to the development of cultivars with vertical resistance. This resistance has, however, been matched by virulent races of *P. pachyrhizi* Syd. and failed soon after their release. Generally for every resistant cultivar that is developed, another race will develop which will match the resistance. Techniques which could be explored to assist in resistance breeding include hand-crossing, the use of male gametocides and genetic modification. Soybeans lend themselves to genetic modification but it would take at least 10 years and millions of rands before a suitable cultivar may be ready for large scale use. Fungicides, therefore, remain the most effective method for control of SBR in the short-term.

The purpose of this research was therefore to systematically investigate all aspects pertaining to chemical control of SBR. Due to the multitude of possibilities for research trials and the limited resources available, it was decided to initially focus the research on certain priority topics. The focal areas of the research were to:

- Determine fungicide efficacy of a group of fungicides which had received emergency registrations. This was expanded to include other fungicides as well.
- Determine the optimum dosage rates for these fungicides.
- Determine the optimum timing of fungicide applications.
- Assess the optimum number of fungicide applications for SBR control.

All research was conducted at Cedara, an agricultural research farm ideally located for fungal disease research as it is situated in a mist-belt, with good rainfall and ideal temperatures for high yielding production. The environment makes Cedara arguably SA's most ideal locale for SBR research. The research results have been collated and presented at farmers' days, congresses (local and international) and published in the popular press. This research has been used as the national guide to SBR control. Following the recent arrival of SBR in the USA, this information has been shared with US plant pathologists, to help them develop fungicide control programmes in the USA.

CHAPTER 1

Literature review

1.1 Introduction

Soybeans (*Glycine max* (L.) Merr.) are a major source of vegetable oil and protein in the world. Argentina, Brazil, China and the USA produce over 90% of the world's soybeans. Although soybean cultivation in Africa has increased in the last four decades from 72 000t on 191 000ha in 1961 to 989 000t on 1 090 000ha in 2002, it accounts for only 0.5% of the annual global production of 179 917 000t (Singh *et al.*, 2004). A Food and Agriculture Organization study in 1978 estimated that 145 million ha is suitable for soybean cultivation in Africa (Anon., 1979). South Africa (SA) produced 202 000t in 2002, with KwaZulu-Natal (KZN) planting less than 25 000ha to soybeans (Scholtemeijer, pers. comm)[1].

In SA, the consumption trends for soybean derived commodities far exceeds the production trends with the result that 600 000 – 800 000t oilcake are imported annually to meet local demand (Joubert, 2004). The Protein Research Foundation (PRF) aims to promote the local production of protein-rich agricultural commodities. The arrival of soybean rust (SBR), a potentially devastating pathogen, in SA was construed as a threat to achieving the goal of increased soybean production. Yield losses of 10 - 80% are recorded for SBR, with extent of reduction related to time of infection, cultivar and environmental conditions. Yield losses in Brazil for the 2004 season were estimated at 4.5 million tons (Anon., 2004), approximately 10% of their annual production. Yield losses in the eastern hemisphere vary from 10 - 15% in some years in southern China (Yu *et al.*, 1980) to 70 - 80% in individual fields in Taiwan (Hsu and Wu, 1968). In SA, yield reductions of 35% have been recorded in experimental research trials (reported in this thesis: Chapter 2, Table 2.10b).

[1] Mr Gerhard Scholtemeijer, Chairman Protein Research Foundation, P.O. Box 671, Parklands, 2121, South Africa

Soybean rust is caused by two described species: *Phakopsora meibomiae* (anamorph *Malupa vignae*), occurring in the New World, and *P. pachyrhizi* (anamorph *M. sojae*) (Hartman *et al.*, 1999). Symptoms of SBR caused by *P. pachyrhizi* and *P. meibomiae* are identical and therefore indistinguishable (Anon., 2002). *Phakopsora pachyrhizi* is, however, more aggressive than *P. meibomiae*. Morphological studies of spore structures and DNA sequencing (Frederick *et al.*, 2002) are the only reliable methods for distinguishing between the two species. The term "Australasian" or its abbreviated form "Asian" have been informally associated with *P. pachyrhizi* since its arrival in South America in 2001 in order to distinguish between the two species.

The rapid spread of *P. pachyrhizi* and its potential for severe yield losses make this pathogen responsible for the most destructive foliar disease of soybean (Miles *et al.*, 2003). Since there are currently no resistant soybean cultivars in SA, the challenge is to manage this disease through other management practices, principally fungicides. Hopefully, in years to come there will be agronomically acceptable cultivars with durable resistance.

This review aims to introduce some of the research that has been conducted on *P. pachyrhizi*, the factors which contribute to SBR epidemics and the steps that have been taken to control the pathogen and limit yield loss associated with SBR infection.

1.2 Pathogen nomenclature and morphology

Soybean rust occurring in SA is caused by the fungus, *P. pachyrhizi* Sydow, belonging to the family Melampsoraceae, order Uredinales. The fungus was first described with this name by H. and P. Sydow in 1914 from the infected leaf of yam bean, *Pachyrhizus erosus* (L.) Urban, collected in 1913 in Taiwan by Y. Fujikuro (Sydow and Sydow, 1914).

The first record of SBR in mycological literature was, however, by P. Hennings in 1903. He described the uredial stage under the name *Uredo sojae* Hennings, collected from *Glycine ussuriensis* (= *G. soja*) in Japan, 1902 (Hennings, 1903). The fungus was also reported under other names. Hiratsuka (1935), Sathe (1972) and Keogh (1974)

have reviewed the early nomenclatural literature associated with the soybean rust fungus and found the following to be synonyms of *P. pachyrhizi*: *Uredo vignae* Bres., *Uredo sojae* Hennings, *Uredo concors* Arthur, *Physopella concors* Arthur, *Physopella vignae* Arthur, *Phakopsora sojae* Fujikuro, *Phakopsora sojae* Sawada, *Uromyces sojae* Miura and *Uromyces sojae* Sawada.

In the life-cycle of rust fungi, five different stages (Stage 0 – Stage IV) are described (Table 1.1). Pycnia (spermogonia) and aecia are unknown for *P. pachyrhizi* (Bromfield, 1984). Uredia (Fig. 1.1) are the most commonly occurring spores in the life-cycle found in nature, with telia occasionally being produced. Basidia have only been produced in the laboratory (Koch and Hoppe, 1987).

Table 1.1 Life-cycle stages described for rust fungi

Stage	Spore-bearing structures	Spore
0	Spermogonia	Spermatia
I	Aecia	Aeciospore
II	Uredia	Urediospore
III	Telia	Teliospore
IV	Basidia	Basidiospore

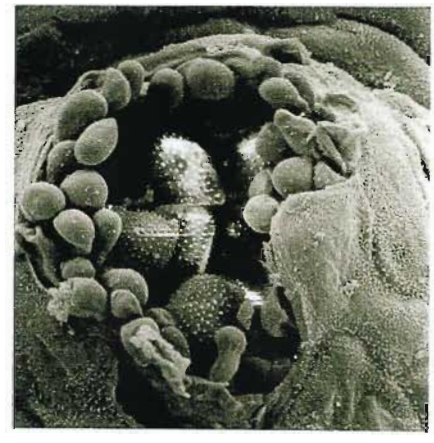

Figure 1.1 Scanning electron micrograph showing erumpent uredium containing urediospores of *Phakopsora pachyrhizi* on soybean (Photograph by Eve du Preez)

The anamorphic fruiting structures (uredia) have a cellular basal peridium terminating in paraphyses. Urediospores (15-24 x 18-34μm) are obovoid to broadly elliptical, with walls that are 1.0μm thick, minutely and densely echinulate, and colourless to pale, yellowish brown. They germinate within 3 – 6h of incubation at 14 - 29°C (Hartman *et al.*, 1999).

Telia (approximately 50 - 150μm in diameter) form subepidermally among uredia and are dark-brown to black at maturity. They are crustose, irregular to round, and sparse to aggregated. Telia of *P. pachyrhizi* contain teliospores arranged in layers of one to seven. Teliospore walls are pale yellowish-brown to colourless and 1μm thick or slightly thicker apically in the uppermost spores (Hartman *et al.*, 1999).

Koch and Hoppe (1987) described the germination of teliospores and the formation of basidia and basidiospores in the laboratory. The inducive treatment is a sequence of wetting and drying which closely resembles environmental conditions in the field. They therefore conclude that the germination of teliospores and formation of basidiospores also occurs in nature, although this has not yet been shown in the field. Information on the infectivity of basidiospores and plant species they can infect is also lacking.

Basidia were described as slightly curved or erect. Basidia with four sterigmata bearing basidiospores were less frequently observed than basidia with one, two or three sterigmata. The length of basidia varied between 23 - 60μm with the average width near the apex being 8.3μm. The length of the sterigmata varied from 6 - 24μm. Basidiospores were ovoid in shape, 6 - 10μm (average of 8.5μm) long and 5 - 6μm (average of 5.4μm) wide (Koch and Hoppe, 1987).

Physiological races

McLean and Byth (1976) demonstrated the presence of races in *P. pachyrhizi* in Australia. The cultivar Wills and accession PI 200492 were used to differentiate races. Race 1 was virulent on Wills and avirulent on PI 200492. Race 2 was virulent on both cultivars. Later, Bromfield *et al.* (1980) identified four different races using three genotypes with known dominant loci for resistance to soybean rust (PI 200492, PI

230970 and PI 462312 = cultivar Ankur) and four different rust isolates from different sources. Presently, there are nine known races of *P. pachyrhizi* (Hartman *et al.*, 1999).

1.3 Symptoms

Infection usually begins on the older, lower leaves of plants at, or after, the flowering stage, so it is generally not noticed until the pods are set, by which time the lower leaves have become chlorotic and infection has progressed to the upper plant canopy. Disease develops first with small, water-soaked lesions, which gradually increase in size, turning from grey to tan or brown. Lesions assume a polygonal shape restricted by leaf veins. Individual lesions may coalesce to present an enlarged lesion eventually reaching a size of 2 - 5mm^2. Within each of the lesions is one to many erumpent, globose uredia. The uredium, with its circular opening, or ostiole, and its extruded urediospores are diagnostic for the rust.

The most commonly observed symptom of SBR is the sporulating lesions on the lower surface of the leaf, although lesions can appear on both leaf surfaces and on petioles, small stems and pods. Lesions on petioles and young stems of soybeans are usually elongated. The number of uredia per lesion increases as lesions age (Sinclair and Hartman, 1996). Under certain conditions, uredia develop in tissue that is not discoloured and does not differ in appearance from tissue not invaded by mycelia of the pathogen.

As the plant matures and sets pods (R3 – R6 growth stages), infection progresses rapidly under the right environmental conditions (moisture, high humidity and heat) to cause severe infection in the middle and upper canopy of the plant. Clouds of spores have been observed within, and or above, canopies of highly infected plant stands (Anon., 2002). When lesion density is high, premature yellowing and leaf abscission occurs. Subsequently, as rust severity increases, premature defoliation and early maturation of plants is common (Sinclair, 1989; Hartman *et al.*, 1999; Miles *et al.*, 2003). Although quantitative data are lacking, it is generally thought that leaf yellowing and abscission are correlated with the number of lesions per leaflet i.e., as the number of lesions per unit area increases, yellowing and defoliation becomes more pronounced.

The rate of severity of these processes may be influenced by the host variety and pathogen isolate involved (Bromfield, 1984).

Depending on the isolate of the pathogen and the soybean strain, either reddish-brown or tan lesions appear; both types may develop on the same leaflet of some cultivars. Tan lesions consist of two or more uredia surrounded by slightly discoloured necrotic areas on leaf surfaces. Uredial pustules become more numerous with advancing infection and often coalesce to form larger pustules which break open, releasing masses of urediospores. The reddish-brown lesions have larger areas of necrosis surrounding a very limited number of uredia (zero, one or two) which usually have a few urediospores visible on the surface (Anon., 2002).

During the early stages of development, before the onset of sporulation, rust lesions may be confused with bacterial pustules caused by *Xanthomonas axonopodis* pv. *phaseoli* (Smith) Vauterin, Hoste, Kersters and Swings. However, the symptoms of the two diseases can be differentiated by the presence of multiple uredia in the rust lesion and by the irregular cracks that usually appear in host tissue with a bacterial pustule lesion (Hartman *et al.*, 1999). Microscopic examination of the symptomatic tissue will further assist identification by revealing bacteria in a bacterial pustule infection, which is not present in SBR infections.

1.4 Host range

Host range studies have been conducted by many researchers around the world. Reviews and additions to the host range list have most notably been made by Sinclair (1982), Tschanz (1982), Bromfield (1984), Rytter *et al.* (1984) and Ono *et al.* (1992). Complications in identifying these alternate hosts have arisen through the renaming of host plant species as well as the different criteria used by researchers in determining a host plant. A plant should only be considered an alternative host if the fungus sporulates on it (Bromfield, 1984). Further factors complicating alternate host range identification include rust pathotype and host species variety tested. The same legume species may support sporulation in one region but not in another due to differences in races of the pathogen. Similarly, if two plants of the same legume species, but from different areas,

are inoculated with the same fungal isolate, different responses may be observed due to varietal differences (Bromfield, 1984). Due to variation in rust pathotypes, differential reactions within host species and factors associated with inoculation and incubation, it is thought that the full host range has not yet been fully identified (Rytter *et al.*, 1984; Miles *et al.*, 2003).

Similar to other rust fungi, *P. pachyrhizi* is an obligate parasite that requires alternate hosts on which to survive unfavourable conditions. Unlike most other rust fungi, which have a narrow host range, *P. pachyrhizi* has a wide host range with reported hosts totalling over 100 species among 44 genera in the family Fabaceae (Table 1.2) (Hershman, 2003). Reports of teliospores in nature are far less common than urediospores. Teliospores have been recorded on eleven host species (Table 1.3).

1.5 History and distribution of soybean rust caused by *Phakopsora pachyrhizi*

Geographical distribution of soybean

China is thought to be the primary gene centre or germplasm pool of soybean (*Glycine max* (L.) Merr.), where it emerged as a cultigen sometime in the 11th century B.C. From the first to the 16th centuries A.D. it was introduced into Burma, north India, Indonesia, Japan, Malaysia, Nepal, the Philippines, Thailand and Vietnam, where landraces of soybean subsequently developed, comprising a secondary gene centre (Hymowitz and Newell, 1980).

Geographical distribution of soybean rust

Soybean rust was first reported in Japan in 1902 (Kitani and Inoue, 1960). Four years later, in 1906, it was reported in India. In 1914 the pathogen was recorded from Papua New Guinea, the Philippines and Taiwan. The first report of soybean rust in Australia was in 1934, from Queensland. The disease was observed subsequently in many Asian countries, including Cambodia, China, Indonesia, Korea, Okinawa, Sri Lanka, Thailand, USSR and Vietnam (Bromfield, 1984).

Table 1.2 Species, other than *Glycine max*, on which *Phakopsora pachyrhizi* has been recorded (after Sinclair, 1982; Tschanz, 1982; Bromfield, 1984; Rytter *et al.*, 1984; Ono *et al.*, 1992)

Alysicarpus glumaceus	*Alysicarpus vaginalis*	*Cajanus cajan*
Cajanus sp.	*Calopogonium mucunoides*	*Canavalia gladiata*
Canavalia maritima	*Canavalia villosa**	*Cassia occidentalis*
Centrosema pubescens	*Clitoria ternatea*	*Coronilla varia*
Coronilla varia 'Emerald'	*Crotalaria anagyroides*	*Crotalaria dissaromoensis*
*Crotalaria incana**	*Crotalaria juncea**	*Crotalaria linifolia*
Crotalaria pallida	*Crotalaria spectablis*	*Crotalaria striata**
*Crotalaria vitellina**	*Crotalaria* sp.	*Delonix regina*
Desmodium discolor	*Desmodium frutescens**	*Desmodium incanum**
Desmodium rhytidophyllum	*Desmodium tortuosum* (formerly *Meibomia supina* and *M. tortuosa*)	*Desmodium triflorum*
Desmodium varians	*Dolichos ascillares* *	*Dolichos axillaris*
*Eriosema crinitium**	*Erythrina glauca** (syn. *E. fusca*)	*Galactia* sp.*
Glycine canescens	*Glycine clandestina*	*Glycine falcata*
Glycine latrobeana	*Glycine soja* (syn. *G. ussuriensis*)	*Glycine tabacina*
Glycine tomentella (syn. *G. tomentosa*)	*Hardenbergi violacea*	*Kennedia coccinea*
Kennedia prostrata	*Kennedia rubicunda*	*Lablab purpureus* (syn. *Dolichos lablab*)
Lespedeza bicolor	*Lespedeza bicolor* forma acutifolia *	*Lespedeza juncea*
Lespedeza stipulaceae	*Lespedeza striata*	*Lotus americana*
Lotus major	*Lotus pedunculatus**	*Lupinus albus**
Lupinus angustifolius	*Lupinus hirsutus*	*Lupinus luteus*
Macroptilium atropurpureum	*M. bracteatum* (syn. *M. bracteatus*)	*Macroptilium lablab**
Macroptilium lathyroides (syn. *Phaseolus lathyroides*)	*Macroptilium siratro**	*Macrotyloma axillare*
Medicago arborea	*Meibomia supina* (now *Desmodium tortuosum*)*	*Melilotus officinalis*
Melilotus speciosus	*Microlespedeza striata**	*Mucuna cochinchinensis*
Neonotonia wightii (syn. *Glycine wightii*; *G. javanica*)	*Pachyrhizus erosus* (syn. *P. angulatis*; *P. bulbosus*)	*Phaseolus coccineus*
*Phaseolus longepedunculatus**	*Phaseolus lunatus* (*P. limensis*)	*Phaseolus macrolepsis**
Phaseolus radiatus var. *flexuous**	*Phaseolus tructeolatus**	*Phaseolus vulgaris*
Pisum sativum	*Psoralea glandulosa**	*Psoralea tenax*
Pueraria lobata (syn. *P. thunbergiana*)	*Pueraria phaseoloides**	*Pueraria triloba**
Rhynchosia minima	*Rhynchosia mollisimia*	*Rhynchosia* sp.
Sesbania exaltata	*Sesbania sericea*	*Sesbania vesicaria*
Sesbania sp.	*Shuteria* sp.*	*Teramnus uncinatus*
Trifolium incarnatum	*Trifolium repens*	*Trigonella foenum-graecum*
*Vicia faba**	*Vicia dasycarpa* (syn. *V. villosa*)	*Vigna angularis**
*Vigna catjang**	*Vigna cylindrical**	*Vigna mungo* (syn. *Phaseolus mungo*)
Vigna radiata (syn. *Phaseolus radiatus*)	*Vigna unguiculata* (syn. *V. sinensis*)	*Voandzeia subterranean**
*Wisteria floribunda**		

*Denotes species on which information regarding sporulation of *Phakopsora pachyrhizi* is uncertain

Table 1.3 Species on which teliospores of *Phakopsora pachyrhizi* have been observed (after Bromfield, 1984)

Host	Location
Canavalia villosa	Guatemala
Crotalaria linifolia	Australia
Desmodium rhytidophyllum	Australia
Glycine max	Japan, Taiwan, Brazil
Glycine wightii	Brazil
Lablab purpureus	Puerto Rico, Brazil
Meibomia supina	Puerto Rico
Pachyrhizi angulatus	Taiwan
Phaseolus lunatus	Brazil
Phaseolus vulgaris	Brazil
Rhynchosia minima	Brazil

Phakopsora pachyrhizi occurred only in the Eastern hemisphere (Asia and Australia) until it was found in Hawaii in May 1994 (Killgore and Heu, 1994). In 1996 it was found to have infected soybeans in Uganda. It then spread southwards to Rwanda, Zimbabwe and Zambia in 1998 (Levy, 2003), and west into Nigeria in 1999 (Akinsanmi and Ladipo, 2001). Soybean rust continued its southward movement to southern Mozambique in early 2000 and into eastern South Africa in 2001 (Pretorius *et al.*, 2001; Levy, 2003).

The first report of rust caused by *P. pachyrhizi* in South America was from Paraguay in March 2001 (Morel *et al.*, 2004). It was subsequently recorded in the state of Paran<, Brazil in 2001 (Yorinori, 2004). In April 2002, SBR was observed in the province of Misiones, Argentina for the first time (Rossi, 2003). According to Yorinori (2004), since its first detection in Paraguay and in the state of Paran<, in 2001, the "Asian" rust has spread to parts of Argentina, all parts of Bolivia, most of Brazil and all parts of Paraguay.

In August 2004, the Agricultural Research Service of the United States Department of Agriculture (USDA) and the Animal Plant Health Inspection Service (APHIS) confirmed a report of SBR in Colombia (Caspers-Simmet, 2004). They reported that it was caused by *P. pachyrhizi*. Colombian authorities said, however, that it was the less aggressive *P. meibomiae* (Anon., 2004). Less than three months later, on 10 November

2004, USDA issued a press release for the first report of SBR on the USA mainland (Rogers and Redding, 2004). SBR now occurs in all major soybean producing countries of the world (Fig. 1.2).

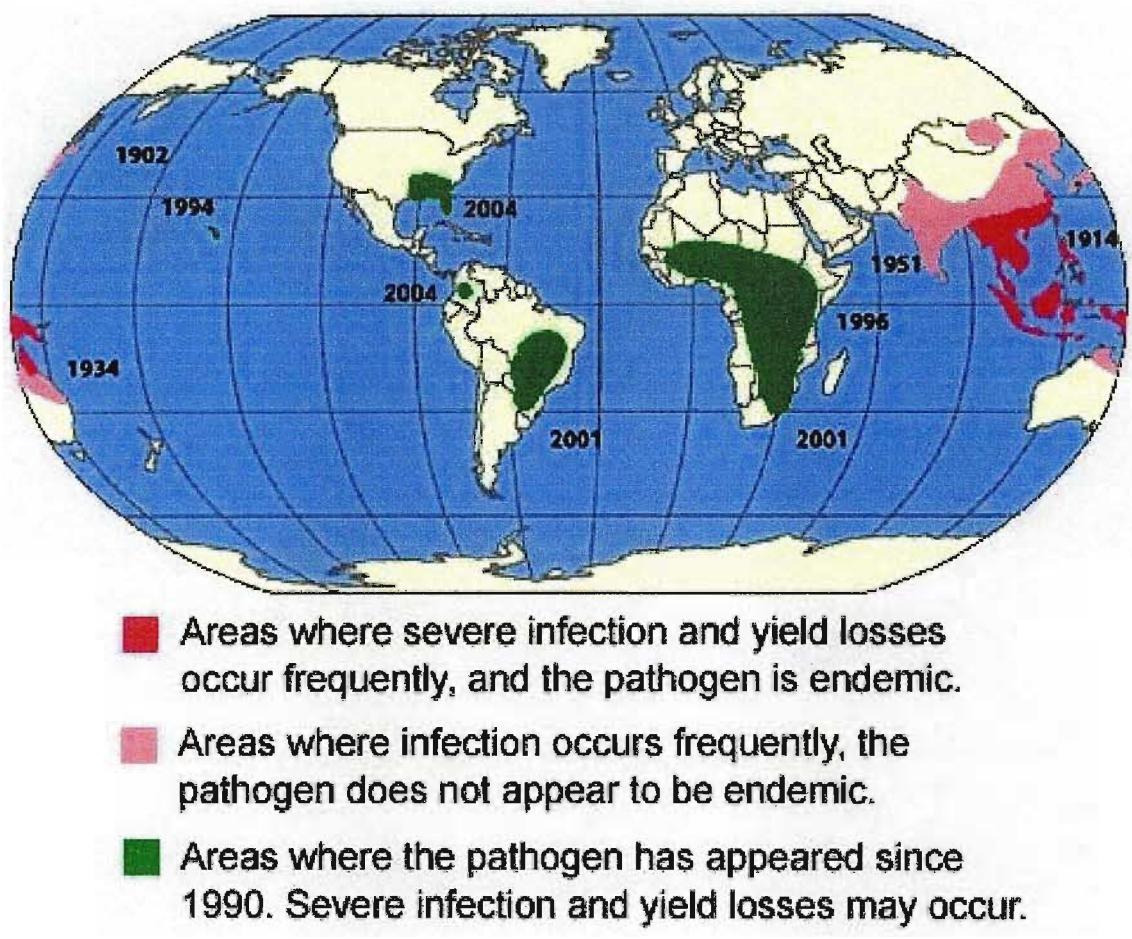

Figure 1.2 Global distribution of *Phakopsora pachyrhizi* showing dates when it was first recorded in each region (adapted from Miles *et al.*, 2003)

1.6 Economic Importance

The genus *Phakopsora* contains more than 90 species recorded on over 150 genera, comprising 30 families of the Angiospermae (Ono *et al.*, 1992). Of the described species, *P. pachyrhizi* is considered the most economically important pathogen of the genus *Phakopsora* (Bromfield, 1984). In the 30 years during which the USA considered SBR an exotic disease, APHIS classified it as the number one threat to soybeans (Hershman, 2003).

Soybean rust lowers soybean yields through premature defoliation and by decreasing yield and yield components: the number of filled pods per plant, the number of normal pods per plant, the number of seeds per plant, the weight of seeds per plant and the 1000-seed weight (Kitani and Inoue, 1960; Ogle *et al.*, 1979; Casey, 1981). It also lowers the oil quality of seeds produced (Ogle *et al.*, 1979). The severity of loss and the particular components of yield affected depend primarily on the time of disease onset and the intensity of disease at specific growth stages of the soybean crop.

Estimations of yield loss in the eastern hemisphere vary from 10 – 15% in some years in southern China (Yu *et al.*, 1980) to 70 – 80% in individual fields in Taiwan (Hsu and Wu, 1968). Seasonal variation in weather conditions results in large variation in yield loss at the same location. A yield loss of 12% is estimated for most years in Taiwan, but in severe years this increases to 50% (Bromfield, 1984).

Experimental determination of yield loss was attempted by Yang (1977) for different cultivars. The yield loss, calculated from the difference for mancozeb sprayed and unsprayed soybean yields, was 23, 24, 32 and 50% for the cultivars Shih-Shih, Tainung #4, Wakajima and Kaohsiung #3, respectively.

Ogle *et al.* (1979) conducted a field trial where soybeans were protected for different lengths of time. Yield reduction in the unsprayed treatment was 67.8% compared with the treatment that was sprayed fortnightly with mancozeb until harvest. Loss was associated with reduction in the number of pods, number of filled seeds, seed mass, seed yield per plant, reduced seed oil content (but not protein) and non-seed dry weight of plants.

In a greenhouse trial conducted by Ogle *et al.* (1979), with the soybean cultivar Wills, treatments were inoculated with urediospores at different plant growth stages and every 10d thereafter up to 10d before harvest. The soybeans inoculated immediately prior to flowering (V5-6 growth stage) yielded only 5.4% (i.e. 94.6% yield reduction) of the yield obtained when plants were inoculated 5wk after flowering (R6-7 growth stage). The treatment inoculated immediately prior to flowering resulted in the lowest number of pods, percentage of filled pods, number of seeds, and percentage of filled seeds. Treatments inoculated for the first time at R3 & R3-4 growth stages resulted in 73%

yield reduction while treatments inoculated at R4 & R5 growth stages resulted in 54% and 27% yield reductions, respectively. In comparison, Casey (1981) reported a 36% yield loss on the cultivar Wills from an infection which initiated just prior to flowering.

The impact of irrigation on yield loss was measured by Casey (1981). Plots that received periodic overhead irrigation experienced yield losses of 59.2 and 61.3% in comparison with comparable non-irrigated plots where yield losses of 9.8 and 30.2%, respectively, were recorded.

An econometric-simulation model used by Kuchler *et al.* (1984) predicted potential damage of US $7.2 billion per annum if a virulent race of *P. pachyrhizi* were to be introduced into the USA. Yield losses in Brazil for the 2004 season were estimated at 4.5 million tons, with an economic value of US $2 billion (Anon., 2004). Since Brazil's annual soybean production is just over half that of the USA, this indicates that Kuchler's model was probably over-predicting the expected yield loss. Even so, the economic importance of SBR remains significant.

1.7 Infection Process

The sequence of events in the development of soybean rust, from the time the urediospore lands on the host until sporulation, is given in Table 1.4 (Marchetti *et al.*, 1975; Bonde *et al.*, 1976; Koch *et al.*, 1983; Koch and Hoppe, 1988).

Phakopsora pachyrhizi is one of only a few rust fungi that is able to penetrate its host directly (Bonde *et al.*, 1976; Koch *et al.*, 1983). In studies by Koch *et al.* (1983), penetration of soybean leaves most frequently occurred directly through the cuticle (Fig. 1.3). Occasionally, however, appressoria formed over stomata. In these cases, penetration occurred through one of the guard cells rather than the stomatal opening (Fig. 1.4).

Table 1.4 The sequence of events over time in the development of soybean rust caused by *Phakopsora pachyrhizi* (after Marchetti *et al.*, 1975; Bonde *et al.*, 1976; Koch *et al.*, 1983; Koch and Hoppe, 1988)

	Sequence of events	Time
1.	Urediospore lands on soybean leaf surface over epidermal cell	0 hpi
2.	Germ-tube development (5-400um)	12 hpi
3.	Appressorium-cone formed	16 hpi
4.	Penetration hyphae formed	16 hpi
5.	First hyphal septum formed	18-20 hpi
6.	Primary hyphae produced	18-20 hpi
7.	Collapse of epidermal cell	24 hpi
8.	Haustorium formed (haustorium encasement)	24-48 hpi
9.	Branching into secondary hyphae	48-72 hpi
10.	Mycelia development inside spongy mesophyll and the intercellular space	3 dpi
11.	Collapse of appressorium and penetration hyphae	4 dpi
12.	Necrotic lesions appear on leaf	6 dpi
13.	Runner hyphae passing through mesophylls	7 dpi
14.	Hyphae aggregate; uredial primordia formed consists of compact layer of 2-3 cell column	9 dpi
15.	Wall thickened (sporogenous tissue, pedicel and urediospore; urediospores mature)	11-12 dpi

hpi – hour post infection

dpi – day post infection

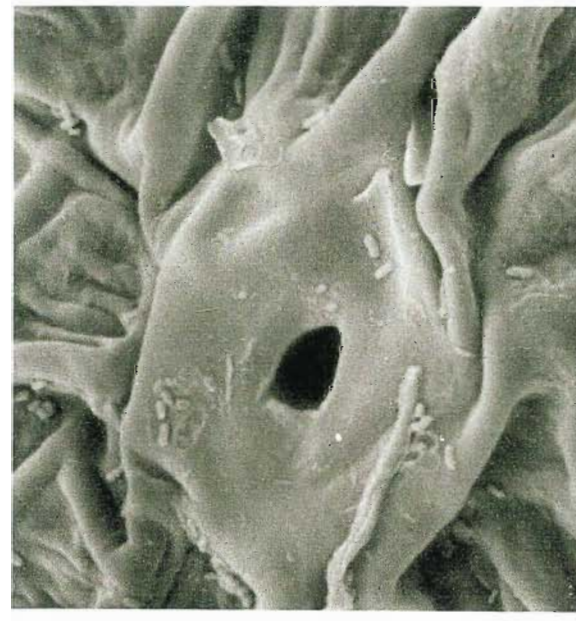

Figure 1.3 Scanning electron micrograph showing a site where direct penetration of the epidermis occurred by *Phakopsora pachyrhizi* on soybean (Photograph by Eve du Preez)

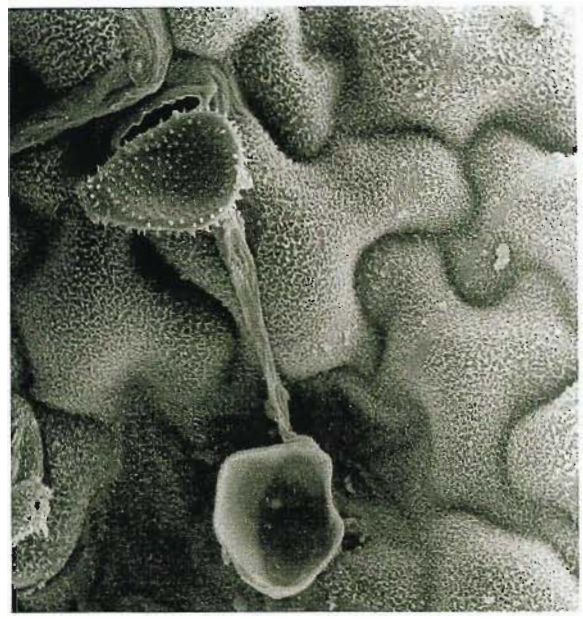

Figure 1.4 Scanning electron micrograph showing germinated urediospore of *Phakopsora pachyrhizi* with appressorium over a stoma on soybean. The urediospore, germ tube, appressorium and epidermal cell around the appressorium have collapsed, indicating that infection occurred at least 4d previously (Photograph by Eve du Preez)

1.8 Epidemiology

The presence of a susceptible host plant, a virulent pathogen, and favourable environmental conditions are essential for successful infection and disease development. Development of the host and pathogen are influenced by environment. Epidemiological studies using cultivars with different levels of resistance, different races of *P. pachyrhizi* and different isolates, which differ in aggressiveness, have been conducted by numerous researchers under differing environmental conditions in order to better understand SBR disease development.

Environmental effects on infection processes have been studied by several researchers. Urediospore germination is adversely affected by light and temperatures <10°C and >28°C, with darkness and temperatures of 15 - 25°C being optimum (Table 1.5).

Table 1.5 Temperature requirements (°C) for urediospore germination of *Phakopsora pachyrhizi*

Temperature range tested	Optimum temperature	No germination at temperatures	Reference
20 (dark)	20	-	Bonde *et al.* (1976)
24 (dark)	24	-	McLean (1981), McLean & Byth (1981)
8-32	21-27	<8 and >32	Kitani & Inoue (1960)
9-26	21	4 and 32	Hsu & Wu (1968)
5-35	15-20	<5 and >35	Keogh (1974)
5.5-31 (light)	12-21	<9 and >28	Melching & Bromfield (1975)
5.5-31 (dark)	15-25	<10 and >28.5	Marchetti *et al.* (1976)

Kitani and Inoue (1960) reported appressorium development at temperatures between 15 and 30°C, with an optimum range of 21 - 28°C. The optimum pH was 4.4 – 8.0. McLean and Byth (1981) found that fungal development (urediospore germination, appressorium formation and penetration) was more rapid within a dew period, but that some urediospores were still able to complete their development, although much slower, in the absence of dew.

Colonization studies conducted by Marchetti *et al.* (1976) on inoculated plants found that maximum infection occurred with 10 - 12h of dew at temperatures of 20 - 25°C and at 15 - 17.5°C with 16 – 18h of dew. The minimum dew period for infection was 6h at 20 - 25°C and 8 – 10h at 15 - 17.5°C. No infection occurred at 27.5°C.

The optimum temperature for uredial development and sporulation is 17°C at night and 27°C during the day (Table 1.6).

Studies on spore production showed that number of spores produced depends on pathogen isolate and cultivar. Melching *et al.* (1979) inoculated the cultivar Wayne with four different rust isolates. The mean total number of spores per lesion over the 39d collection period was 2028, 3768, 6268 and 6600 for the Australian, Indian, Indonesian and Taiwanese isolates, respectively. Furthermore, Melching *et al.* (1979) observed that new uredia continued to form on Wayne for 56d after inoculation. Yeh *et al.* (1982) inoculated soybean plants of TK 5 and PI 230971 and collected spores over 36d. The

mean total spore production per lesion was 12646 and 9396 for TK 5 and PI 230971, respectively.

Table 1.6 Temperature requirement (°C) for uredial development (UD) and sporulation (S) of *Phakopsora pachyrhizi*

Temperature °C (h duration)		RH	Time to UD and S (d)	Reference
Day	Night			
25 (12)	20 (12)	-	9	Keogh (1974)
20 (12)	15 (12)	-	18	Keogh (1974)
27 (13)	17 (11)	75-80%	9	Kochman (1979)
17 (13)	7 (11)	75-80%	14	Kochman (1979)
22 (13)	12 (11)	75-80%	11	Kochman (1979)
32 (13)	22 (11)	75-80%	11	Kochman (1979)

Field research on the rate of spread of SBR was conducted by Kitani and Inoue (1960) and Casey (1979). A mean rate of spread of $1m.d^{-1}$ was calculated in the trial by Kitani and Inoue. Casey collected data over three seasons from 1974 to 1977. The rate of disease spread varied from $0.15m.d^{-1}$ in the least favourable season to $0.45m.d^{-1}$ in the season most conducive for disease development. Casey (1979) concluded that "extended periods of leaf surface wetness of approximately $10h.d^{-1}$ and moderate temperatures (18 - 26°C) were necessary for the development of a severe epidemic. Extreme temperatures (>30°C and/or <15°C) and/or dry conditions retarded development of the rust. Furthermore, prolonged temperatures above 27°C appeared to inhibit the fungus even when leaf-surface wetness was theoretically adequate".

Glasshouse studies conducted by Melching in 1974 on the cultivar Wayne, inoculated at the V5 - V6 growth stage, showed that 50% defoliation occurred 44 – 46d post inoculation (dpi). Plants were brittle and dead 57dpi and a yield loss of 60% was recorded. In these trials rust lesions were first detected 11dpi. Assessments made 14 - 16dpi indicated averages of 1660 - 2500 rust lesions per plant. An average of 600 lesions per leaflet was recorded 44 - 46dpi (cited by Bromfield, 1984).

Tschanz communicated a hypothesis to Bromfield that the rate of rust development is affected by physiological age of the soybean plant (Bromfield, 1984). The consequence of this is that early data concerning dynamics of epidemics, selection of 'resistant'

soybean cultivars, yield components and yield loss reported on prior to this theory, may be flawed.

1.9 Disease management

Successful SBR management can be expected from the skilful utilization of appropriate fungicides applied as necessary, the development of effective biocontrol agents, the orchestration of appropriate practices of good husbandry, and deployment of disease-resistant and tolerant varieties.

1.9.1 Chemical approaches

All of the early research on the use of fungicides for the control of SBR was conducted in the Eastern Hemisphere where the disease has been a problem for many years. Kitani and his colleagues pioneered research into chemical control of SBR, investigating the effectiveness of lime sulphur, Bordeaux mixture, mercurials and zineb for the control of SBR in Japan (Kitani *et al.*, 1960a; Kitani *et al.*, 1960b; Kitani *et al.*, 1960c). In the two decades following Kitani's research, a wide array of protectant and eradicant fungicides were tested (Hung and Liu, 1961; Liu, 1966; Jan and Wu, 1971; Sangawongse, 1973; Yang *et al.* 1974; Torres and Quebral, 1976; Sinclair, 1977; Quebral, 1977; Yang, 1977; Sinclair, 1978).

No one class of fungicide emerged as uniquely effective in suppressing *P. pachyrhizi*, although formulations of the zinc ion-maneb complex, applied periodically throughout the growing season, were most effective. The problem with the use of the mostly protectant fungicides tested is that to be effective, at least 3 – 5, or more, fungicide applications are required on a weekly or 10d basis, making spraying both time-consuming and expensive. For these reasons, the use of fungicides is only warranted where losses of 80% are expected, with expected losses of 10 - 15% not justifying the time and cost of spraying. It is difficult, however, to predict at the start of the season what the expected yield losses might be. In addition, the occurrence and severity of SBR varies among regions, from season to season, and even within regions in the same season.

In the 1980s and 1990s more effective, systemic fungicide classes were developed with the emergence of the triazoles in the sterol biosynthesis inhibitors (SBIs) and the strobilurins. Fungicides from these classes are currently used for the control of rust in many crops around the world. In a survey conducted in Minnesota and North Dakota, USA during 1996, for disease control in drybeans, Glogoza (1998) reported that fungicides used for rust control were propiconazole, chlorothalonil and maneb. By 2004, in the same areas, fungicides used for drybean rust control also included azoxystrobin, pyraclostrobin and boscalid (Bradley, 2004). Similarly, in wheat crops in the USA, registrations for the control of rust diseases (leaf, stripe and stem rust) include protectants such as mancozeb plus systemics in the triazole and strobilurin classes. Registrations include pyraclostrobin, propiconazole, azoxystrobin and propiconazole + trifloxystrobin (Anon., 2005).

Patil and Anahosur (1998) were the first researchers to publish results on the use of triazole fungicides for SBR control. Two sprays were applied at a 15d interval, with the first application at the appearance of rust pustules on lower leaves of the plants. Hexaconazole (25% disease; 30.31q.ha^{-1}), propiconazole (33% disease; 27.36q.ha^{-1}) and triadimefon (33% disease; 28.02q.ha^{-1}) treatments provided the best disease control and resulted in the highest seed yields. Lowest yield was obtained from the unsprayed control (92% disease; 16.60q.ha^{-1}) which was not significantly different from mancozeb (79% disease; 18.11q.ha^{-1}), tridemorph (73% disease; 19.47q.ha^{-1}) and chlorothalonil (72% disease; 18.90q.ha^{-1}) treatments.

Fungicide trials conducted in Zimbabwe by Dr Clive Levy, after the arrival of SBR in 1998, included evaluations of triazole fungicides (Levy, pers. comm., 2001)[2]. A lack of registered fungicides in Zimbabwe, however, meant that no research was conducted on the use of strobilurins and certain triazole fungicides. Levy's recommendation for fungicide control of SBR involves making the first fungicide application at flowering and subsequent sprays at 20d intervals with a total of either two or three fungicide sprays in a season.

[2] Dr Clive Levy, Commercial Farmers Union of Zimbabwe, PO Box WTG390, Westgate, Harare, Zimbabwe

More recently, the USDA has conducted fungicide efficacy trials at various locations in Paraguay, South Africa and Zimbabwe (Levy, 2004; Miles *et al.*, 2004). Evaluations of 23 fungicides from principally triazole and strobilurin classes have been conducted, with the first application at growth stage R1 and subsequent sprays at 20d intervals. Trials were split for two and three fungicide applications. Both triazole and strobilurin fungicides and the mixes of the two are effective in reducing SBR severity. Miles also commented that the triazoles have a shorter residual period, but can kill rust infections within a leaf. The strobilurins, however, have a longer residual period, but are not as effective in controlling established infections.

Even though fungicides are available which are effective in controlling SBR, the consensus is that still much more research needs to be conducted in order to optimise fungicide use in SBR control. "In view of the marked differences among soybean-growing areas in weather patterns, cropping systems, availability of labour, availability of specific types of application equipment, cost of materials, availability of capital, and other relevant economic, technological, and environmental factors, it is apparent that specific recommendations for effective chemical control must be developed for and tailored to specific locales" (Bromfield, 1984). "More work must be done on timing, rates, number of applications, plant age and other factors affecting the use of fungicides for control of this disease" (Sinclair and Hartman, 1996). "Additional research on the timing of application and rotation of triazoles and strobilurin fungicides is needed. With the single site mode of action from each group of fungicides it is necessary to limit their use to one application per season for each class in order to reduce selection pressure and hence the development of a fungicide-resistant *P. pachyrhizi* population. The relative curative ability of the triazoles and the interaction between application rates and residual effects need to be evaluated" (Miles *et al.*, 2004).

With an improved understanding of the epidemiology of *P. pachyrhizi* and the advanced technological equipment available, optimization of fungicide timing cannot be mentioned without acknowledging the efforts which are being made in the forecasting of SBR epidemics for the development of an effective preventative fungicide spray-program. Collaborative research is being conducted by South African, South American and North American researchers into developing models for the prediction of SBR

epidemics. This work, which will result in the minimum use of fungicide and labour, is however, still in the early stages of development.

1.9.2 Cultural approaches

Changes in cultural practices can be implemented in order to reduce disease incidence or delay the onset of disease. The following practices can be used alone or in combination, to manage SBR (Bromfield, 1984; Sinclair and Hartman, 1996).

Modification of planting date

SBR occurs at different times of the year in different regions with epidemics being determined by weather patterns. This needs to be understood precisely and the information used to determine planting dates, to minimise severe losses. Keogh (1978) suggests that the rust can be avoided in coastal New South Wales by timing the critical stages in the development of the soybean crop so that none coincides with conditions most suitable for the disease. In his experience, the period between February and June (late summer to early winter) was most favourable for rust development. Pupipat (1977) suggests that severe rust can be avoided in the Chiang Mai area of northern Thailand if the crop is planted between the latter part of December and January 10th. Trials conducted in SA showed that the effect of planting date was not sufficient as a control strategy to reduce disease severity to levels that would reduce significant yield losses (Caldwell and McLaren, 2004).

Utilization of early-maturing cultivars with a short pod-filling stage

If the disease appears late in the growing season, soybeans may escape severe losses if they mature before an epidemic. This practice has been followed in northern KwaZulu-Natal, SA with success (personal observation).

Spacing

Little is known about the effects of spacing on the epidemiology of soybean rust. Trials in SA showed that a wider row-spacing (90cm) allowed for earlier appearance of symptoms than narrower row-spacing (45cm) but once SBR was present it appeared to develop quicker in the narrow rows, presumably due to a more favourable microclimate and the inability of fungicides to effectively penetrate the canopy (Caldwell and

McLaren, 2004). Despite these differences, it was apparent that row spacing as a practice to reduce disease to below thresholds, was ineffective.

Control of weed hosts

The host range of *P. pachyrhizi* includes a number of wild legume species. Control of alternate hosts with herbicides or other means in the vicinity of soybean production areas should be practised. Until more is known about the aerial transport and over-wintering of the spores of this fungus, the extent of the area in which weed hosts must be controlled will be based on observations and good judgement.

Control of cultivated hosts

Hosts include a number of cultivated legume species, such as common bean and lima bean. These crops should be avoided near soybean production areas in regions where the pathogen is endemic. In Queensland (Anon., 1974), it is recommended that, wherever possible, soybeans should be grown well away from pastures containing glycine (*Neonotonia wightii*), a common pasture legume known to be a host for *P. pachyrhizi*.

Crop rotation

Crop rotation has been effective for the control of other plant diseases and, because of the short-lived nature of the urediospores, it is presumed that crop rotation may be an effective means for control of this disease (Sinclair and Hartman, 1996). However, since SBR does not survive in crop debris, crop rotation between years will not be effective. The usefulness of crop rotation as a control method would be in the avoidance of continuous soybean cultivation *i.e.* when two or three crops are produced successively in the same year.

Intercropping

There is no evidence that intercropping will provide disease control, but studies need to be made to test this possibility (Sinclair and Hartman, 1996).

Transport of host material

The long-range transport of host plant material other than seeds, whether alternate species or alternate hosts, should be forbidden until there is proof that the fungus is not

carried in these tissues. The long-range transport of seeds of the various hosts should be regulated, except in the case of soybeans, for which there is evidence that the fungus is not seedborne (Sinclair and Hartman, 1996).

Planting of non-host barriers

In endemic areas, non-host plant barriers should be planted around soybean fields or larger production areas to reduce the amount of airborne inoculum spreading from one area to another (Bromfield, 1984). However, considering the ease with which spores are disseminated and the amount of inoculum produced this suggestion is considered to be of little value in SBR control.

Sanitation measures

All plant debris of hosts of this fungus should be destroyed after harvest, by burning, ploughing, chemical treatment or other such means, until the extent to which this fungus can overseason in the tissues of its various hosts is known (Sinclair and Hartman, 1996).

Surveillance and inspection

Constant surveillance of epidemic areas and inspection of exported and imported host material, including seeds, is necessary for local, regional and international control of this pathogen.

As knowledge of *P. pachyrhizi* increases, specific recommendations for specific cultural control methods in specific localities are being formulated and integrated with other control measures to improve management of the disease.

1.9.3 Biological approaches

Blakeman and Fokkema (1982) reported on the increased research attention paid to the investigation of fungi, bacteria, viruses and virus-like particles as biocontrol agents for plant pathogens. More than 30 genera of fungi have been found inhabiting sori on rust-infected plants (Littlefield, 1981), but it is uncertain as to how many of these are truly parasitic on the rust fungus. Blakeman and Fokkema (1982) listed *Eudarluca caricis* (Fr.) OE Erikss. (anamorph *Darluca filum* (Biv.-Bern. Ex Fr.) Cast), *Tuberculina vinosa*

and *Lecanicillium lecanii* (Zimm.) Gams and Zare (= *Verticillium lecanii*) as the most important hyperparasitic fungi on rusts.

Eudarluca caricis has not been reported on *P. pachyrhizi*, but Naidu (1978) has reported its parasitism of *P. elettariae* (Racib.) Cummins, the causal agent of cardamon rust, in India. Pothidee *et al.* (1980) have reported the parasitism of *P. pachyrhizi* by two species of *Tuberculina* in Thailand. Although sporulation of more than 80% was obtained on the uredia for the two *Tuberculina* species, germination of the conidia produced was poor.

Pon *et al.* (1954) described a soilborne bacterium, *Xanthomonas parasitica*, disseminated by rain splash, which parasitizes uredia of various cereal rust fungi and causes urediospore lysing. Bacteria in the genus *Bacillus* have also been implicated in urediospore lysing and in the inhibition of urediospore germination (Littlefield, 1981). Observations made by Bromfield (1984) in Indonesia and Thailand led him to conclude that in those countries, at least, sori of *P. pachyrhizi* are being adversely affected by unidentified bacterial hyperparasites. Personal observation of simple testing of a *Bacillus* species, foliar-sprayed onto soybean plants in South Africa, appeared to keep leaves greener and delay leaf abscission when compared to plants which were not treated with the *Bacillus* formulation.

Virus-like particles occur widely in fungi and have been observed in various stages of several rust organisms. Nothing is known about the presence of virus-like particles in *P. pachyrhizi* (Bromfield, 1984).

Even though advances have been made in biocontrol, there are none yet available for SBR disease management. Another factor which makes biocontrol difficult to implement is the length of time required to kill the pathogen in comparison with the immediate knockdown effect of agrochemicals and hence changing farmer mindset to adopt biocontrol technology.

1.9.4 Genetic approaches

Soybean is a self-pollinated legume with natural outcrossing of <0.5 – 1%. Plant breeding procedures such as backcrossing, single pod descent, pedigree breeding and bulk population breeding are some of the more common procedures used to develop improved soybean varieties. All of these procedures involve making crosses by hand pollination (Sleper and Shannon, 2003).

Breeding for durable soybean rust resistance combined with other desirable agronomic traits including yield is the major goal of soybean breeders. Plant pathologists and plant breeders recognise specific (vertical) and general (horizontal) resistance to, and tolerance of, soybean rust in soybeans. They further recognise that these responses to the pathogen are under genetic control and subject to manipulation by plant breeders.

Vertical resistance

In the early years, soybean breeders in Taiwan, Australia, India and many SE Asian countries developed cultivars which have specific resistance (Table 1.7). Specific resistance, usually under the control of one or a few major genes, is generally expressed as a hypersensitive response and reactions of this type can be readily evaluated. Variability for soybean rust reactions exists in wild annual and perennial *Glycine* species. To date, only one line of *G. soja* has been shown to have specific resistance. *Glycine max* and *G. soja*, both annual species from the subgenus *Soja*, each have a diploid chromosome number of 40 and cross readily. However, the transfer of rust resistant genes from perennial *Glycine* species from the subgenus *Glycine* is more difficult (Bromfield, 1984).

Table 1.7 Soybean cultivars developed using specific rust resistant alleles against *Phakopsora pachyrhizi*

Cultivar name	Country	Resistant source
Tainung No. 3	Taiwan (1968)	PI 200492
Tainung No. 4	Taiwan (1971)	PI 200492
Kaohsiung No. 3	Taiwan (1971)	PI 200492
Ankur	India	PI 462312
Dowling	U.S.A. (1978)	PI 200492

Cheng and Chan (1968), Shanmugasundaram (1976), Singh and Thapliyal (1977), McLean (1981), McLean and Byth (1981), Hartwig and Bromfield (1983) and Hartwig (1986) have all conducted research using different races to identify resistant genes. To date, four dominant, independently inherited genes for resistance to *P. pachyrhizi* – Rpp_1, Rpp_2, Rpp_3, Rpp_4 – have been identified in plant introductions (PI) 200492, 230970, 462312 (Ankur), and 459025, respectively. These lines, as well as seven others, are suspected to contain genes for resistance. PI 239718A and PI 239871B (*G. soja*), PI 230971 and PI 459024, and the cultivars Taita Koahsiung-5, Tainung-4, and Wayne have been used as differentials to identify nine races at the Asian Vegetable Research and Development Centre, Taiwan. The predominant race was compatible with nine of the eleven differentials, indicating that some races already possess multiple virulence factors to known and suspected genes for resistance. Subsequently, researchers have released other lines for evaluating resistance to *P. pachyrhizi* (Sinclair and Hartman, 1996).

Historically the utilization of single gene resistance has given rise to cultivars whose resistance is short-lived, with resistance developing often before or soon after the cultivar is released for commercial use. Loss of resistance is due to the occurrence of a new race or races due to the change in the virulence of an existing race.

Presently, the emphasis of vertical resistance breeding is on identifying genetic markers near resistant genes. If a genetic marker is linked to a resistant gene, it could be converted to a restriction fragment length marker that would provide a means for screening for resistant genes (Sinclair and Hartman, 1996). If new resistant genes are identified they could be stacked to avoid single gene exposure and hence also delay the rapid breakdown of resistance.

Horizontal resistance

General resistance, assumed to be controlled by many genes, each having a small effect, reduces the amount of rust and the rate of rust development even though the infection type produced is the same as that produced on a fully susceptible host. Evaluation of general resistance requires periodic assessment of rust prevalence and severity throughout a cropping season for a number of years. The detection and utilization of

lines with general resistance lag behind the detection and utilization of lines with specific resistance in soybean development programs (Bromfield, 1984).

Tolerance

Tolerance is the major approach used by most breeders. The term 'tolerance' is applied to those varieties or lines that yield well even though severely rusted. Tolerance, in this sense, cannot be identified by visual observation but only by measuring the effect of the rust on yield. The variety Shih-Shih, grown in Taiwan, may possess true tolerance because it yields well even when heavily rusted (Bromfield, 1984). Trials conducted in different seasons show the instability of some of the genotypes in their rust tolerance and yield potential. Therefore, it is important to evaluate in different seasons and possibly in different locations before arriving at major conclusions.

1.10 Conclusions

In the last three years the world's largest soybean producing areas, North and South America, have been affected by the virulent SBR pathogen, *P. pachyrhizi*. It is likely that research into the biology, factors affecting epidemiology, and various control measures will be stepped up in order to ultimately find the most economic and environmentally friendly method of managing this devastating disease.

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CHAPTER 2

Fungicide efficacy in soybean rust (*Phakopsora pachyrhizi* Syd.) control on soybeans (*Glycine max* (L.) Merr.)

Abstract

Trials were conducted over three seasons from 2001 - 2004 at Cedara (29°32'S, 30°16'E and alt. 1071m), 20km north-west of Pietermaritzburg, to evaluate the efficacy of fungicides for the control of soybean rust. Fungicides were applied three times during the growing season, at 21d intervals, from flowering until physiological maturity of the soybean crop. The most meaningful results were achieved in the warm and wet third season, as a mid-season drought developed in the first season and the second season was hot and dry. All fungicides reduced disease and increased yield and seed mass compared with the unsprayed treatment. Even though all fungicides were effective, trends showed that single-component fungicides were not as effective as fungicides containing mixtures of products. Fungicides containing carbendazim generally resulted in higher yields than others, whilst strobilurins on their own performed poorly. When strobilurins were mixed with a triazole fungicide, an increase in efficacy was observed. The best disease control over the season was attained with the use of Early Impact, Folicur and 'Impact with carbendazim', which had zero disease, while use of Capitan and Punch C resulted in very low levels of disease (0.047 and 0.085 standardised area under disease progress curve (SAUDPC), respectively). However, the best disease control did not always translate directly into high yield. 'Impact' with carbendazim (4973kg.ha^{-1}), Early Impact (4854kg.ha^{-1}) and Capitan (4677kg.ha^{-1}) were top yielding, but Punch Xtra (4845kg.ha^{-1}) and Fungicide X (4737kg.ha^{-1}) yielded better than Folicur (4544kg.ha^{-1}) and Punch C (4455 kg.ha^{-1}). Amistar, Dithane M-45 and Denarin with SAUDPCs of 2.433, 1.902 and 1.731, respectively, had the highest disease severity, but poor disease performance did not always translate into low yield. Denarin (4447kg.ha^{-1}) yielded better than Amistar (3884kg.ha^{-1}) and Dithane M-45 (4088kg.ha^{-1}). Bayfidan, however, which had an SAUDPC of 0.627, yielded lower than expected (3873kg.ha^{-1}) and resulted in the lowest yields of all fungicides tested.

2.1 Introduction

For nearly a century soybean rust (SBR), caused by *Phakopsora pachyrhizi* (Syd.), on soybeans (*Glycine max* (L.) Merr.) was a disease restricted to the tropics of the eastern hemisphere. However, in the last decade SBR has become established in Africa, South America and, more recently, North America (Killgore and Heu, 1994; Akinsanmi and Ladipo, 2001; Pretorius *et al.*, 2001; Levy, 2003; Rossi, 2003; Yorinori *et al.*, 2003; Morel *et al.*, 2004; Rogers and Redding, 2004). Although many speculations have been made, reasons for the sudden expansion in distribution of the pathogen are still not clearly understood.

The rapid spread of *P. pachyrhizi* and potential for severe yield losses make this the most destructive foliar disease of soybeans (Miles *et al.*, 2003). Urediospores are wind dispersed and believed to have spread through Africa on the inter-tropical convergence zone (Levy, pers. comm.)[1], whilst the recent U.S. infection is suspected to have blown in with Hurricane Ivan (Rogers and Redding, 2004).

SBR lowers yields through premature defoliation and by decreasing the number of filled pods per plant, the number of normal pods per plant, the number of seeds per plant, the mass of seeds per plant, and the 1000-seed mass (Kitani *et al.*, 1960a). It also lowers the quality of the seeds produced (Ogle *et al.*, 1979). The severity of loss and the particular components of yield affected depend primarily on the time of disease onset and the intensity of disease at particular growth stages of the soybean crops, with losses of up to 91% having been reported (Bromfield, 1984; Shanmugasundaram *et al.*, 2004).

A pathogen as destructive as this has to be controlled. Traditionally, the approach is to breed resistant cultivars. Research from the East, however, shows that cultivars with genetic resistance have not been stable or provided durable resistance in the field (Shanmugasundaram *et al.*, 2004). Cultivars with resistance genes are also not always the most productive, possessing traits such as shattering, small seed size and virus susceptibility, which make them undesirable (Poonpolgul, 2004). For this reason it is not uncommon for farmers to prefer planting high yielding cultivars and apply

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fungicides, rather than to use cultivars with resistance. This trend is common worldwide on any number of crops (Ward *et al.*, 1999). Therefore, without any stable resistant cultivars in South Africa (SA), fungicides remain the only effective method for controlling SBR caused by *P. pachyrhizi*.

Research on the use of fungicides to control SBR dates back to the 1960s (Kitani *et al.*, 1960a, 1960b, 1960c; Chu and Chuang, 1961; Huang and Kiu, 1961; Wang, 1961) when protectant fungicides such as Bordeaux mixture, copper, dichlone, dinocap, lime-sulphur, mancozeb, maneb, wettable sulphur and zineb were tested. Later, systemic fungicides such as oxycarboxin from the oxathiin chemical group and benomyl from the benzimidazole group were evaluated, but reports on their efficacy were mixed (Jan and Wu, 1971; Sangawongse, 1973; Thapliyal and Singh, 1974).

In the 1970s and 1980s the systemic fungicides triforine and triadimefon, from the sterol biosynthesis inhibitor (SBI) chemical group, were tested (Sangawongse, 1973; Hu and Yang, 1977; Osathaphant *et al.*, 1980; Pupipat and Choonhawong, 1981; Maiti *et al.*, 1982; Pupipat *et al.*, 1982; Maiti *et al.*, 1983; Junqueria *et al.*, 1984; Chen and Nguyen, 1988). In the last two decades changes in the chemistry of fungicides have been remarkable, with the result that very little information is available to guide the direction of fungicide control of SBR with the chemistry available today. At the time of planning research trials in SA Patil and Anahosur as well as Levy were the only researchers to have evaluated modern triazole fungicides for SBR control. Patil and Anahosur (1998) conducted trials with the hexaconazole and propiconazole triazole fungicides in India. Research in Zimbabwe (Levy, pers. comm.) also showed that fungicides from the triazole group had activity against the SBR pathogen.

Zimbabwe has limited registration of fungicides, as well as a different growing season length, with different climatic conditions to SA. Due to differences in cropping systems, weather patterns, availability of labour, fungicides and fungicide application equipment, amongst others, Bromfield (1984) proposed that specific recommendations must be developed for specific locales. For these reasons SA has had to expand the research into controlling SBR with fungicides.

By the 2003/2004 soybean growing season, chemical companies held emergency registrations for nine fungicides to control SBR in SA (Nel, pers. comm.)[2]. All products contained chemicals from the SBI group of fungicides, also known as the demethylation inhibitors. Seven of the nine fungicides are single-component chemicals with site-specific modes of action, mainly from the triazole sub-group of SBIs. The other two fungicide mixtures are from the SBI and benzimidazole fungicide groups.

This study was undertaken to determine the efficacy of the registered fungicides in controlling SBR, as well as to examine the efficacy potential of fungicides from different chemical groups.

2.2 Materials and methods

Trial site

Trials were conducted at Cedara agricultural research farm (29°32'S, 30°16'E and alt. 1071m), 20km north-west of Pietermaritzburg. Evaluations of different fungicides were conducted over three seasons, in 2001/2002, 2002/2003 and 2003/2004. The 2001/2002 and 2002/2003 trials were conducted on the same land, previously planted to potatoes. To reduce the build-up of soil pathogens and for good agricultural practice, the 2003/2004 trial was moved to a new land which had previously been planted to maize.

Land preparation

Soil samples were taken of the topsoil (0-15cm) and fertilizer was supplied according to Fertrec recommendations from the Cedara Fertilizer Advisory Service (Farina and Channon, 1988). Phosphorus was band-applied in the rows at planting to supply 21kg.ha^{-1} in 2001/2002 and 10.5kg.ha^{-1} in 2002/2003 (source: superphosphate (10.5%)). In 2003/2004, nitrogen, phosphorus and potassium were broadcast and disced in prior to planting to supply 46kg.ha^{-1}, 69kg.ha^{-1} and 92kg.ha^{-1}, respectively (source: 2:3:4 (40)).

[2] Ms Annette Nel, National Department of Agriculture, Technical Advisor to the Registrar Act 36/1947, Private Bag X343, Pretoria, 0001, South Africa

Climatic data

Automatic weather stations based at Cedara were used to collect information on rainfall and temperature over the growing seasons. Long-term monthly rainfall and temperature averages at Cedara were supplied by the Institute for Soil, Climate and Water[3].

Trial design

Two separate fungicide efficacy trials (Trials 1a & b) were planted in the 2001/2002 season. Since the trials were designed as efficacy x rate trials the treatment number was too large for reliable analysis in a single trial. Trials were laid out in randomised complete block design, replicated three times. Plots comprised four 5m rows spaced 45cm apart, except in 2003/2004, when six rows were planted per plot. The number of rows was increased to make the distribution of inoculum among plots more uniform. The two central rows of each plot were used as data rows for disease evaluation and yield determination. The unsprayed outer rows of the plots are referred to as border rows.

Planting

Trials were hand-planted with the soybean cultivar LS666 (cultivar reactions to SBR had not been determined at the time of planting since the pathogen had only just arrived in SA). Planting date, seeding rate and final plant population are shown in Table 2.1 Soybeans were inoculated with *Bradyrhizobium japonicum* ((Kirchner) Jordan) at planting to enhance good nodule formation. Normal pest and weed control practices for the area were followed.

Table 2.1 Planting date, cultivar, seeding rate and plant population of soybean trials planted at Cedara in the 2001 – 2004 growing seasons

Season	Planting date	Cultivar & expected germination	Seeding rate (seeds.ha^{-1})	Plant population (plants.ha^{-1})
2001/2002	21 December 2001	LS666 (70-79% germination)	350 000	215 000 (61% germination)
2002/2003	13 November 2002	LS666 (>90% germination)	300 000	270 000 (90% germination)
2003/2004	12 November 2003	LS666 (>90% germination)	300 000	260 000 (87% germination)

[3] Institute for Soil, Climate and Water, Agromet Section, Private Bag X79, Pretoria, 0001, South Africa

Fungicide treatments

Fungicide treatments were selected from commercially available products which had received emergency registration in South Africa for the purpose of controlling SBR. These were from the triazole and benzimidazole chemical groups. These groups were chosen because they were known to be effective against SBR (Levy, pers. comm., 2001). Fungicides from the protectant and strobilurin chemical classes were also evaluated. Punch C was used as a performance standard against which other fungicides could be compared, as it was one of the first fungicides to receive emergency registration when SBR was identified in SA. Fungicides evaluated in 2001/2002 and 2002/2003 included Amistar, Bayfidan, Capitan, Denarin, Dithane M-45 applied with Bond (a sticker), Folicur, Impact, Punch Xtra, Score and an experimental triazole/strobilurin premix (Fungicide X). In 2003/2004, at the request of the manufacturers, Early Impact and another experimental triazole/strobilurin premix (Fungicide Y) were evaluated. Shavit, a generic of Bayfidan, was also evaluated. Rates of fungicides used were those suggested by the manufacturers. In 2001/2002 the first two fungicide applications were applied at double the suggested rate due to a calibration error. This was corrected for the third fungicide application. Fungicides evaluated are listed in Table 2.2.

Three applications were made at 21d intervals (Table 2.3). In 2001/2002 the initial application was made at early flowering (R1 growth stage). In 2002/2003 the initial application was made just before flowering (V11 growth stage), while in 2003/2004 the first application was made at the late flowering stage (R2 – R3 growth stage).

From 2001 – 2003, full cover fungicide sprays of 160ℓ.ha^{-1} at 200kPa pressure were applied to the central two rows of each plot, leaving the outer rows as border rows to reduce interplot interference. Fungicide spray solutions were applied using a CO_2-pressured back-pack sprayer with a horizontal spray-boom comprising two Spraying Systems TeeJet No8001 nozzles spaced 45cm apart. In 2003/2004, all fungicide spray solutions were applied with a CO_2-pressured backpack sprayer with a horizontal spray-boom and two Albuz ATR 80 hollow cone nozzles spaced 45cm apart. Full cover sprays of 420ℓ.ha^{-1} at 200kPa pressure were applied to the two central rows.

Table 2.2 Fungicides evaluated at Cedara from 2001 to 2004 for the control of soybean rust caused by *Phakopsora pachyrhizi*

Active ingredient (a.i.)	Concentration & formulation	Trade name	Manufacturer	Application rate (.ha^{-1})		Total amount of active ingredient applied during season		
				ml	g a.i.	2001/2002†	2002/2003	2003/2004
azoxystrobin	250 SC	Amistar	Syngenta	300	75	375	225	225
carbendazim & flusilazole‡	250 SC 125	Punch Xtra	Du Pont	800	200 100	1000 500	600 300	450 225
carbendazim & flutriafol	150 SC 94	Early Impact	Cheminova	1200	180 112.8	-	-	540 338.40
difenoconazole	250 EC	Score	Syngenta	325	81.25	406.25	243.75	243.75
flusilazole	250 EW	Capitan	Du Pont	400	100	500	300	300
flusilazole & carbendazim	250 SC 125	Punch C	Du Pont	400	100 50	500 250	300 150	300 150
flutriafol (& carbendazim)°	125 SC (117.5) (250)	Impact	Cheminova	1000	125	625	375	367.50 (250)
mancozeb	800 WP	Dithane M-45	Algro-Chem	2kg	1600	8000	4800	4800
tebuconazole	250 EW	Folicur	Bayer	750	187.5	937.5	562.5	562.50
triadimenol	250 DC	Bayfidan	Bayer	500	125	625	375	375
triadimenol	250 EC	Shavit	Makhteshim-Agan	500	125	-	-	375
triforine	190 EC	Denarin	BASF	1500	285	1425	855	855
triazole & strobilurin §	225 SC	Fungicide X	BASF	700	157.5	785	383.25	405
triazole & strobilurin	125 SC 200	Fungicide Y	Syngenta	375	46.88 75	-	-	140.63 225

† The first two applications received double the chemical dose. This error was corrected for the third application.

‡ In 2003/2004 this dosage rate was lowered to 600ml.ha^{-1}, as the manufacturers had reduced the registered application rate.

° In 2003/2004, due to the wrong product being supplied, the first application was made with flutriafol (117.5g) + carbendazim (250g). Second and third applications were made with flutriafol (125g).

§ In 2002/2003 a formulation containing 182.5g was used at a rate of 700ml.ha^{-1}.

In 2003/2004, the 225g formulation was applied at a rate of 600ml.ha^{-1}, as requested by the manufacturer.

Artificial inoculation

In an effort to ensure even inoculum pressure throughout the trials, field plots were artificially inoculated with *P. pachyrhizi*. In 2001/2002, freshly collected *P. pachyrhizi* spores from early-infected trap plants were sprayed in a solution containing 0.01% household dishwashing liquid, using a knapsack sprayer, over the plots on 4 and 11

March at R3 growth stage (72 and 79d after planting (DAP), respectively). In 2002/2003, soybeans were planted in plastic bags (one plant per bag) and grown in tunnels, where favourable conditions allowed them to become naturally infected. On 19 February (99 DAP), these bags were placed in every second plot within the trial. In 2003/2004, five infected leaves were stapled singly, at approximately1m intervals, onto plants in the first row of each six-row plot, 98 DAP.

Table 2.3 Time (days after planting) of flowering of soybeans and fungicide applications for trials conducted at Cedara from 2001 - 2004 for the control of soybean rust caused by *Phakopsora pachyrhizi*

Season	First flower (DAP)*	Fungicide applications (DAP)		
		1st spray	2nd spray	3rd spray
2001/2002	60	63	84	105
2002/2003	70	64	85	106
2003/2004	71	82	103	124

* DAP = days after planting

Disease assessment

Disease severity assessments were made regularly, at twice-weekly intervals on plants in the data rows, from flowering (R1) until physiological maturity (R7) of the crop. A plot rating was given after examining at least ten plants in the central two rows. Disease was assessed according to a rating scale developed at Cedara (Table 2.4). The scale uses the position of rust pustules on the plant, pustule density, chlorosis and defoliation as parameters for determining disease severity. Data were used for calculating the area under disease progress curve (AUDPC), which summarises the disease epidemic. The AUDPC was calculated using a trapezoidal integration program (Berger, 1981) and was standardised (SAUDPC) by dividing the AUDPC value by the duration of the epidemic. The SAUDPC allows for comparisons of disease from one season to another.

Harvesting

Leaving a 0.5m border on either end of each of the 5m rows, the central 4m of the central two rows in each plot, were hand-harvested. Grain yields were adjusted to a moisture content of 12.5% and expressed as $kg.ha^{-1}$. Seed mass (g) was determined for 100 seeds and corrected for moisture content. Protein and oil analyses were conducted

on the seed using the Dumas combustion method (Dumas, 1831) and supercritical fluid extraction, respectively.

Table 2.4 Rating scale used for assessing soybean rust caused by *Phakopsora pachyrhizi* on soybeans in trials at Cedara

Rating	Percentage	Description
0	0%	no visible pustules (disease)
1	< 5%	light density pustules (with flecking) of lower leaves
2	5 – 19%	medium density of pustules and chlorosis of lower 1/3 leaves
3	20 -29%	lower 1/3 leaves chlorotic with medium/ dense pustules above bottom third & light infection to top of plant
4	30 – 39%	severe pustules with chlorosis on lower 50% of plant; bottom leaves starting to defoliate
5	40 – 59%	high density of pustules with chlorosis of lower 2/3 of leaves; progressive defoliation
6	60 – 69%	only 1-2 green leaves left on plant, although infected
7	70 – 79%	severe pustules and spread of chlorosis over entire plant (leaves still turgid and firm)
8	80 – 94%	widespread defoliation (leaves shrivelled/ mummified and withered)
9	≥ 95%	complete defoliation due to disease and early maturation - only stalks and pods remain

Statistical analyses

Statistical analyses of trial data (final disease severity, SAUDPC, yield, seed mass, protein and oil content) were conducted by analysis of variance (ANOVA) using Genstat 6.1. Mean separations were based on the least significant differences (LSD) or Duncan's multiple range test (DMRT) at the 5% level of probability. The LSD test is used when planned pair comparisons are being made and when the treatment number is not too large (less than six). DMRT is used when the treatment number exceeds five (Steel and Torrie, 1981).

2.3 Results

Flowering, first sign of disease and physiological maturity of the crop are presented in Table 2.5.

Table 2.5 Time (days after planting) of flowering, first sign of disease and physiological maturity for soybean rust trials, caused by *Phakopsora pachyrhizi*, conducted on soybeans at Cedara from 2001 - 2004

Season	First flower (DAP)*	First sign of disease (DAP)	Physiological maturity (DAP)
2001/2002	60	89	123
2002/2003	70	105	138
2003/2004	71	106	141

* DAP = days after planting

Climatic data

Although rainfall was good early in the season, the 2001/2002 season was characterised by a mid-season drought, which persisted from flowering (mid-February) until physiological maturity of the soybean trial crop. The 2002/2003 season was warm, but very dry, for the duration of the growing season. In contrast, the 2003/2004 season was warm, with well-distributed rainfall throughout the growing season (Table 2.6).

Table 2.6 Rainfall and temperature data at Cedara for the 2001 - 2004 soybean growing seasons

	November	December	January	February	March	April
Rainfall (mm)						
2001/2002	126	158	153	38	59	61
2002/2003	52	84	47	66	95	44
2003/2004	104	103	162	154	89	4
Mean monthly *	111	132	135	123	111	52
Mean temperature (°C)						
2001/2002	18.5	18.7	20.7	18.6	19.5	17.6
2002/2003	16.7	19.1	19.8	20.8	19.7	17.5
2003/2004	17.6	18.8	19.7	19.3	18.5	17.3
Mean monthly *	18.0	19.5	20.2	20.2	19.3	16.7

* Long-term mean from 01/07/1914 to 30/06/2004

2.3.1 2001/2002 season

Final disease severity reached 28% in unsprayed plots. The yield average for Trial 1a (3751kg.ha^{-1}) was lower than that of Trial 1b (4233kg.ha^{-1}). Similarly, seed mass was also lower in Trial 1a (16.43g.100 seed^{-1}) than Trial 1b (16.94g.100 seed^{-1}). Protein and oil means were 36.48% and 19.63%, respectively in Trial 1a and 35.65% and 20.25%, respectively, in Trial 1b.

Trial 1a

Final disease severity

Fungicide treatments reduced final disease severity (Table 2.7a). The unsprayed control resulted in a mean final disease severity of 28% compared with 2% or less in fungicide sprayed treatments. There were no differences among the fungicide treatments (Table 2.7b).

Standardised area under disease progress curve (SAUDPC)

Fungicide treatments reduced SAUDPC. There were no differences among the fungicides (Tables 2.7b).

Yield

The mean of fungicide sprayed treatments (3860kg.ha^{-1}) resulted in a higher yield than unsprayed treatments (2991kg.ha^{-1}). ANOVAs are presented in Table 2.7a. There were differences among fungicide treatments. Capitan, Dithane M-45, Folicur, Punch C and Punch Xtra grouped together, producing the highest yields (Fig. 2.1). Applications of Bayfidan and Score resulted in yields not different from the highest yielding treatments, although they were also not different from the untreated control (Table 2.7b).

Seed mass

The mean of fungicide sprayed treatments (16.54g.100 seed^{-1}) resulted in a higher seed mass than unsprayed treatments (15.65g.100 seed^{-1}). ANOVAs are presented in Table 2.7a. Applications of Dithane M-45 resulted in the highest seed mass of all fungicides tested, although it was not significantly different from Folicur, Punch C and Punch Xtra, which resulted in the next highest seed mass, followed by Score (Fig. 2.2). Bayfidan

Table 2.7a ANOVA of soybean rust final disease severity (percentage), log transformed standardised area under disease progress curve (SAUDPC), yield (kg.ha^{-1}), seed mass (g.100 seed^{-1}), protein and oil content (percentage) for fungicides evaluated in Trial 1a (2001/2002)

	Main effect	Degrees of freedom	Final disease severity	SAUDPC	Yield	Seed mass	Protein	Oil
Mean square	Fungicide	7	273.851	1.207	455159	0.728	1.423	0.466
	Unsprayed vs Sprayed	1	1913.625	8.401	1978003	2.096	7.757	0.829
	Residual	14	1.887	0.040	133665	2.858	2.531	0.962
F-value	Fungicide		145.13	30.54	3.41	3.57	0.56	0.48
	Unsprayed vs Sprayed		1014.16	212.64	14.80	10.27	3.07	0.86
F-probability	Fungicide		<0.001 **	<0.001 **	0.024 *	0.021 *	0.774	0.831
	Unsprayed vs Sprayed		<0.001 **	<0.001 **	0.002 **	0.006 **	0.102	0.369
% c.v.			29.2	61.7	9.7	2.8	4.4	5.0
Level of significance			P = > 0.05 n.s.		P = ≤ 0.05 *		P = ≤ 0.01 **	

Table 2.7b Table of means for soybean rust final disease severity (percentage), log transformed standardised area under disease progress curve (SAUDPC), yield (kg.ha^{-1}), seed mass (g.100 seed^{-1}), protein and oil content (percentage) for fungicides evaluated in Trial 1a (2001/2002)

Treatment	Final disease severity * (%)		SAUDPC * (log)		Yield * (kg.ha^{-1})		Seed mass * (g.100 seed^{-1})		Protein (%)	Oil (%)
Bayfidan	1.33	a	0.19	a	3463	ab	16.04	bc	35.83	19.42
Capitan	1.67	a	0.12	a	3729	a	16.12	bc	36.37	19.77
Dithane M-45	2.00	a	0.09	a	4091	a	17.19	a	36.50	19.29
Folicur	1.33	a	0.06	a	4098	a	16.90	ab	36.77	19.66
Punch C	1.33	a	0.10	a	4102	a	16.60	ab	35.81	20.27
Punch Xtra	0.67	a	0.03	a	3911	a	16.55	ab	36.20	20.07
Score	1.00	a	0.09	a	3622	ab	16.38	abc	36.39	19.39
Unsprayed	28.33	b	1.89	b	2991	b	15.65	c	37.99	19.13

* Means followed by a different letter in the same column are significantly different at P = 0.05, using Duncan's multiple range test

and Capitan resulted in the lowest seed masses of the fungicide treatments. Applications of Bayfidan, Capitan and Score resulted in seed masses which were not significantly different from the unsprayed control (Table 2.7b).

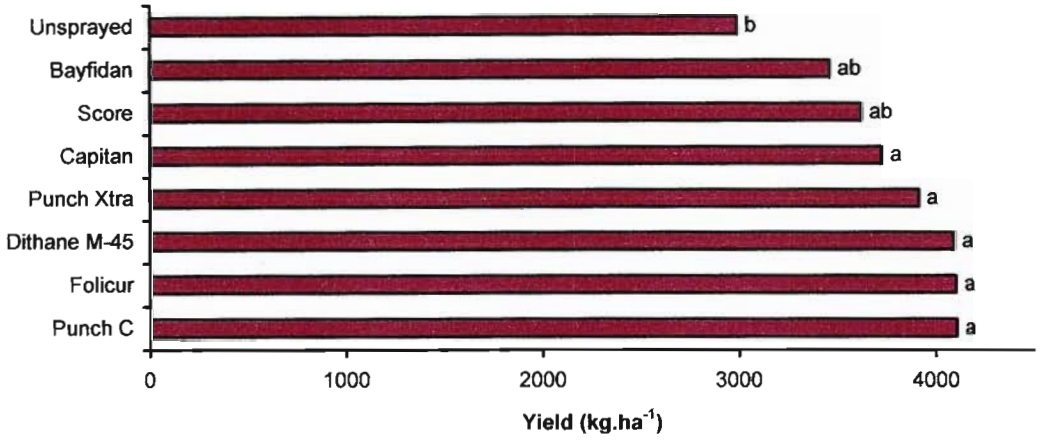

Figure 2.1 Soybean yield (kg.ha^{-1}) response to fungicides evaluated for the control of SBR caused by *Phakopsora pachyrhizi* in Trial 1a at Cedara (2001/2002)

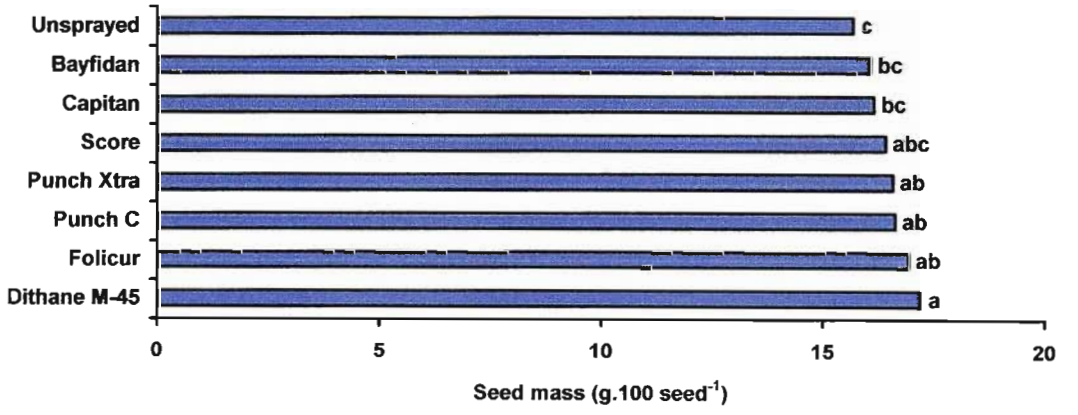

Figure 2.2 Soybean seed mass (g.100 seed^{-1}) response to fungicides evaluated for the control of SBR caused by *Phakopsora pachyrhizi* in Trial 1a (2001/2002)

Protein

There were no significant differences among fungicide treatments or between the mean of the fungicide sprayed and the unsprayed control (Table 2.7a).

Oil

There were no significant differences among fungicide treatments or between the mean of the fungicide sprayed and the unsprayed control (Table 2.7a).

Trial 1b

Final disease severity

The coefficient of variation was high. A square root transformation of the data resulted in a lower, yet still high, coefficient of variation (43.3%). The ANOVA is presented for the transformed data in Table 2.8a. For comparison across other seasons both the percentage and the transformed data are presented in the table of means (Table 2.8b). Means separation letters are, however, only given for the transformed data. Fungicide treatments reduced final disease severity (Table 2.8a). The unsprayed control resulted in a mean final disease severity of 27%, compared with 3.5% or less in fungicide sprayed treatments. There were no differences among the fungicide treatments (Table 2.8b).

Standardised area under disease progress curve (SAUDPC)

Fungicide treatments reduced SAUDPC (Table 2.8a). There were no differences among the fungicides (Table 2.8b).

Yield

There were no significant yield differences among fungicides, but the mean of the fungicides (4361kg.ha^{-1}) resulted in significantly higher yields than the unsprayed control (3593kg.ha^{-1}).

Seed mass

The mean of fungicide treatments (17.18g.100 seed^{-1}) resulted in a significantly higher seed mass than the unsprayed control (15.76g.100 seed^{-1}). The ANOVA is presented in Table 2.8a. Fungicide X resulted in the highest seed mass, but was not significantly different from Amistar, Denarin and Punch C, which grouped together with the next highest seed mass (Fig. 2.3). Impact, which resulted in the lowest seed mass of all the fungicide treatments, was not significantly different from Amistar, Denarin and Punch C, but was also not significantly different from the unsprayed control (Table 2.8b).

Table 2.8a ANOVA of soybean rust final disease severity (square root transformed), log transformed standardised area under disease progress curve (SAUDPC), yield (kg.ha^{-1}), seed mass (g.100 seed^{-1}), protein and oil content (percentage) for fungicides evaluated in Trial 1b (2001/2002)

	Stratum	Degrees of freedom	Final disease severity	SAUDPC	Yield	Seed mass	Protein	Oil
Mean square	Fungicide	5	7.895	1.379	463315	1.435	2.953	0.569
	Unsprayed vs Sprayed	1	38.178	6.833	1471976	5.012	7.738	1.781
	Residual	10	0.657	0.049	230274	0.345	3.447	0.893
F-value	Fungicide		12.02	27.90	2.01	4.16	0.86	0.64
	Unsprayed vs Sprayed		58.11	138.25	6.39	14.54	2.24	1.99
F-probability	Fungicide		<0.001 **	<0.001 **	0.162	0.026 *	0.541	0.677
	Unsprayed vs Sprayed		<0.001 **	<0.001 **	0.030 *	0.003 **	0.165	0.188
% c.v.			43.3	48.0	11.3	3.5	5.2	4.7
Level of significance			P = > 0.05 n.s.		P = ≤ 0.05 *		P = ≤ 0.01 **	

Table 2.8b Table of means for final disease severity (percentage and square root transformed), log transformed standardised area under disease progress curve (SAUDPC), yield (kg.ha^{-1}), seed mass (g.100 seed^{-1}), protein and oil content for (percentage) fungicides evaluated in Trial 1b (2001/2002)

Treatment	Final disease severity *			SAUDPC *		Yield	Seed mass *		Protein	Oil
	(%)	(sqrt)		(log)		(kg.ha^{-1})	(g.100 seed^{-1})		(%)	(%)
Amistar	3.50	1.72	a	0.30	a	4673	17.26	ab	34.66	20.65
Denarin	1.67	1.28	a	0.17	a	4169	17.24	ab	35.24	20.41
Fungicide X	2.00	1.15	a	0.18	a	4241	17.76	a	35.27	19.95
Impact	1.00	0.80	a	0.10	a	4103	16.59	bc	36.65	20.27
Punch C	2.00	1.15	a	0.18	a	4618	17.04	ab	34.97	20.67
Unsprayed	26.67	5.13	b	1.84	b	3593	15.76	c	37.12	19.55

* Means followed by a different letter in the same column are significantly different at P = 0.05, using Duncan's multiple range test

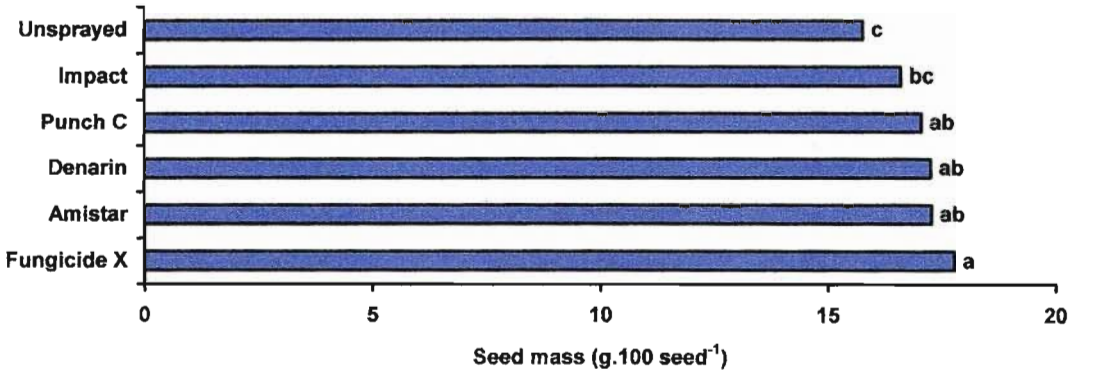

Figure 2.3 Soybean seed mass (g.100 seed^{-1}) response to fungicides evaluated for the control of SBR caused by *Phakopsora pachyrhizi* in Trial 1b (2001/2002)

Protein

There were no significant differences among fungicide treatments or between the mean of the fungicide sprayed and the unsprayed control (Table 2.8a).

Oil

There were no significant differences among fungicide treatments or between the mean of the fungicide sprayed and the unsprayed control (Table 2.8a).

2.3.2 2002/2003 season

The very hot and dry conditions throughout the season resulted in unfavourable conditions for disease development. Disease severity was low and coefficients of variation were very high for all disease parameters. Transformations were not helpful and therefore the final disease severity is presented as a percentage for comparison across other seasons. Final disease severity was only 27.5% in the unsprayed control. Coefficients of variation were low for yield components (yield and seed mass) and seed quality (protein and oil content) but F-test probabilities were high, with no significant differences. Differences between sprayed and unsprayed treatments are therefore not presented. The mean yield and seed mass for the season was 3952kg.ha^{-1} and

18.69g.100 seed^{-1}, respectively. Protein and oil contents were 35.48% and 20.33%, respectively.

Final disease severity

There were no significant differences in final disease severity (Table 2.9a). Final disease severity only reached 27.5% in the unsprayed control. Dithane M-45 and Amistar treated soybeans resulted in final disease severities of 5.7% and 3.5%, respectively.

Standardised area under disease progress curve (SAUDPC)

ANOVA tables are presented in Table 2.9a. Dithane M-45 and Amistar treatments were not significantly different from the unsprayed control (Table 2.9b & Fig. 2.4).

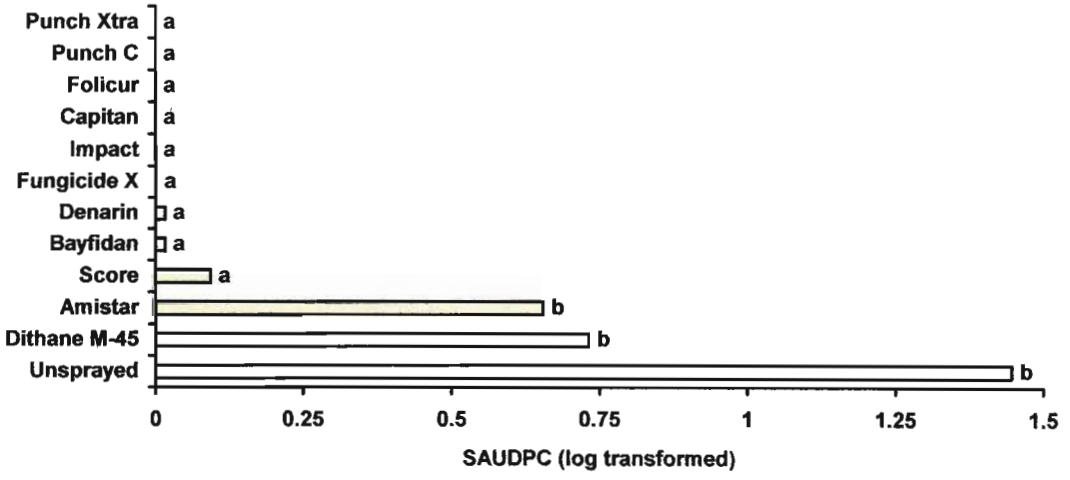

Figure 2.4 Standardised area under disease progress curve (SAUDPC log transformed) response to fungicides evaluated for the control of SBR caused by *Phakopsora pachyrhizi* in 2002/2003

Yield

There were no yield increases from fungicide applications (Table 2.9a).

Seed mass

There were seed mass benefits to fungicide spraying (Table 2.9a).

Table 2.9a ANOVA of soybean rust final disease severity (percentage), log transformed standardised area under disease progress curve (SAUDPC), yield (kg.ha^{-1}), seed mass (g.100 seed^{-1}), protein and oil content (percentage) for fungicides evaluated in 2002/2003

	Stratum	Degrees of freedom	Final disease severity	SAUDPC	Yield	Seed mass	Protein	Oil
Mean square	Fungicide	11	184.25	0.637	119190	1.060	4.228	1.762
	Residual	22	85.91	0.210	169849	1.220	4.573	0.910
F-value	Fungicide		2.14	3.03	0.70	0.87	0.92	1.94
F-probability	Fungicide		0.062	0.013 *	0.724	0.581	0.535	0.090
% c.v.			282.4	185.7	10.4	5.9	6.0	4.7
Level of significance			P = > 0.05 n.s.		P = ≤ 0.05 *		P = ≤ 0.01 **	

Table 2.9b Table of means for soybean rust final disease severity (percentage), log transformed standardised area under disease progress curve (SAUDPC), yield (kg.ha^{-1}), seed mass (g.100 seed^{-1}), protein and oil content (percentage) for fungicides evaluated in 2002/2003

Treatment	Final disease severity (%)	SAUDPC * (log)		Yield (kg.ha^{-1})	Seed mass (g.100 seed^{-1})	Protein (%)	Oil (%)
Amistar	3.5	0.66	b	4043	19.48	34.48	20.87
Bayfidan	0.3	0.02	a	4111	18.12	36.02	20.65
Capitan	0.0	0.00	a	4102	18.67	34.61	20.86
Denarin	0.3	0.02	a	3736	19.02	37.15	19.97
Dithane M-45	5.7	0.73	b	4137	18.49	36.17	20.49
Fungicide X	0.0	0.00	a	4175	19.38	34.72	20.64
Folicur	0.0	0.00	a	3623	19.08	36.89	18.18
Impact	0.0	0.00	a	3878	18.47	34.99	20.32
Punch C	0.0	0.00	a	4170	17.74	37.17	20.22
Punch Xtra	0.0	0.00	a	3703	19.43	33.43	21.28
Score	2.0	0.09	a	3956	18.57	35.05	20.27
Unsprayed	27.5	1.45	b	3785	17.88	35.11	20.21

* Means followed by a different letter in the same column are significantly different at P = 0.05, using Duncan's multiple range test

Protein

There were no significant differences among treatments (Table 2.9a).

Oil

There was no significant difference in oil content (Table 2.9a). Orthogonal contrasts showed, however, that the oil content for Folicur (18.18%) treatments was significantly lower than Denarin (19.97%) and hence all other fungicides as well (Fig. 2.5).

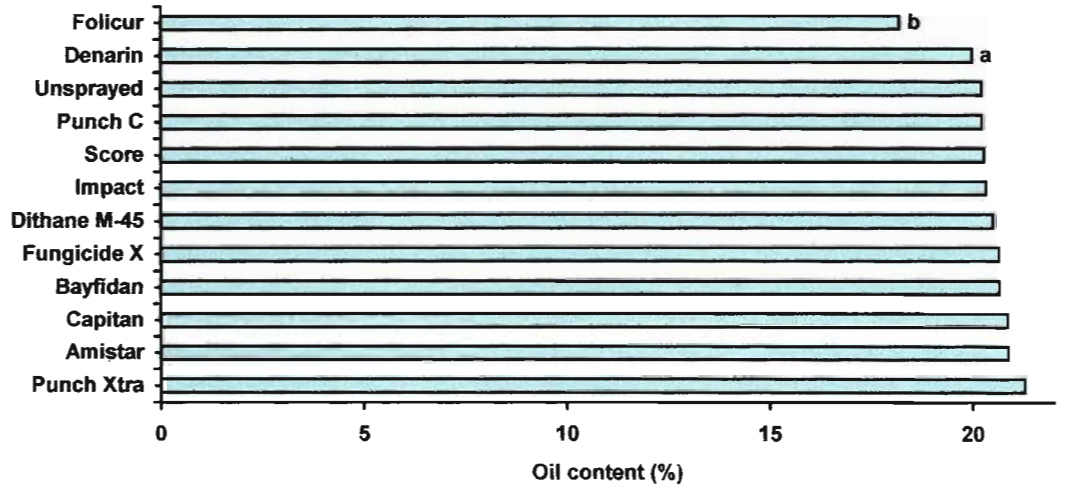

Figure 2.5 Soybean oil content (%) response to fungicides evaluated for the control of SBR caused by *Phakopsora pachyrhizi* in 2002/2003

2.3.3 2003/2004 season

The consistent rainfall led to conditions that were not only good for disease development, but also for plant growth. Final disease severity reached 83% in the unsprayed control, compared with 28% and 3% in 2001/2002 and 2002/2003, respectively. Despite the high levels of disease, with the application of fungicides high yields and seed masses were obtained. The average yield and seed mass were 4395kg.ha^{-1} and 20.30g.100 seed^{-1}, respectively. Protein and oil contents were 38.32% and 16.09%, respectively.

Final disease severity

Fungicides reduced final disease severity, with the mean of the sprayed treatments resulting in a final disease severity of 8% (Tables 2.10a and b). Final disease severity was 83% in the unsprayed control. Amistar, Dithane M-45 and Bayfidan treatments all resulted in final disease severities of 40%, 27% and 17%, respectively, with all other fungicides resulting in final disease severities of 10% or less (Table 2.10b). Early Impact, Folicur and 'Impact with carbendazim' treated plots had no visible disease at the final assessment.

Standardised area under disease progress curve (SAUDPC)

Fungicides reduced SAUDPC (Table 2.10a). Although significantly better than the unsprayed control, Amistar and Dithane M-45 treatments gave the least disease control of all the fungicide treatments (Table 2.10b). Early Impact, Folicur and 'Impact with carbendazim' treatments gave complete disease control for the duration of the growing period.

Yield

All fungicide treatments resulted in higher yields than the unsprayed control (3123kg.ha^{-1}). Amistar and Bayfidan treatments, amongst others, resulted in the lowest yields of all the fungicides tested (Fig. 2.6), while 'Impact with carbendazim', amongst others, resulted in the highest yield (Fig. 2.7). Fungicides which supplied 250g, or more, of carbendazim as active ingredient in the season, in combination with a fungicide having another mode of action, resulted in higher yields compared to all other fungicides.

Seed mass

All fungicide treatments resulted in higher seed mass than the unsprayed control (15.53g.100 seed^{-1}). The ANOVA is presented in Table 2.10a. Amistar (19.05g.100 seed^{-1}), amongst others, resulted in the lowest seed mass, although it was significantly higher than the unsprayed control (Table 2.10b). 'Impact with carbendazim', amongst others, resulted in the highest seed mass (Fig. 2.7).

Table 2.10a ANOVA of soybean rust final disease severity (square root transformed), log transformed standardised area under disease progress curve (SAUDPC), yield (kg.ha^{-1}), seed mass (g.100 seed^{-1}), protein and oil content (percentage) for fungicides evaluated in 2003/2004

	Stratum	Degrees of freedom	Final disease severity	SAUDPC	Yield	Seed mass	Protein	Oil
Mean square	Fungicide	14	20.984	3.223	709263	6.644	0.508	0.177
	Residual	28	0.622	0.125	157922	0.916	1.301	0.591
F-value	Fungicide		33.76	25.82	4.49	7.25	0.39	0.30
F-probability	Fungicide		<0.001 **	<0.001 **	<0.001 **	<0.001 **	0.966	0.990
% c.v.			31.6	43.2	9.0	4.7	3.0	4.8
Level of significance			P = > 0.05 n.s.		P = ≤ 0.05 *		P = ≤ 0.01 **	

Table 2.10b Table of means for final disease severity (percentage and square root transformed), log transformed standardised area under disease progress curve (SAUDPC), yield (kg.ha^{-1}), seed mass (g.100 seed^{-1}), protein and oil content (percentage) for fungicides evaluated in 2003/2004

Treatment	Final disease severity *			SAUDPC *		Yield *		Seed mass *		Protein	Oil
	(%)	(sqrt)		(log)		(kg.ha^{-1})		(g.100 seed $^{-1}$)		(%)	(%)
Amistar	40.00	6.23	g	2.43	d	3884	c	19.05	c	38.23	15.67
Bayfidan	8.33	2.49	cd	0.63	ab	3873	c	20.46	abc	37.89	16.21
Capitan	0.68	0.74	ab	0.05	a	4677	ab	20.67	abc	38.11	16.11
Denarin	16.67	3.90	ef	1.73	c	4447	abc	20.20	abc	38.27	16.00
Dithane M-45	26.67	5.14	fg	1.90	cd	4088	bc	19.96	bc	38.05	15.99
Early Impact	0.00	0.00	a	0.00	a	4854	ab	20.99	ab	38.44	15.95
Fungicide X	0.67	0.67	ab	0.16	ab	4737	ab	21.25	ab	38.62	16.54
Fungicide Y	5.00	2.24	cd	0.38	ab	4472	abc	20.68	abc	38.26	16.18
Folicur	0.00	0.00	a	0.00	a	4544	abc	20.97	ab	37.41	15.98
Impact	0.00	0.00	a	0.00	a	4973	a	22.06	a	38.35	16.15
Punch C	0.35	0.41	a	0.09	a	4455	abc	20.80	abc	38.74	16.46
Punch Xtra	2.33	1.41	abc	0.36	ab	4845	ab	21.31	ab	38.11	16.39
Score	10.00	2.98	de	0.82	b	4283	abc	20.43	abc	38.78	15.99
Shavit	4.33	2.07	bcd	0.40	ab	4665	ab	20.09	bc	38.44	16.00
Unsprayed	83.33	9.12	h	3.32	e	3123	d	15.53	d	39.15	15.73

* Means followed by a different letter in the same column are significantly different at P = 0.05, using Duncan's multiple range test

For the most part, seed mass and yield correlated positively. A notable exception to this was Bayfidan, which, for the low yield produced, had a higher than expected seed mass (Fig. 2.7). Conversely, Shavit had a higher yield than expected from its low seed mass.

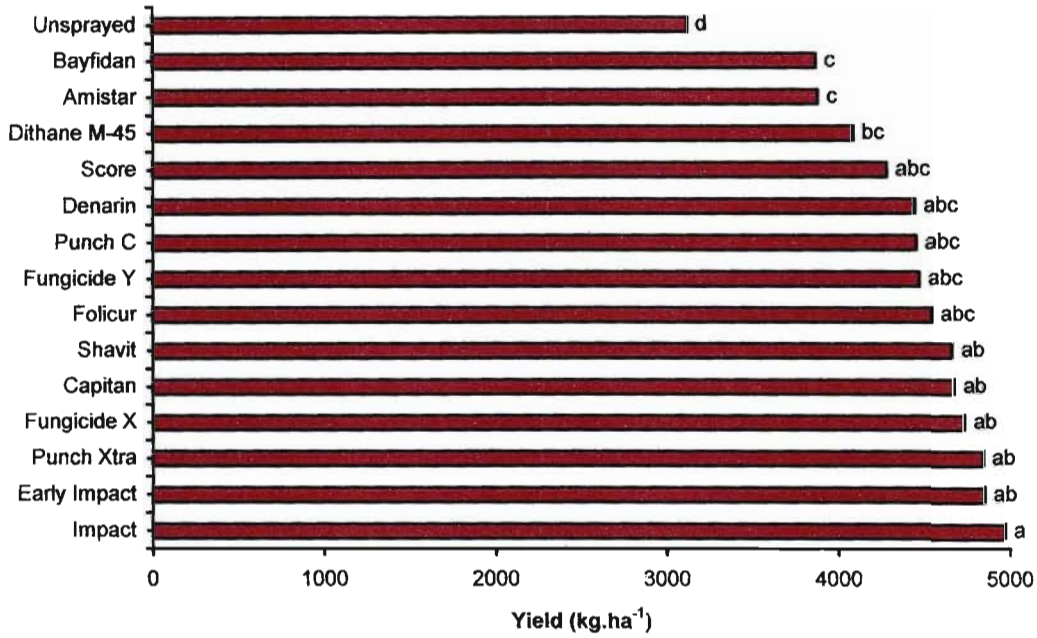

Figure 2.6 Soybean yield ($kg.ha^{-1}$) response to fungicides evaluated for the control of SBR caused by *Phakopsora pachyrhizi* in 2003/2004

Protein

There were no significant differences among treatments (Table 2.10a).

Oil

There were no significant differences among treatments (Table 2.10a).

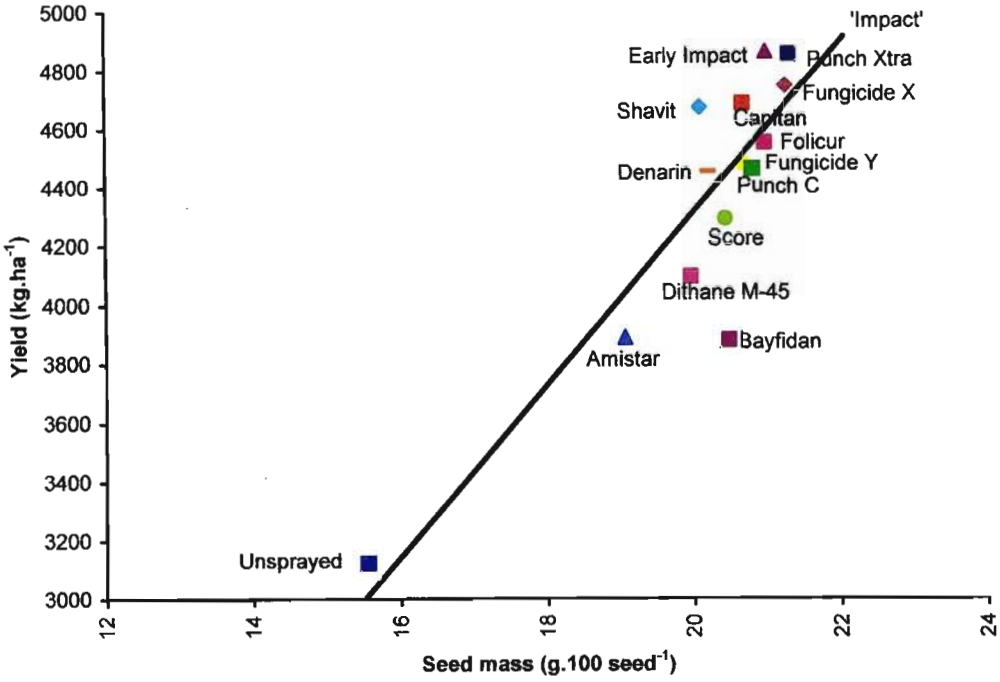

Figure 2.7 Relationship between soybean yield (kg.ha^{-1}) and seed mass (g.100 seed^{-1}) for fungicides evaluated for the control of SBR caused by *Phakopsora pachyrhizi* in 2003/2004

2.4 Discussion

Providing uniform inoculum levels across the trials proved to be the biggest challenge in this research. Spores are wind-dispersed, with new infections beginning when spores blow into the crop from alternate hosts scattered around outside the soybean field. This results in focal areas of disease within trials. If the epidemic begins at one point in a trial, the inoculum spreads outwards from that focal point, resulting in some plots with high levels of disease, and others with very little. This is different from other wind-dispersed diseases like grey leaf spot in maize (*Zea mays* (L.)) caused by *Cercospora zeae-maydis* (Tehon and Daniels) where new seasonal infections begin from infected maize stover within maize lands, and result in uniform levels of infection throughout the plant stand. Statistical analysis requires that there is homogeneity in the experimental units, which is, as explained, difficult to achieve with diseases that are wind-dispersed. The high coefficients of variation for disease ratings highlight this problem.

Various attempts were made to normalise inoculum levels throughout the trial plots. The reasons for their failure or success are discussed here. In 2001/2002, two attempts were made to spray freshly collected spores in a soapy solution over the plots. This approach was unsuccessful due to extremely high temperatures (>30°C without rainfall) for several days following application of spores. In 2002/2003, soybeans were planted in plastic bags and grown in tunnels, where favourable conditions allowed them to become naturally infected. These bags were then placed in every second plot within the trial. Although supplementary irrigation was provided, the hot and dry conditions resulted in overall low infection (28% in the unsprayed treatment). Unlike the previous season, this technique provided longer exposure, and if it had been used in 2001/2002 may have had a good chance of success, but the unfavourable environmental conditions of the 2002/2003 season also failed to provide favourable conditions for infection. In 2003/2004, infected leaves were stapled onto the border rows of plants in the plots. Plot sizes were also increased from four to six rows, resulting in larger unsprayed areas to increase inoculum levels. Weather conditions were more favourable than the previous seasons for disease development, with the result that the coefficient of variation for disease was lowest in the 2003/2004 season.

Fungicides containing a mixture of products with different modes of action are the key to fungicide resistance management where more than one application is needed. The pathogen has to develop resistance to fungicides at more than one site, which is harder than overcoming a fungicide which works by disrupting fungus physiology at a single site. The 2003/2004 season, with favourable disease conditions, showed the benefits of using fungicide mixtures rather than single component fungicides. The fungicides which gave the best disease control were mostly those which contained fungicides from two chemical classes which have different modes of action. Fungicides which resulted in poor disease control were all single component fungicides. Amistar, a strobilurin fungicide, was not effective on its own. However, when mixed with a triazole (Fungicide Y), disease levels were significantly lower. Triforine, the active ingredient of Denarin, is one of the oldest sterol biosynthesis inhibitor (SBI) fungicides. It was first tested for use, as Saprol, against SBR in Thailand in the late 1970s (Osathaphant *et al.*, 1980). Triforine is in the piperazine group of fungicides, developed before the triazole group. Piperazines and triazoles both belong to the SBI class of fungicides. All current

SA registrations for SBR control are triazoles, except for triforine. As a piperazine fungicide, Denarin did not control SBR as effectively as triazole fungicides.

The best disease control data resulted from the 2003/2004 trial. Amistar, a strobilurin fungicide with single-site activity, was less effective than Dithane M-45, a broad-spectrum contact fungicide. Amistar is used effectively against rusts in cereal crops, however. The lack of disease control with Amistar in these trials may be related to an ineffective dosage rate. Another observation is that although Amistar treatments resulted in higher disease levels than other fungicide treatments, yield was not always the lowest. In 2001/2002, Amistar's high yield was, however, most probably due to a greater total amount of active ingredient being applied than in other seasons. More active ingredient resulted in better yield potential. In 2003/2004, Bayfidan and Amistar resulted in a final disease severity of 8% and 40% and SAUDPC of 0.6 and 2.4, respectively. However, yields were not significantly different. Bayfidan also resulted in poor yields in 2001/2002 suggesting that even though it kept disease levels reasonably low, yield was compromised. Shavit, a generic of Bayfidan, was only evaluated in the last season. Disease levels were not significantly different from Bayfidan, although yield was significantly higher in Shavit treatments. Seed mass was not significantly different between Bayfidan and Shavit.

The mixture of unrelated fungicides with different modes of action form the basis of fungicide resistance management (Delp, 1988). In 2003/2004 the highest yields resulted from treatments containing carbendazim. More specifically, treatments which contained a total of 250g or more of carbendazim yielded best. Punch C, which contained a total of 150g carbendazim as an active ingredient, yielded approximately 400–500 kg less than Punch Xtra, Early Impact and the 'Impact with carbendazim' treatments. The erroneous application of the 'Impact with carbendazim' treatment supports the role carbendazim appears to have in fungicide efficacy. In 2001/2002 the Impact treatment resulted in lower yields than those from other fungicide treatments that year. The enhanced efficacy may also have been due to the supply of fresh Impact in 2003/2004 as the product which was supplied for the first two seasons was not thought to be recently manufactured.

Yields and seed masses in 2003/2004 were generally higher than for other years, even though disease pressure was highest in this season. This may be because the wet, warm conditions which favour disease development are also more favourable for soybean plant growth. The other difference was that the initial fungicide application was moved from early flowering in 2001/2002 and 2002/2003 to late flowering in 2003/2004. This provided the benefits of the triazole fungicides, but without interrupting the sensitive stage of flower development or causing flower drop, which ultimately impacts on yield. The unprotected time between the breakdown of fungicide from the last application and physiological maturity was also decreased.

Analysis of quality parameters such as protein and oil show no significant differences between sprayed and unsprayed treatments, although oil content was slightly higher in soybeans protected from SBR through fungicide applications. Ogle *et al.* (1979) reported that rust infection resulted in no significant effect on protein content of the seed, whereas oil content was markedly reduced in treatments exposed longest and most severely to rust. Patil and Anahosur (1998) found that oil content was lower in unprotected than fungicide-treated soybeans, but that the difference was significant at only one of the two trial sites. Protein content was also not significant, confirming the results of Ogle *et al.* (1979) and the present research findings. Howell (1960) noted that nitrogen accumulates in the seed at a uniform rate, but that oil accumulation is related to dry matter accumulation in the seed, with a rapid increase during a two week period following the time when seed is approximately 30mg. This development phase coincides with the R4-5 to R6 growth stage interval.

The only significant difference obtained among fungicides was in oil content for Folicur in 2002/2003. Folicur treatments were the only ones to exhibit phytoxicity symptoms, expressed as leaf necrosis. The result was lower yield than the unsprayed treatment. Perhaps the leaf necrosis decreased the oil content since it negatively affected the yield. The effect is therefore suspected to be related to chemical toxicity.

In all other cases, across all seasons, there were no differences among fungicide treatments for seed quality parameters. This is in line with Patil and Anahosur's (1998) findings, where oil and protein contents were not different as a result of different fungicide treatments. Hexaconazole (16.8% and 38.4%), propiconazole (16.8% and

38.6%) and triadimefon (16.7% and 38.6%) resulted in oil and protein contents that were similar. These results were in line with the 2003/2004 results where mean oil and protein contents of 16.1% and 38.3%, respectively, were realised.

Protein content was higher and oil content lower (38.32% and 16.09%, respectively) in 2003/2004 than in other years (35.87% and 20.07% mean values, respectively). Hurburgh *et al.* (1987) found oil concentration to be higher in hot, dry years. The protein concentration was lower under these environmental factors, with the reduction in protein greater than the increase in oil. In these trials, however, the increase in oil was greater than the decrease in protein. The wet 2003/2004 season may explain the higher protein and lower oil contents for that year compared with the results from 2001/2002 and 2002/2003, during which dry conditions prevailed. Yaklich *et al.* (2002) analysed 51y of seed protein and oil data for soybeans in the U.S.A. and noted differences dependent upon location and maturity group. He also found that changes in protein levels were not as noticeable as oil concentrations. He was unable to determine the reason for change in oil concentrations, although he indicated that genetics was not the cause.

Since Amistar is not as effective as triazole fungicides in SBR control, and more expensive in SA than any of the triazoles, it would not be recommended for use as the sole fungicide in a spray program for SBR management. All triazole-containing fungicides are, however, effective in controlling SBR when compared to unsprayed controls. There is very little difference among the triazole-containing fungicides and hence type of fungicide sprayed is not critical. In conclusion, fungicide price may therefore be the main factor in fungicide choice for SBR control.

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CHAPTER 3

Rate of fungicide application for soybean rust (*Phakopsora pachyrhizi* Syd.) control on soybeans (*Glycine max* (L.) Merr.)

Abstract

Evaluation of the rates of fungicide applications required to control soybean rust (SBR) was conducted in several trials over three seasons from 2001 – 2004 at Cedara (29°32'S, 30°16'E and alt. 1071m), 20km north-west of Pietermaritzburg. Ten fungicides were evaluated in the first two seasons at half, full and double the registered or target rate. Three fungicide applications were made at 21d intervals, commencing at flowering. In 2003/2004, four of the ten fungicides were evaluated at four different rates of application, including the target rate, one increment higher and two increments lower than target rate. A drought in 2002/2003 resulted in very low and uneven disease severity, limiting the value of the results. High disease pressure resulted in the 2003/2004 season generating the most meaningful results. Results showed that rate as a main effect was seldom significant. Fungicide as a main effect had many more significant responses. The rate by fungicide interaction was only significant in 2003/2004. Folicur showed no response to rate of application when tested at 450, 600, 750 and 900ml.ha^{-1}. Amistar appeared to have lowest disease and best yield components at a rate of 400ml.ha^{-1}. Fungicide X at 700ml.ha^{-1} resulted in no disease and highest yield components. An increasing response to increased rate of application highlighted a need for this fungicide to be evaluated at even higher rates. Variable yield data made it difficult to interpret the optimal rate for application of Punch Xtra. Seed mass was highest at 500ml.ha^{-1}, while yield was highest at 700ml.ha^{-1}. Applications at 600ml.ha^{-1} resulted in more disease and lower yield components than at 500ml.ha^{-1}.

3.1 Introduction

Soybean rust (SBR), caused by *Phakopsora pachyrhizi* Syd., is the most destructive foliar pathogen of soybeans. The speed with which *P. pachyrhizi* has spread around the Western Hemisphere in the last few years and the yield losses that have been incurred following its establishment make effective management of this pathogen a necessity. First reports of SBR were made in Zimbabwe in 1998, South Africa (SA) in 2001, Paraguay and Brazil in 2001, Argentina in 2002, Bolivia in 2003, Colombia and mainland U.S.A. in 2004 (Pretorius *et al.*, 2001; Levy, 2003; Rossi, 2003; Morel *et al.*, 2004; Rogers and Redding, 2004; Yorinori, 2004). Yield losses in Brazil for the 2004 season were estimated by Embrapa to be 4.5 million tons, with an economic value of US $2 billion (Anon., 2004a).

Rust fungi are obligate parasites, *i.e.*, they survive only on living plant hosts. They usually have five distinct fruiting structures in the life cycle, each with its own spore form, which appear in a distinct sequence. The life-cycle of *P. pachyrhizi* is not fully understood, with no records of spermatia or aeciospores for the pathogen (Bromfield, 1984). New infections are caused by urediospores. Teliospores serve as the overwintering stage but are not often seen in the field. They germinate to produce the basidium which produces basidiospores. Basidia and basidiospores have only been produced from the germination of telia in the laboratory (Koch and Hoppe, 1987). Spermagonia develop from basidiospores, to produce spermatia which, in turn, produce aecia and aeciospores. Uredia and urediospores develop from aeciospores. Telia eventually develop from uredia when plant hosts approach maturity. Only basidiospores, aeciospores and urediospores are able to infect host plants (Agrios, 1997).

Understanding the biology of the pathogen usually helps in disrupting the life-cycle and hence managing the disease. Some rust fungi can complete their life-cycle on a single host plant, while others require two different or alternate host plants. In cases where an alternate host is integral to the life-cycle, control is achieved through removal of the alternate host. Although *P. pachyrhizi* is known to have more than 90 alternate hosts, it is not certain whether any are essential for completing the life-cycle of the fungus. Rust control is generally achieved through the use of resistant cultivars, chemical sprays or

removal of the alternate host. Since much of the biology of *P. pachyrhizi* is not fully understood, removal of all alternate hosts is not practical. Management of the disease is therefore dependent upon the use of resistant cultivars and fungicides.

The use of resistant cultivars is easier, safer and less expensive than other methods of disease control. To date, four dominant, independently inherited genes for resistance to *P. pachyrhizi* have been identified (Hartwig, 1986). Several cultivars were developed using these resistance genes, but resistance was not durable in the field. Cultivars with vertical resistance have a few resistance genes which are usually resistant to some of the races of the pathogen. A partial differential set containing eleven, of 14 possible genetic combinations, was developed at the Asian Vegetable Research Development Centre (AVRDC). Researchers at the AVRDC identified nine races of *P. pachyrhizi*. All races were, however, virulent on three or more of the differentials. The predominant race was virulent on ten of the eleven differentials, indicating that some races possess multiple virulence factors to the resistant genes (Sinclair and Hartman, 1996). New races of airborne pathogens, such as *P. pachyrhizi*, virulent to resistant cultivars, can easily be introduced and quickly become widespread. Resistance in the cultivar is matched by novel virulent races and the old cultivar should be replaced by a cultivar which has different genes for resistance. Depending on several factors which contribute towards the pathogen matching resistance genes in the host, new resistant cultivars need to be replaced every three to ten years. The use of fungicides, however, which reduce the exposure of the resistant cultivar to large pathogen populations, could increase resistant cultivars' lifespans.

The effective use of fungicides remains crucial to managing SBR in the absence of resistant cultivars, as is the case in SA. An integral component of fungicide efficacy is dosage rate. Too low a dosage rate results in the fungicide being ineffective and promotes fungicide resistance development in the pathogen. Too high a dosage rate results in phytotoxicity and wastage of fungicide, which is costly. At the time of planning these trials no known research had been conducted anywhere in the world on optimisation of triazole or strobilurin fungicide dosage rates for SBR control.

Evaluation of dosage rates assist in determining the optimal amount of chemical that is required to control the pathogen. It also allows for the testing of chemical residues, to

determine whether or not the dosage rate exceeds the maximum permissible residue. Since no cultivars with resistance to SBR exist and fungicides remain the only effective tool for controlling SBR it is essential that pathogen resistance to fungicides does not develop through the use of non-optimal dosage rates. It is therefore of critical importance to determine the optimal dosage rate for each fungicide. The present study was conducted to assess dosage rates which should be recommended for registration of fungicides for the control of SBR in SA.

3.2 Materials and methods

Trial site

Trials were conducted at Cedara agricultural research farm (29°32'S, 30°16'E and alt. 1071m), 20km north-west of Pietermaritzburg. Evaluations of different fungicide rates were conducted in 2001/2002, 2002/2003 and 2003/2004. The 2001/2002 and 2002/2003 trials were conducted on the same land, previously planted to potatoes. The 2003/2004 trial was planted on land that had previously been planted to maize.

Land preparation

Soil samples were taken of the topsoil (0-15cm) and fertilizer was supplied according to Fertrec recommendations from the Cedara Fertilizer Advisory Service (Farina and Channon, 1988). Phosphorus was band-applied in the rows at planting to supply 21kg.ha^{-1} in 2001/2002 and 10.5kg.ha^{-1} in 2002/2003 (source: superphosphate (10.5%)). In 2003/2004, nitrogen, phosphorus and potassium were broadcast and disced in prior to planting to supply 46kg.ha^{-1}, 69kg.ha^{-1} and 92kg.ha^{-1}, respectively (source: 2:3:4 (40)).

Climatic data

Automatic weather stations based at Cedara were used to collect information on rainfall and temperature over the growing seasons. Long term monthly rainfall and temperature averages at Cedara were supplied by the Institute for Soil, Climate and Water[1].

[1] Institute for Soil, Climate and Water, Agromet Section, Private Bag X79, Pretoria, 0001, South Africa

Trial design

Two separate fungicide rates trials (Trials 1a & b) were planted in the 2001/2002 season. Since the trials were designed as efficacy x rate trials the treatment number was too large for reliable analysis in a single trial. A factorial design with randomised complete block was used in 2001/2002. In 2002/2003 and 2003/2004 trials were designed as factorials with a split-plot design, with fungicide rate as the whole plot and fungicide the sub-plot. All trials contained three replications. Plots comprised four 5m rows spaced 45cm apart, except in 2003/2004, where the plots consisted of six rows. The number of rows was increased to make the distribution of inoculum among plots more uniform. The two central rows of each plot were used as data rows for disease evaluation and yield determination. The unsprayed outer rows of the plots are referred to as border rows.

Planting

Trials were hand-planted with the soybean cultivar LS666. Planting dates, seeding rates and final plant populations are shown in Table 3.1 Soybeans were inoculated with *Bradyrhizobium japonicum* ((Kirchner) Jordan) at planting to enhance good nodule formation. Normal pest and weed control practices for the area were followed.

Table 3.1 Planting date, cultivar, seeding rate and plant population of soybean trials planted at Cedara in the 2001 - 2004 growing seasons

Season	Planting date	Cultivar & expected germination	Seeding rate (seeds.ha^{-1})	Plant population (plants.ha^{-1})
2001/2002	21 December 2001	LS666 (70-79% germination)	350 000	215 000 (61% germination)
2002/2003	13 November 2002	LS666 (>90% germination)	300 000	270 000 (90% germination)
2003/2004	12 November 2003	LS666 (>90% germination)	300 000	260 000 (87% germination)

Fungicide treatments

In 2001/2002 and 2002/2003 the effect of half the registered or target rate, the full rate and double the rate were evaluated for nine of ten fungicides. At the manufacturer's request, Amistar was evaluated at a target rate of 300ml.ha^{-1} and lower and higher rates of 200 and 400ml.ha^{-1}, respectively (Tables 3.2a). No unsprayed control was included in

any of the rate trials because the comparisons were between different rates of each individual fungicide. The fungicide efficacy trials in Chapter 2 show that the fungicides are effective in controlling SBR.

Table 3.2a Fungicides evaluated and their target application rates for the control of soybean rust caused by *Phakopsora pachyrhizi* in trials at Cedara in 2001/2002 and 2002/2003

Active ingredient (a.i.)	Concentration & formulation	Trade name	Manufacturer	Target application rate (.ha^{-1})					
				ml			g a.i.		
				Half	Full	Double	Half	Full	Double
azoxystrobin	250 SC	Amistar	Syngenta	200	300	400	50	75	100
carbendazim	250 SC	Punch	Du Pont	400	800	1600	100	200	400
& flusilazole	125	Xtra					50	100	200
difenoconazole	250 EC	Score	Syngenta	162.5	325	650	40.625	81.25	162.5
flusilazole	250 EW	Capitan	Du Pont	200	400	800	50	100	200
flusilazole	250 SC	Punch C	Du Pont	200	400	800	50	100	200
& carbendazim	125						25	50	100
flutriafol	125 SC	Impact	Cheminova	500	1000	2000	62.5	125	250
tebuconazole	250 EW	Folicur	Bayer	375	750	1500	93.75	187.5	375
triadimenol	250 DC	Bayfidan	Bayer	250	500	1000	62.5	125	250
triforine	190 EC	Denarin	BASF	750	1500	3000	142.5	285	570
triazole	225 SC[a]	Fungicide	BASF	375	700	1400	78.75	157.5	315
& strobilurin	182.5 SC[b]	X		375	700	1400	63.875	127.75	255.5

a Formulation used in 2001/2002

b Formulation used in 2002/2003

Calibration of the spray equipment was based on a walking speed of 1m.s^{-1}. After the first two applications in 2001/2002, it was realised that the actual walking speed in the canopied plants was slower, at 0.5m.s^{-1}. Effectively, this resulted in double the fungicide being applied for these two applications. The spray volume was recalculated on the slower walking speed and the corrected target application rates were applied for the third spray in 2001/2002. The actual fungicide rates applied are recorded in Table 3.2b.

In 2003/2004, the trial was reduced to the evaluation of four dosage rates of four fungicides (Table 3.3). These fungicides were selected as a representative of the different chemical classes and mixtures of classes being used to control SBR. Amistar was the single product strobilurin and Folicur the single product triazole. Fungicide X

was the strobilurin/triazole mixture and Punch Xtra the triazole/benzimidazole mixture. The registered or target rate was evaluated, as were two rates lower and one higher than the target rate. Rather than checking for efficacy and phytotoxicity with half and double rates respectively, this trial aimed to determine an optimal dosage rate for each fungicide evaluated.

Table 3.2b Actual application rates of fungicides applied for the control of soybean rust caused by *Phakopsora pachyrhizi* at Cedara in 2001/2002 and 2002/2003

Trade Name	Application Rate (.ha^{-1})											
	Total g a.i. (after 3 applications)						ml					
	2001/2002†			2002/2003			2001/2002†			2002/2003		
	Half	Full	Double	Half	Full	Double	Half	Full	Double	Half	Full	Double
Amistar	250	375	500	150	225	300	333	500	667	200	300	400
Punch Xtra	500 + 250	1000 + 500	2000 + 1000	300 + 150	600 + 300	1200 + 600	667	1333	2667	400	800	1600
Score	203.13	406.25	812.50	121.88	243.75	487.50	271	542	1083	162.5	325	650
Capitan	250	500	1000	150	300	600	333	667	1333	200	400	800
Punch C	250 + 500	500 + 1000	1000 + 2000	150 + 300	300 + 600	600 + 1200	333	667	1333	200	400	800
Impact	312.50	625	1250	187.50	375	750	833	1667	3333	500	1000	2000
Folicur	468.75	937.50	1875	281.25	562.50	1125	625	1250	2500	375	750	1500
Bayfidan	312.50	625	1250	187.50	375	750	417	833	1667	250	500	1000
Denarin	712.50	1425	2850	427.50	855	1710	1250	2500	5000	750	1500	3000
Fungicide X ‡	393.75	787.5	1575	191.63	383.25	766.50	583	1167	2333	350	700	1400

† The first two applications received double the chemical dose. This error was corrected for the third application.

‡ Two different formulations were used in the two seasons. The 2001/02 formulation contained 225g a.i. while the 2002/03 formulation contained 182.5g.

Table 3.3 Application rates of fungicides evaluated for the control of soybean rust caused by *Phakopsora pachyrhizi* in trials at Cedara in 2003/2004

Trade Name	Target rate (ml.ha^{-1})				Application rate (Total g a.i. applied.ha^{-1})			
	Low	Med	Reg	High	Low	Med	Reg	High
Amistar	200	300	400	500	150	225	300	375
Punch Xtra	400	500	600	700	300 + 150	375 + 187.5	450 + 225	525 + 262.5
Fungicide X	400	500	600	700	270	337.5	405	472.5
Folicur	450	600	750	900	337.5	450	562.5	675

Three applications were made, at 21d intervals (Table 3.4). In 2001/2002 the initial application was made at early flowering. In 2002/2003 the initial application was made just before flowering, while in 2003/2004 the first application was made at the late flowering stage.

Table 3.4 Time (days after planting) of flowering of soybeans and fungicide applications for trials conducted at Cedara from 2001 – 2004 for the control of soybean rust caused by *Phakopsora pachyrhizi*

Season	First flower (DAP)*	Fungicide applications (DAP)		
		1st spray	2nd spray	3rd spray
2001/2002	60	63	84	105
2002/2003	70	64	85	106
2003/2004	70	82	103	124

* DAP = days after planting

From 2001 – 2003 full cover fungicide sprays of 160ℓ.ha^{-1} at 200kPa pressure were applied to the central two rows of each plot, leaving the outer rows as border rows to reduce interplot interference. Fungicides were applied using a CO_2-pressured back-pack sprayer with a horizontal spray-boom comprising two Spraying Systems TeeJet No8001 nozzles, spaced 45cm apart. In 2003/2004, all fungicide spray solutions were applied with a CO_2-pressured backpack sprayer with a horizontal spray-boom and two Albuz ATR 80 hollow cone nozzles, spaced 45cm apart. Full cover sprays of 420ℓ.ha^{-1} at 200kPa pressure were applied to the two central rows.

Artificial inoculation

To ensure even inoculum pressure throughout the trials, field plots were artificially inoculated with *P. pachyrhizi*. In 2001/2002, freshly collected *P. pachyrhizi* spores from early-infected trap plants were sprayed in a solution containing 0.01% household dishwashing liquid, using a knapsack sprayer, over the plots on 4 and 11 March at R3 growth stage (72 and 79d after planting (DAP), respectively). In 2002/2003, soybeans were planted in plastic bags (one plant per bag) and grown in tunnels, where favourable conditions allowed them to become naturally infected. On 19 February (99 DAP), these bags were placed in every second plot within the trial. In 2003/2004, five infected

leaves were stapled singly, at approximately1m intervals, onto plants in the first row of each six-row plot, 98 DAP.

Disease assessment

Disease severity assessments were made regularly, at twice-weekly intervals on plants in the data rows, from flowering (R1) until physiological maturity (R7) of the crop. A plot rating was given after examining at least ten plants in the central two rows. Disease was assessed according to a rating scale developed at Cedara (see Table 2.4 of Chapter 2). The scale uses the position of rust pustules on the plant, pustule density, chlorosis and defoliation as parameters for determining disease severity. Data were used to calculate the area under disease progress curve (AUDPC), which summarises the disease epidemic. The AUDPC was calculated using a trapezoidal integration program (Berger, 1981) and standardised (SAUDPC) by dividing the AUDPC value by the duration of the epidemic. The SAUDPC allows for comparisons of disease from one season to another.

Harvesting

Leaving a 0.5m border on either end of each of the 5m rows, the central 4m of the central two rows in each plot, were hand-harvested. Grain yields were adjusted to a moisture content of 12.5% and expressed as $kg.ha^{-1}$. Seed mass (g) was determined for 100 seeds and corrected for moisture content. Protein and oil analyses were conducted on the seed using the Dumas combustion method (Dumas, 1831) and supercritical fluid extraction, respectively.

Statistical analyses

Statistical analyses of trial data (final disease severity, SAUDPC, yield, seed mass, protein and oil content) were conducted by analysis of variance (ANOVA), using Genstat 6.1. Mean separations were based on the least significant differences (LSD) or Duncan's multiple range test (DMRT) at the 5% level of probability. The LSD test is used when planned pair comparisons are being made and when the treatment number is not too large (less than six). DMRT is used when the treatment number exceeds five (Steel and Torrie, 1981). Linear and quadratic responses to rates of fungicide applications were analysed.

3.3 Results

Climatic data

Although rainfall was good early in the season, the 2001/2002 season was characterised by a mid-season drought, which persisted from flowering (mid-February) until physiological maturity of the soybean crop. The 2002/2003 season was warm, but very dry throughout the duration of the trial. In contrast, the 2003/2004 season was warm, with well-distributed rainfall throughout the growing season (Table 3.5).

Table 3.5 Rainfall and temperature data at Cedara for the 2001 - 2004 soybean growing seasons

	November	December	January	February	March	April	Total
Rainfall (mm)							
2001/2002	126	158	153	38	59	61	469 †
2002/2003	52	84	47	66	95	44	344 ‡
2003/2004	104	103	162	154	89	4	612 ‡
Mean monthly §	111	132	135	123	111	52	-
Mean temperature (°C)							
2001/2002	18.5	18.7	20.7	18.6	19.5	17.6	-
2002/2003	16.7	19.1	19.8	20.8	19.7	17.5	-
2003/2004	17.6	18.8	19.7	19.3	18.5	17.3	-
Mean monthly §	18.0	19.5	20.2	20.2	19.3	16.7	-

† Total rainfall is for the growing period (01/12 to 30/04)

‡ Total rainfall is for the growing period (01/11 to 31/03)

§ Long-term mean from 01/07/1914 to 30/06/2004

Flowering, first signs of disease and physiological maturity of the crop are presented in Table 3.6.

Table 3.6 Dates of various soybean plant growth stages in trials at Cedara from 2001 - 2004

Event	2001/2002		2002/2003		2003/2004	
	Date	DAP †	Date	DAP	Date	DAP
Planting date	21 December	0	13 November	0	13 November	0
Flowering	19 February	60	22 January	70	22 January	70
First sign of disease in trial	27 March	97	26 February	105	2 March	110
Physiological maturity	22 April	123	31 March	138	2 April	141

† DAP = days after planting

3.3.1 2001/2002 season

Trial 1a

Final disease severity

No factors were significant (Table 3.7a). The coefficient of variation was high (74.8%).

Standardised area under disease progress curve (SAUDPC)

No factors were significant (Table 3.7a). The coefficient of variation was high (109.8%).

Yield

Fungicide was highly significant, but rate and fungicide x rate interaction were not significant (Table 3.7a). Coefficient of variation was 8.6%. Bayfidan resulted in significantly lower yields than all other fungicides (Table 3.7b and Fig. 3.1).

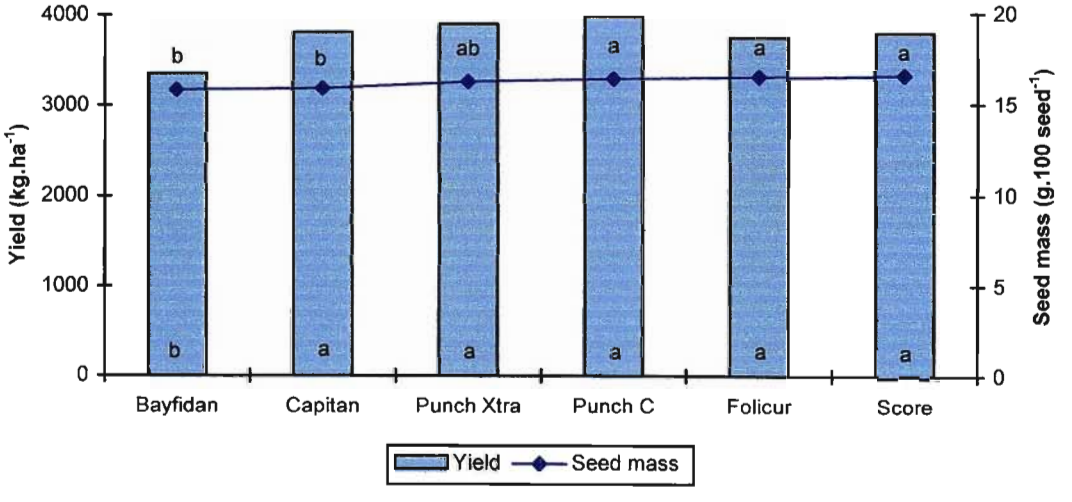

Figure 3.1 Soybean yield (kg.ha^{-1}) and seed mass (g.100 seed^{-1}) response to fungicides evaluated for the control of SBR caused by *Phakopsora pachyrhizi* in Trial 1a at Cedara (2001/2002)

Seed mass

Fungicide was highly significant, but rate and the fungicide x rate interaction were not significant (Table 3.7a). Coefficient of variation was low, at 2.9%. Bayfidan and Capitan treatments resulted in the lowest seed mass. Folicur, Punch C and Score

Table 3.7a ANOVA of soybean rust final disease severity (square root transformed), log transformed standardised area under disease progress curve values (SAUDPC), yield ($kg.ha^{-1}$), seed mass ($g.100\ seed^{-1}$), protein and oil content (percentage) for rate of fungicide application in Trial 1a (2001/2002)

	Main effects and interaction	Degrees of freedom	Final disease severity	SAUDPC	Yield	Seed mass	Protein	Oil
Mean square	Fungicide	5	0.408	0.036	417799	0.896	1.679	0.806
	Rate	2	0.564	0.052	78396	0.434	0.040	0.814
	linear	1	0.829	0.053	76326	0.573	0.068	0.021
	quadratic	1	0.299	0.050	80465	0.295	0.011	1.606
	Fungicide.Rate	10	0.572	0.027	114183	0.208	2.357	0.948
	fungicide.linear	5	0.577	0.040	91146	0.227	3.646	0.732
	fungicide.quadratic	5	0.566	0.014	137221	0.190	1.069	1.165
	Residual	34	0.469	0.021	105212	0.224	2.360	1.035
F-value	Fungicide		0.87	1.66	3.97	4.00	0.71	0.78
	Rate		1.20	2.42	0.75	1.94	0.02	0.79
	linear		1.77	2.49	0.73	2.56	0.03	0.02
	quadratic		0.64	2.34	0.76	1.32	0.00	1.55
	Fungicide.Rate		1.22	1.25	1.09	0.93	1.00	0.92
	fungicide.linear		1.23	1.85	0.87	1.01	1.54	0.71
	fungicide.quadratic		1.21	0.65	1.30	0.85	0.45	1.13
F-probability	Fungicide		0.511	0.171	0.006 **	0.006 **	0.619	0.572
	Rate		0.313	0.104	0.482	0.159	0.983	0.464
	linear		0.192	0.124	0.400	0.119	0.866	0.887
	quadratic		0.430	0.135	0.388	0.259	0.947	0.221
	Fungicide.Rate		0.314	0.296	0.400	0.518	0.464	0.530
	fungicide.linear		0.316	0.129	0.514	0.426	0.202	0.622
	fungicide.quadratic		0.326	0.665	0.285	0.525	0.808	0.366
% c.v.			74.8	109.8	8.6	2.9	4.2	5.2
Level of significance			P = > 0.05 n.s.		P = ≤ 0.05 *		P = ≤ 0.01 **	

Table 3.7b Table of means for soybean rust final disease severity (square root transformed), log transformed standardised area under disease progress curve values (SAUDPC), yield (kg.ha^{-1}), seed mass (g.100 seed^{-1}), protein and oil content (percentage) for rate of fungicide application in Trial 1a (2001/2002)

Treatment	Final disease severity (sqrt)				SAUDPC (log)			
	Half	Full	Double	Mean	Half	Full	Double	Mean
Bayfidan	2.07	0.91	0.81	1.26	0.41	0.19	0.06	0.22
Capitan	1.05	1.00	0.47	0.84	0.17	0.12	0.07	0.12
Folicur	0.33	0.94	0.67	0.65	0.02	0.06	0.06	0.05
Punch C	1.16	0.94	0.91	1.00	0.29	0.10	0.19	0.19
Punch Xtra	1.63	0.47	0.81	0.97	0.25	0.03	0.09	0.12
Score	0.47	0.81	1.05	0.78	0.04	0.09	0.17	0.10
Mean	1.12	0.85	0.78	0.92	0.20	0.10	0.11	0.13
LSD (0.05)	n.s.				n.s.			
Treatment	Yield (kg.ha^{-1})				Seed mass (g.100 seed^{-1})			
	Half	Full	Double	Mean *	Half	Full	Double	Mean *
Bayfidan	3234	3463	3359	3352 b	15.60	16.04	16.01	15.88 b
Capitan	3819	3729	3868	3805 a	15.95	16.12	15.81	15.96 b
Folicur	3715	4098	3394	3736 a	16.48	16.90	16.27	16.55 a
Punch C	4024	4102	3791	3972 a	16.62	16.60	16.14	16.45 a
Punch Xtra	3781	3911	3987	3893 a	16.56	16.55	15.85	16.32 ab
Score	3997	3622	3736	3785 a	16.86	16.38	16.71	16.65 a
Mean	3762	3821	3689	3757	16.34	16.43	16.13	16.30
LSD (0.05)	n.s.			DMRT	n.s.			DMRT
Treatment	Protein (%)				Oil (%)			
	Half	Full	Double	Mean	Half	Full	Double	Mean
Bayfidan	35.79	35.83	35.73	35.78	18.74	19.42	19.75	19.31
Capitan	35.68	36.37	35.83	35.96	19.33	19.77	18.78	19.29
Folicur	34.23	36.77	37.35	36.12	20.42	19.66	19.85	19.97
Punch C	36.56	35.81	35.81	36.06	19.41	20.27	19.11	19.59
Punch Xtra	37.73	36.20	36.61	36.84	18.37	20.07	19.20	19.21
Score	37.29	36.39	36.49	36.72	19.98	19.39	19.84	19.73
Mean	36.21	36.23	36.30	36.25	19.37	19.76	19.42	19.52
LSD (0.05)	n.s.				n.s.			

* Means followed by a different letter in the same column are significantly different at P = 0.05, according to Duncan's multiple range test (DMRT)

treatments resulted in the highest seed mass. Punch Xtra treatments resulted in seed mass which was not significantly different from the lowest or the highest seed masses (Table 3.7b and Fig. 3.1).

Protein

No factors were significant (Table 3.7a). The coefficient of variation was low (4.2%).

Oil

No factors were significant (Table 3.7a). The coefficient of variation was low (5.2%).

Trial 1b

Final disease severity

Fungicide was the only significant effect (Table 3.8a). Amistar and Denarin treatments resulted in the highest final disease severity (Table 3.8b). The coefficient of variation was high (60.9%).

Standardised area under disease progress curve (SAUDPC)

Fungicide was not significant (P = 0.055) and neither was rate or the fungicide x rate interaction (Table 3.8a). The coefficient of variation was high (93.5%).

Yield

Fungicide and rate were significant, although their interaction was not (Table 3.8a). The coefficient of variation was 10.1%. Double rates resulted in yields that were significantly lower than full or half rates (Table 3.8b and Fig. 3.2). Amistar and Punch C treatments resulted in the highest yields, while Denarin and Impact treatments resulted in the lowest yields. Fungicide X was not significantly different from the highest yielding fungicides but was also not different from the lowest yielding fungicides (Table 3.8b and Fig. 3.3).

Table 3.8a ANOVA of soybean rust final disease severity (square root transformed), log transformed standardised area under disease progress curve values (SAUDPC), yield ($kg.ha^{-1}$), seed mass ($g.100\ seed^{-1}$), protein and oil content (percentage) for rate of fungicide application in Trial 1b (2001/2002)

	Main effects and interaction	Degrees of freedom	Final disease severity	SAUDPC	Yield	Seed mass	Protein	Oil
Mean square	Fungicide	4	2.343	0.174	749363	1.506	4.780	0.286
	Rate	2	0.461	0.086	729255	0.391	1.374	2.439
	linear	1	0.005	0.001	1297479	0.207	1.045	0.011
	quadratic	1	0.916	0.171	161031	0.576	1.703	4.867
	Fungicide.Rate	8	0.908	0.097	76347	0.063	3.491	0.246
	fungicide.linear	4	1.418	0.151	113629	0.036	1.865	0.129
	fungicide.quadratic	4	0.399	0.043	39065	0.091	5.117	0.363
	Residual	28	0.746	0.066	181789	0.379	3.112	0.663
F-value	Fungicide		3.14	2.64	4.12	3.98	1.54	0.43
	Rate		0.62	1.30	4.01	1.03	0.44	3.68
	linear		0.01	0.01	7.14	0.55	0.34	0.02
	quadratic		1.23	2.59	0.89	1.52	0.55	7.34
	Fungicide.Rate		1.22	1.47	0.42	0.17	1.12	0.37
	fungicide.linear		1.90	2.28	0.63	0.09	0.60	0.19
	fungicide.quadratic		0.53	0.65	0.21	0.24	1.64	0.55
F-probability	Fungicide		0.030 *	0.055	0.009 **	0.011 *	0.219	0.785
	Rate		0.546	0.288	0.029 *	0.369	0.647	0.038 *
	linear		0.935	0.915	0.012 *	0.465	0.567	0.899
	quadratic		0.277	0.119	0.355	0.228	0.466	0.011 *
	Fungicide.Rate		0.325	0.213	0.899	0.994	0.379	0.927
	fungicide.linear		0.138	0.085	0.649	0.984	0.666	0.939
	fungicide.quadratic		0.711	0.629	0.928	0.913	0.191	0.702
% c.v.			60.9	93.5	10.1	3.6	5.0	4.1
Level of significance			P = > 0.05 n.s.		P = ≤ 0.05 *		P = ≤ 0.01 **	

Table 3.8b Table of means for soybean rust final disease severity (square root transformed), log transformed standardised area under disease progress curve values (SAUDPC), yield (kg.ha^{-1}), seed mass (g.100 seed^{-1}), protein and oil content (percentage) for rate of fungicide application in Trial 1b (2001/2002)

Treatment	Final disease severity (sqrt)				SAUDPC (log)			
	Half	Full	Double	Mean	Half	Full	Double	Mean
Amistar	2.81	1.72	1.63	2.05 c	0.70	0.30	0.26	0.42
Denarin	2.00	1.28	2.40	1.89 bc	0.40	0.17	0.71	0.43
Fungicide X	1.52	1.15	0.47	1.05 a	0.33	0.18	0.10	0.21
Impact	0.67	0.80	1.63	1.03 a	0.05	0.10	0.23	0.13
Punch C	0.80	1.15	1.22	1.06 ab	0.16	0.18	0.25	0.20
Mean	1.56	1.22	1.47	1.42	0.33	0.19	0.31	0.28
LSD (0.05)	n.s.			0.834	n.s.			

Treatment	Yield (kg.ha^{-1})				Seed mass (g.100 seed^{-1})				
	Half	Full	Double	Mean *	Half	Full	Double	Mean *	
Amistar	4523	4673	4341	4512 a	17.15	17.26	16.88	17.10	abc
Denarin	4094	4169	3683	3982 b	17.22	17.24	17.09	17.18	ab
Fungicide X	4610	4241	3756	4202 ab	17.36	17.76	17.51	17.54	a
Impact	3875	4103	3793	3924 b	16.61	16.59	16.44	16.54	c
Punch C	4691	4618	4316	4542 a	16.53	17.04	16.38	16.65	bc
Mean **	4359 a	4361 a	3978 b	4232	16.97	17.18	16.86	17.00	
LSD (0.05)	318.9			411.7	n.s.			0.594	

Treatment	Protein (%)				Oil (%)			
	Half	Full	Double	Mean	Half	Full	Double	Mean
Amistar	34.52	34.66	35.02	34.73	19.99	20.65	19.96	20.20
Denarin	34.84	35.24	34.54	34.87	19.25	20.41	19.66	19.77
Fungicide X	37.51	35.27	36.35	36.38	19.84	19.95	20.08	19.96
Impact	33.17	36.65	34.57	34.80	19.56	20.27	19.46	19.76
Punch C	33.75	34.97	35.18	34.64	19.73	20.67	19.40	19.93
Mean **	34.76	35.36	35.13	35.08	19.67 b	20.39 a	19.71 b	19.93
LSD (0.05)	n.s.				0.609			n.s.

* Means followed by a different letter in the same **column** are significantly different at P = 0.05, according to Fisher's LSD

** Means followed by a different letter in the same **row** are significantly different at P = 0.05, according to Fisher's LSD

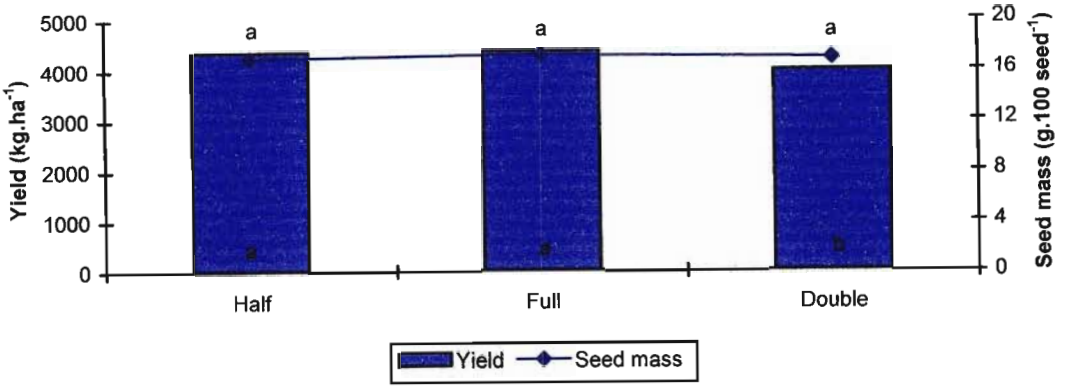

Figure 3.2 Yield (kg.ha^{-1}) and seed mass (g.100 seed^{-1}) response to different rates of fungicide evaluated for the control of soybean rust caused by *Phakopsora pachyrhizi* in Trial 1b at Cedara (2001/2002)

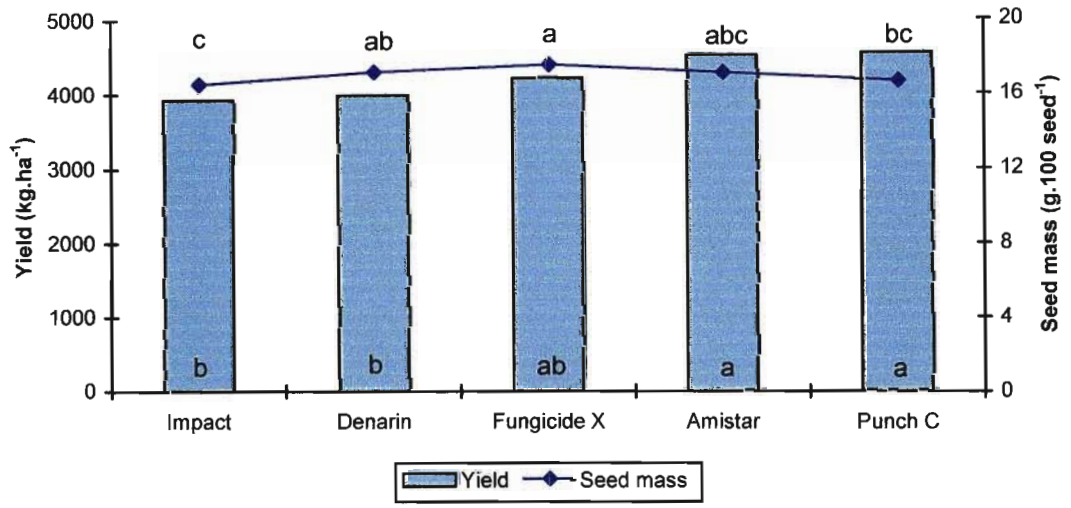

Figure 3.3 Yield (kg.ha^{-1}) and seed mass (g.100 seed^{-1}) response to different fungicides evaluated for the control of soybean rust caused by *Phakopsora pachyrhizi* in Trial 1b at Cedara (2001/2002)

Seed mass

Fungicide was significant, but rate and the fungicide x rate interaction were not (Table 3.8a). The coefficient of variation was low at 3.6%. Fungicide X resulted in the highest seed mass, while Impact resulted in the lowest seed mass (Table 3.8b and Fig. 3.3).

Protein

No factors were significant (Table 3.8a). The coefficient of variation was low (5.0%).

Oil

Rate of fungicide application was significant with a quadratic effect (Table 3.8a). Oil content was highest at the full rate of fungicide application (Table 3.8b and Fig. 3.4). Coefficient of variation was low at 4.1%. Fungicide and the interaction between fungicide and rate were not significant.

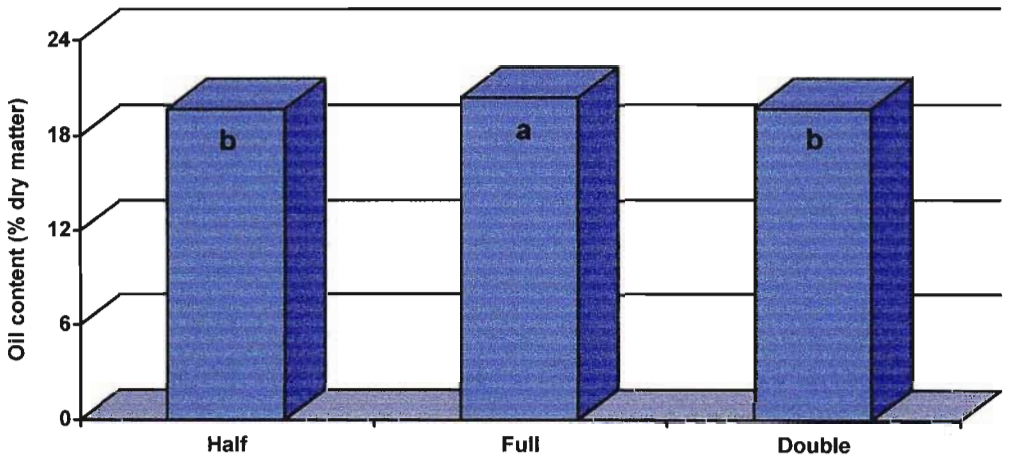

Figure 3.4 Soybean oil content (%) resulting from different rates of fungicide application in Trial 1b at Cedara (2001/2002)

3.3.2 2002/2003 season

Final disease severity

Fungicide type was significant, but a very high coefficient of variation (469.7%) makes analysis of the ANOVA invalid.

Standardised area under disease progress curve (SAUDPC)

As with final disease severity, fungicide was significant, but the very high coefficient of variation (368.8%) invalidates interpretation of the ANOVA.

Yield

The coefficient of variation was low (8%). However, no factors were significant (Tables 3.9a & b).

Table 3.9a ANOVA of soybean rust final disease severity (percentage), log transformed standardised area under disease progress curve values (SAUDPC), yield (kg.ha^{-1}), seed mass (g.100 seed^{-1}), protein and oil content (percentage) for rate of fungicide application in 2002/2003

	Main effects and interaction	Degrees of freedom	Final disease severity	SAUDPC	Yield	Seed mass	Protein	Oil
Mean square	Rate	2	12.048	0.041	202765	1.513	41.037	1.391
	linear	1	9.087	0.004	18	2.206	66.118	1.179
	quadratic	1	15.010	0.077	405512	0.820	15.956	1.602
	Residual	4	14.484	0.084	149870	1.169	3.022	0.548
	Fungicide	9	19.916	0.184	157159	0.974	5.803	1.221
	Fungicide.Rate	18	7.858	0.027	140715	0.489	1.309	0.871
	Residual	54	8.451	0.056	101142	0.859	1.995	0.819
F-value	Rate		0.83	0.49	1.35	1.29	13.58	1.70
	linear		0.63	0.05	0.00	1.89	21.88	1.44
	quadratic		1.04	0.93	2.71	0.70	5.28	1.96
	Residual		1.71	1.48	1.48	1.36	1.51	0.67
	Fungicide		2.36	3.27	1.55	1.13	2.91	1.49
	Fungicide.Rate		0.93	0.47	1.39	0.57	0.66	1.06
F-probability	Rate		0.499	0.646	0.356	0.369	0.016 *	>0.05
	linear		0.473	0.831	0.992	0.241	0.009 **	>0.05
	quadratic		0.366	0.391	0.175	0.449	0.083	>0.05
	Fungicide		0.025 *	0.003 **	0.153	0.356	0.007 **	0.175
	Fungicide.Rate		0.548	0.866	0.174	0.906	0.837	0.411
% c.v.			469.7	368.8	8.0	5.0	3.8	4.7
Level of significance			P = > 0.05 n.s.		P = ≤ 0.05 *		P = ≤ 0.01 **	

Table 3.9b Table of means for soybean rust final disease severity (percentage), log transformed standardised area under disease progress curve values (SAUDPC), yield (kg.ha^{-1}), seed mass (g.100 seed^{-1}), protein and oil content (percentage) for rate of fungicide application in 2002/2003

Treatment	Final disease severity (%)				SAUDPC (log)			
	Half	Full	Double	Mean *	Half	Full	Double	Mean *
Amistar	10.50	1.33	2.50	4.78	0.69	0.24	0.44	0.46
Bayfidan	0.02	0.00	0.00	0.01	0.00	0.00	0.00	0.00
Capitan	0.00	0.02	0.00	0.01	0.00	0.00	0.00	0.00
Denarin	0.02	0.00	1.33	0.45	0.01	0.00	0.24	0.08
Folicur	0.33	0.00	0.00	0.11	0.02	0.00	0.00	0.01
Fungicide X	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Impact	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Punch C	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00
Punch Xtra	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Score	2.50	0.02	0.00	0.84	0.27	0.01	0.00	0.09
Mean	1.34	0.14	0.38	0.62	0.10	0.03	0.07	0.06
LSD (0.05)	n.s.				n.s.			
Treatment	Yield (kg.ha^{-1})				Seed mass (g.100 seed^{-1})			
	Half	Full	Double	Mean	Half	Full	Double	Mean
Amistar	4402	3722	3979	4034	18.81	18.83	18.21	18.62
Bayfidan	3891	3987	4140	4006	18.17	18.89	17.86	18.31
Capitan	4048	3977	3815	3947	18.03	17.87	17.18	17.70
Denarin	3838	3687	3976	3834	19.42	18.03	18.59	18.68
Folicur	4054	3992	3868	3971	19.27	18.48	18.77	18.84
Fungicide X	4491	3848	4112	4150	18.90	17.85	18.27	18.34
Impact	3960	3596	3545	3700	18.39	18.07	18.85	18.44
Punch C	3800	4295	4176	4090	19.19	18.56	18.27	18.68
Punch Xtra	3957	3924	4108	3996	18.93	18.68	18.59	18.73
Score	3768	3624	4169	3854	18.13	18.50	18.42	18.35
Mean	4021	3865	3989	3958	18.72	18.38	18.30	18.47
LSD (0.05)	n.s.				n.s.			
Treatment	Protein (%)				Oil (%)			
	Half	Full	Double	Mean **	Half	Full	Double	Mean
Amistar	39.24	39.44	36.03	38.24 ab	19.47	19.45	19.24	19.39
Bayfidan	39.91	38.37	37.16	38.48 a	20.80	19.76	19.42	19.99
Capitan	37.15	37.60	35.73	36.83 bc	18.25	19.51	19.04	18.93
Denarin	38.63	39.05	37.57	38.42 a	19.01	18.71	18.60	18.77
Folicur	39.89	39.45	36.54	38.63 a	18.59	19.08	19.25	18.97
Fungicide X	38.24	37.87	35.96	37.36 abc	19.56	19.51	18.07	19.05
Impact	38.95	37.17	36.11	37.41 abc	18.35	19.73	18.25	18.78
Punch C	37.11	39.01	36.24	37.45 abc	18.48	19.16	19.09	18.91
Punch Xtra	37.26	37.33	36.08	36.89 bc	19.75	18.85	19.36	19.32
Score	37.13	36.67	35.10	36.30 c	19.51	19.41	18.63	19.18
Mean ***	38.35 a	38.19 a	36.25 b	37.60	19.18	19.32	18.89	19.13
LSD (0.05)	1.246			DMRT	n.s.			

* c.v. % too high for valid results

** Means followed by a different letter in the same column are significantly different at P = 0.05, using Duncan's multiple range test

*** Means followed by a different letter in the same row are significantly different at P = 0.05, using Fisher's LSD

Seed mass

No factors were significant (Table 3.9a). The coefficient of variation was low (5%).

Protein

The coefficient of variation was low (3.8%). Fungicide was significant (Fig. 3.5). Bayfidan, Denarin and Folicur, amongst others, resulted in the highest protein content, while Score, amongst others, resulted in the lowest protein content. Rate of fungicide application was significant with a linear response. Protein content decreased as the rate of application increased (Table 3.9b and Fig. 3.6). The interaction between rate and fungicide was not significant (Table 3.9a).

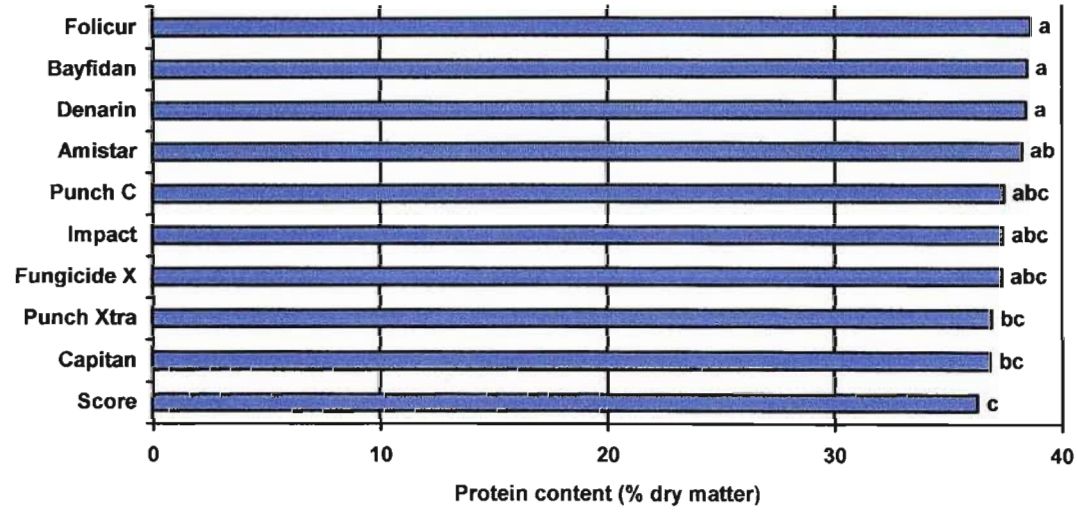

Figure 3.5 Soybean protein content (%) for different fungicides evaluated for the control of soybean rust caused by *Phakopsora pachyrhizi* at Cedara in 2002/2003

Oil

No factors were significant (Table 3.9a and Fig. 3.6). The coefficient of variation was low (4.7%).

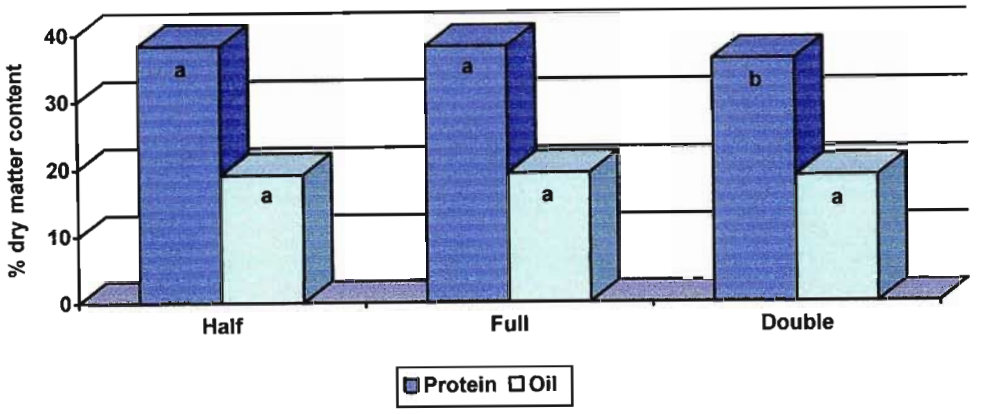

Figure 3.6 Percentage soybean protein and oil response to rate of fungicide application evaluated for the control of soybean rust caused by *Phakopsora pachyrhizi* at Cedara in 2002/2003

3.3.3 2003/2004 season

Final disease severity

The coefficient of variation was 25.7%. Rate was not significant (P = 0.06), although there was a significant linear response to the rate of fungicide application, with final disease severity decreasing as the rate of application increased (Tables 3.10a & b). Fungicide type was also significant, but more importantly the interaction between rate and fungicide was significant (Table 3.10a). There was no response to rate for Amistar and Folicur. Amistar had high final disease levels, with a mean of 39%. Folicur had very low final disease levels, with a mean of 0.68%. Fungicide X had a significantly higher final disease severity at the medium rate. Punch Xtra had the highest final disease severity at the lowest application rate (Fig. 3.7).

Standardised area under disease progress curve (SAUDPC)

The coefficient of variation was 22.4%. Rate of application was not significant, although there was a significant linear response to SAUDPC (Table 3.10a). SAUDPC decreased as rate of fungicide application increased (Table 3.10b). Fungicide and the interaction between fungicide and rate of application were significant (Table 3.10a). There was no response to rate of application for Folicur. Amistar and Fungicide X had

Table 3.10a ANOVA of soybean rust final disease severity (angular transformed), log transformed standardised area under disease progress curve (SAUDPC), yield (kg.ha^{-1}), seed mass (g.100 seed^{-1}), protein and oil content (percentage) for rate of fungicide application in 2003/2004

	Main effects and interaction	Degrees of freedom	Final disease severity	SAUDPC	Yield	Seed mass	Protein	Oil
Mean square	Rate	3	62.51	0.113	203080	2.330	2.026	1.225
	linear	1	179.85	0.290	68624	2.485	0.412	0.046
	quadratic	1	7.56	0.022	316722	4.499	3.971	2.558
	Residual	6	14.39	0.035	363711	1.209	1.371	0.517
	Fungicide	3	3656.89	14.698	180001	3.446	1.167	0.118
	Fungicide.Rate	9	49.03	0.066	145400	0.722	0.810	0.181
	Residual	22 (2)	12.00	0.023	188383	0.262	0.409	0.511
F-value	Rate		4.34	3.27	0.56	1.93	1.48	2.37
	linear		12.50	8.37	0.19	2.06	0.30	0.09
	quadratic		0.53	0.65	0.87	3.72	2.90	4.95
	Residual		1.20	1.49	1.93	4.62	3.35	1.01
	Fungicide		304.63	631.04	0.96	13.18	2.85	0.23
	Fungicide.Rate		4.08	2.82	0.77	2.76	1.98	0.35
F-probability	Rate		0.060	0.101	0.662	0.226	0.312	0.169
	linear		0.012 *	0.028 *	0.679	0.202	0.603	0.776
	quadratic		0.496	0.452	0.387	0.102	0.140	0.068
	Fungicide		<0.001 **	<0.001 **	0.431	<0.001 **	0.061	0.874
	Fungicide.Rate		0.003 **	0.023 *	0.643	0.025 *	0.092	0.945
% c.v.			25.7	22.4	10.0	2.4	1.6	4.3
Level of significance			P = > 0.05 n.s.		P = ≤ 0.05 *		P = ≤ 0.01 **	

Table 3.10b Table of means for soybean rust final disease severity (angular transformed), log transformed standardised area under disease progress curve (SAUDPC), yield (kg.ha^{-1}), seed mass (g.100 seed^{-1}), protein and oil content (percentage) for rate of fungicide application in 2003/2004

Treatment	Final disease severity (angular) *					SAUDPC (log) *				
	Low	Med	Reg	High	Mean	Low	Med	Reg	High	Mean
Amistar	38.24 a	40.18 a	39.33 a	38.24 a	39.00	2.33 ab	2.54 b	2.22 a	2.23 a	2.33
Folicur	2.71 a	0.00 a	0.00 a	0.00 a	0.68	0.03 a	0.00 a	0.00 a	0.00 a	0.01
Fungicide X	4.25 a	11.94 b	0.14 a	0.00 a	4.08	0.08 a	0.35 b	0.00 a	0.00 a	0.11
Punch Xtra	17.47 b	7.15 a	12.37 ab	3.83 a	10.20	0.57 c	0.18 ab	0.32 bc	0.03 a	0.27
Mean	15.67	14.82	12.96	10.52	13.49	0.75	0.77	0.64	0.57	0.68
LSD (0.05)	5.945					0.2713				
Treatment	Yield (kg.ha^{-1})					Seed mass (g. 100 seed^{-1}) *				
	Low	Med	Reg	High	Mean	Low	Med	Reg	High	Mean
Amistar	3754	4376	4617	4107	4213	20.25 b	20.87 ab	22.09 a	20.46 b	20.92
Folicur	4237	4603	4136	4186	4291	21.51 a	22.37 a	22.20 a	21.71 a	21.95
Fungicide X	4471	4527	4463	4549	4502	21.45 b	21.91 ab	22.18 ab	22.84 a	22.10
Punch Xtra	4214	4444	4137	4484	4320	21.17 b	22.56 a	21.95 ab	21.84 ab	21.88
Mean	4169	4487	4338	4332	4331	21.09	21.93	22.10	21.71	21.71
LSD (0.05)	n.s.					1.2312				
Treatment	Protein (%)					Oil (%)				
	Low	Med	Reg	High	Mean	Low	Med	Reg	High	Mean
Amistar	39.01	39.10	38.65	39.19	38.99	16.86	16.14	16.67	16.42	16.52
Folicur	38.64	39.24	39.39	39.81	39.27	16.86	15.73	16.31	16.55	16.37
Fungicide X	38.60	39.94	39.54	38.68	39.19	16.91	16.35	16.10	16.50	16.47
Punch Xtra	37.79	39.73	38.75	38.03	38.57	16.68	16.05	16.70	16.97	16.60
Mean	38.51	39.51	39.08	38.93	39.01	16.83	16.07	16.44	16.61	16.49
LSD (0.05)	n.s.					n.s.				

* Means followed by a different letter in the same row are significantly different at P = 0.05, according to Fisher's LSD

the highest SAUDPCs at the medium rate. The highest SAUDPC was at the lowest application rate for Punch Xtra (Table 3.10b and Fig. 3.8).

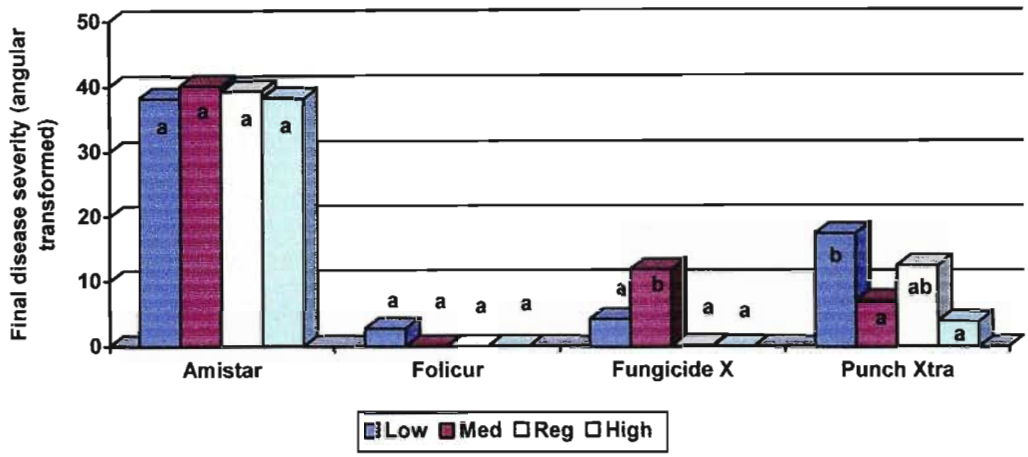

Figure 3.7 Final disease severity response to rate of application for different fungicides evaluated for the control of soybean rust caused by *Phakopsora pachyrhizi* at Cedara in 2003/2004

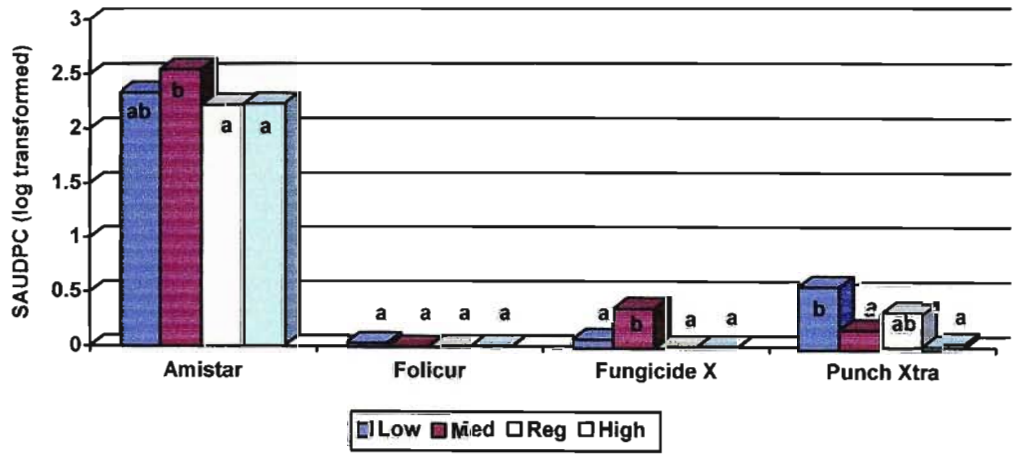

Figure 3.8 Standardised area under disease progress curve (SAUDPC) response to rate of application for different fungicides evaluated for the control of soybean rust caused by *Phakopsora pachyrhizi* at Cedara in 2003/2004

Yield

The coefficient of variation was 10%. No factors were significant (Table 3.10a).

Seed mass

Coefficient of variation was low at 2.4%. Rate of application was not significant. Fungicide and, more importantly, the interaction between fungicide and rate of application were significant (Table 3.10a). The highest seed mass for Amistar resulted from the application rate of 400 ml.ha^{-1}. There was no response to rate of application for Folicur. The highest seed mass for Fungicide X was at the highest application rate. The highest seed mass for Punch Xtra was obtained through the application rate of 500ml.ha^{-1} (Table 3.10b and Fig. 3.9).

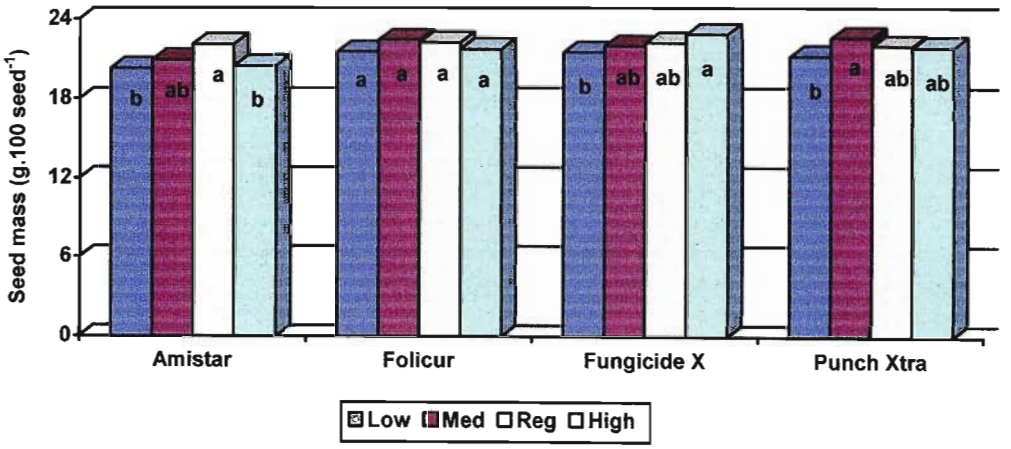

Figure 3.9 Soybean seed mass (g.100 seed^{-1}) response to rate of application for different fungicides evaluated for the control of soybean rust caused by *Phakopsora pachyrhizi* at Cedara in 2003/2004

Protein

The coefficient of variation was very low (1.6%). Fungicide was not significant (P = 0.061) and the interaction between fungicide and rate of application was not significant (P = 0.092). Rate of application was also not significant (Table 3.10a).

Oil

The coefficient of variation was low (4.3%). Rate of application was not significant, although trends show a nearly significant (P = 0.068) quadratic response to application rate (Table 3.10a and b). Fungicide and the fungicide x rate interaction were also not significant (Table 3.10a).

3.3.4 Response of individual fungicides to rate of application for all trials

The responses of individual fungicides to rates applied are illustrated graphically (Fig. 3.10 – 3.19). When the disease continues to decline with increasing rates, the optimum rate is not indicated. Optimum rates are indicated when the disease curve flattens between rates. Similarly, optimum yields are not achieved when the yield continues to climb with increasing rates.

Amistar

Disease control increased as dosage rate increased (Fig 3.10). Application rates of 400ml.ha^{-1} and greater resulted in the most effective disease control. There was no response to disease control at rates greater than 500ml.ha^{-1}. The highest yield was realised through application of Amistar at a rate of 400ml.ha^{-1}.

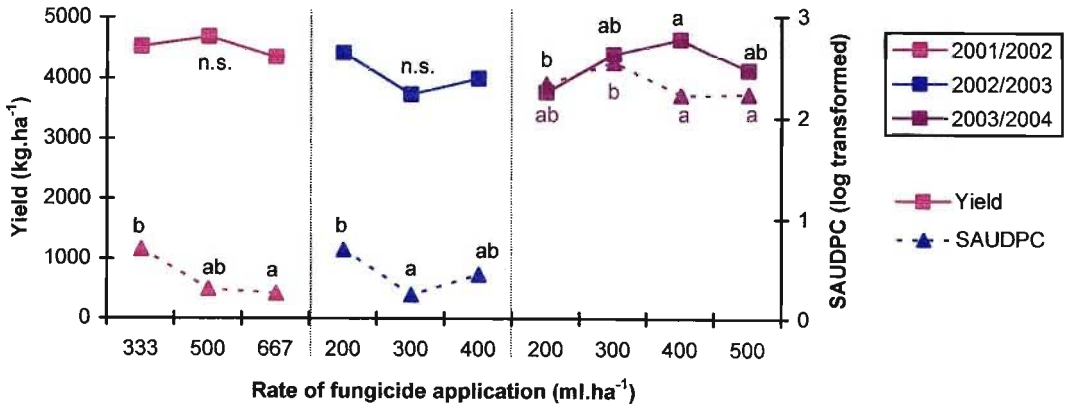

Figure 3.10 Relationship between yield and standardised area under disease progress curve (SAUDPC) for Amistar in the control of soybean rust caused by *Phakopsora pachyrhizi* from 2001 – 2004

Folicur

There was no significant response between disease control and dosage rate. All rates evaluated, from 450 - 2500ml.ha^{-1}, controlled disease (Fig. 3.11). Yield decreased as dosage rate increased, with phytotoxicity symptoms visible at many of the dosage rates in 2001/2002 and 2002/2003, when fungicides were applied at low spray volumes. At the registered rate of 750ml.ha^{-1} yields were lower than at lower dosage rates, suggesting that the registered rate is not optimal.

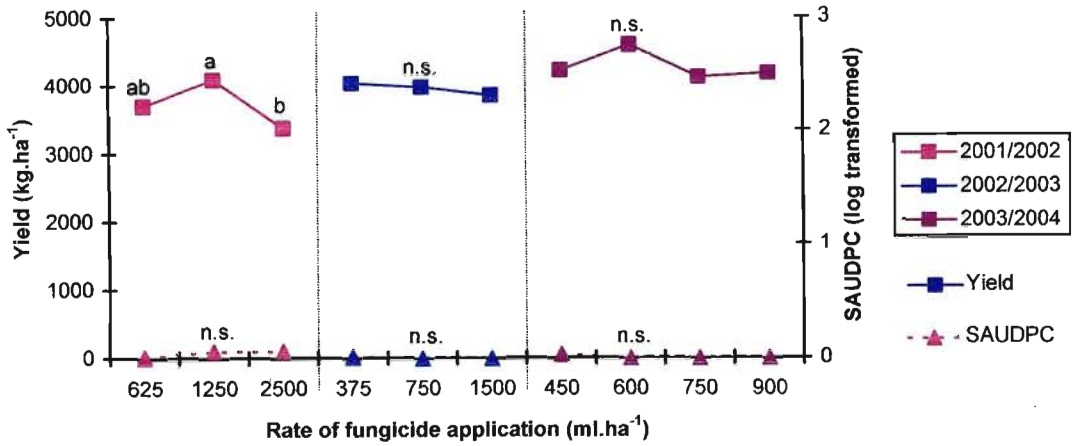

Figure 3.11 Relationship between yield and standardised area under disease progress curve (SAUDPC) for Folicur in the control of soybean rust caused by *Phakopsora pachyrhizi* from 2001 – 2004

Fungicide X

At high dosage rates, Fungicide X caused phytotoxicity symptoms, evident both in the low yields that resulted and expressed in the plants with leaves curling under and stunting of the soybean plants. This was especially evident in 2001/2002 when rates of over 2l.ha^{-1} were applied. Generally, disease was controlled across all rates evaluated, from 350 – 2333ml.ha^{-1} (Fig. 3.12). All rates from 350 – 700ml.ha^{-1} resulted in high yields, with adequate disease control. In 2002/2003 yields decreased between 350 and 700ml.ha^{-1}, suggesting that a rate lower than 700ml.ha^{-1} is optimal.

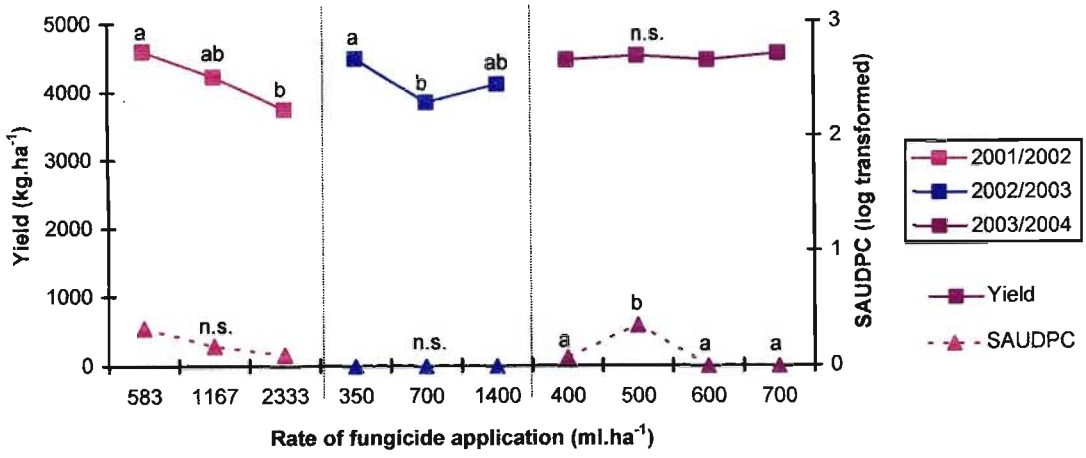

Figure 3.12 Relationship between yield and standardised area under disease progress curve (SAUDPC) for Fungicide X in the control of soybean rust caused by *Phakopsora pachyrhizi* from 2001 – 2004

Punch Xtra

Disease control was good, with control improving as dosage rates increased from 400 – 700ml.ha^{-1} (Fig. 3.13). At rates of >800ml.ha^{-1} there was no further response to disease control. Although not significant, yield improved as dosage rate increased. The optimum dosage rate lies between 500 – 800ml.ha^{-1}.

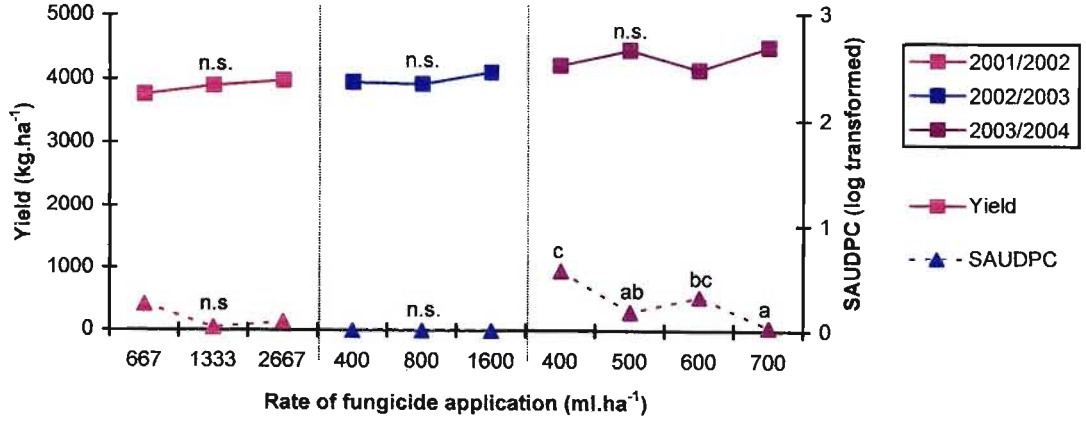

Figure 3.13 Relationship between yield and standardised area under disease progress curve (SAUDPC) for Punch Xtra in the control of soybean rust caused by *Phakopsora pachyrhizi* from 2001 – 2004

Punch C

At high dosage rates of 1333ml.ha^{-1}, yield decreased, as is evident in the 2001/2002 trials (Fig. 3.14). From 333 – 667ml.ha^{-1} there was no yield response to an increase in dosage rate. Similarly, between 400 – 800ml.ha^{-1}, there was no increase in yield with the higher dosage rate. Yield increased with an increase in dosage rate from 200 – 400ml.ha^{-1}. These results indicate that the registered rate of 400ml.ha^{-1} is optimal. Disease was controlled at all dosage rates evaluated.

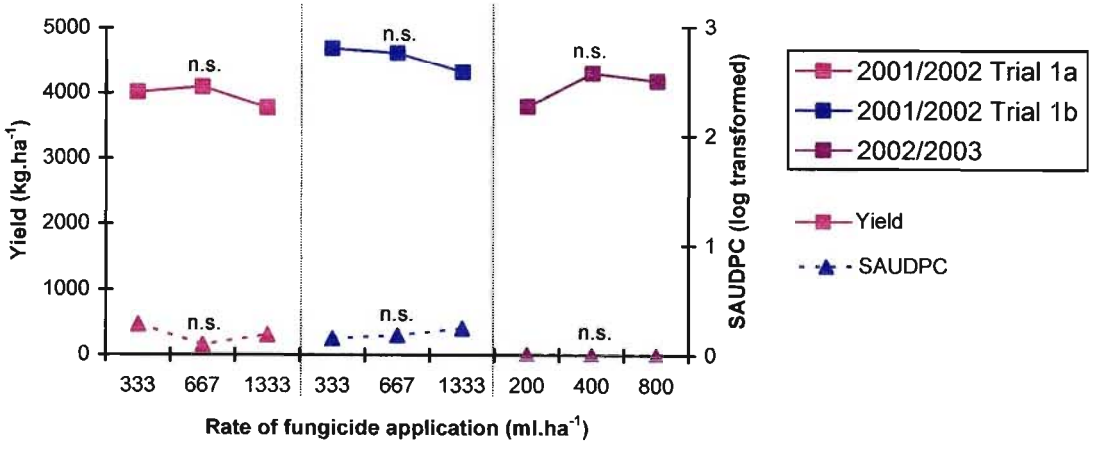

Figure 3.14 Relationship between yield and standardised area under disease progress curve (SAUDPC) for Punch C in the control of soybean rust caused by *Phakopsora pachyrhizi* from 2001 – 2003

Capitan

Disease control was good for all rates evaluated (Fig. 3.15). There was no distinct yield response, either increase or decrease, to rate of fungicide, across the range evaluated, 200 – 1333ml.ha^{-1}. The registered rate of 400ml.ha^{-1} is optimal.

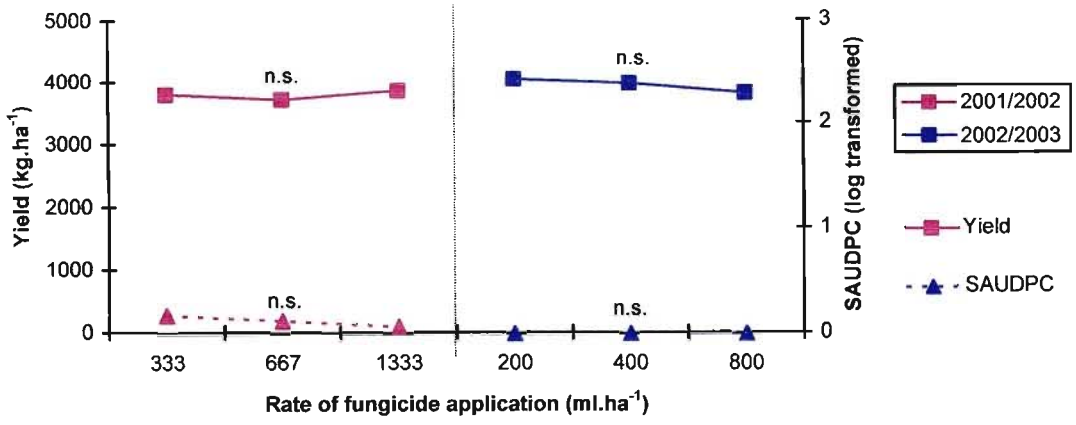

Figure 3.15 Relationship between yield and standardised area under disease progress curve (SAUDPC) for Capitan in the control of soybean rust caused by *Phakopsora pachyrhizi* from 2001 – 2003

Bayfidan

Disease control improved with increasing dosage rate (Fig. 3.16). Yield increased with increased dosage rates of 417 – 833ml.ha^{-1} and 500 – 1000ml.ha^{-1}. The registered dosage rate of 500ml.ha-1 does not appear to be optimal. A higher rate of between 800 – 1000ml.ha^{-1}, may be more effective.

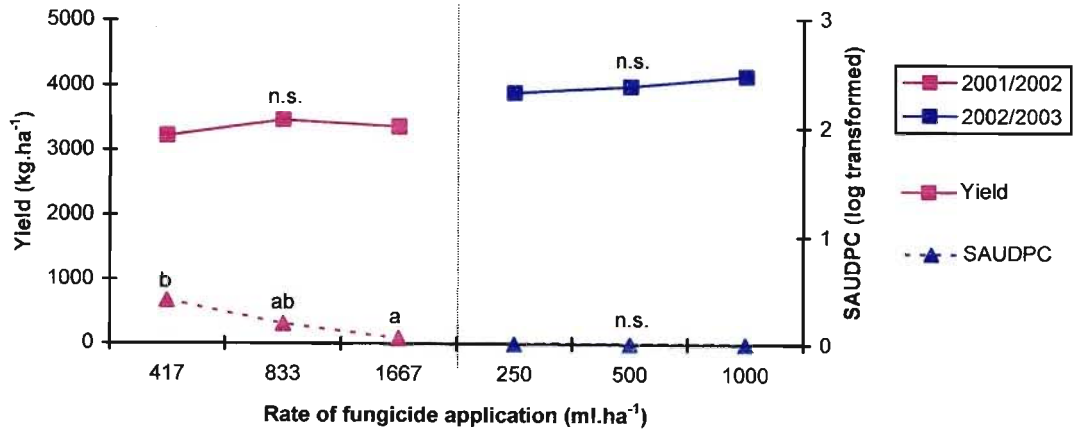

Figure 3.16 Relationship between yield and standardised area under disease progress curve (SAUDPC) for Bayfidan in the control of soybean rust caused by *Phakopsora pachyrhizi* from 2001 – 2003

Score

All dosage rates evaluated controlled disease (Fig. 3.17). Yield increased as dosage rate increased from 325 – 650ml.ha^{-1} and increased slightly from 542 – 1083ml.ha^{-1}. This suggests that the registered dosage rate of 325ml.ha^{-1} is not optimal. A rate of 500 – 750ml.ha^{-1} may result in more optimal yields.

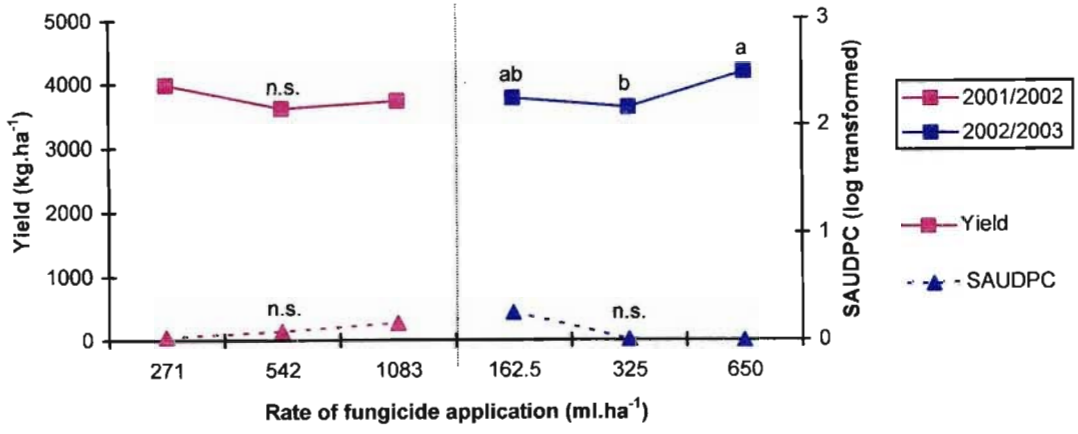

Figure 3.17 Relationship between yield and standardised area under disease progress curve (SAUDPC) for Score in the control of soybean rust caused by *Phakopsora pachyrhizi* from 2001 – 2003

Denarin

Disease was not controlled at 5000ml.ha^{-1} (Fig. 3.18). Yield decreased when dosage rates were increased from 2500 – 5000ml.ha^{-1}, probably as a result of poor disease control and phytotoxicity. Yield increased when dosage rates were increased from 1250 – 2500ml.ha^{-1} and from 1500 – 3000ml.ha^{-1}. This suggests that the optimal rate is higher than the registered rate of 1500ml.ha^{-1}.

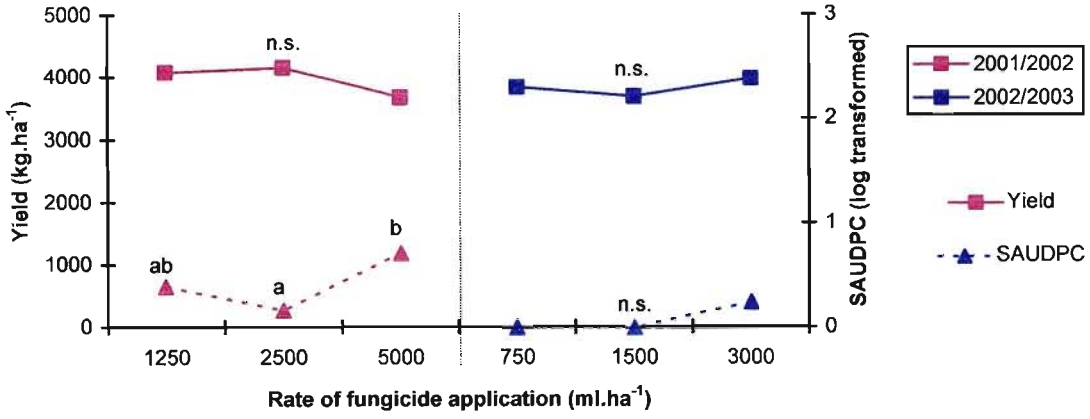

Figure 3.18 Relationship between yield and standardised area under disease progress curve (SAUDPC) for Denarin in the control of soybean rust caused by *Phakopsora pachyrhizi* from 2001 – 2003

Impact

Disease control was good for all dosage rates evaluated (Fig. 3.19). Yield increased as dosage rate increased from 833 – 1667ml.ha^{-1}, but decreased when dosage rate increased from 1667 – 3333ml.ha^{-1}. In 2002/2003, when there was very little disease development, yield decreased as dosage rate increased. The optimum rate would appear to be the registered rate of 1000ml.ha^{-1}, as in high disease pressure years, 500ml.ha^{-1} would not be expected to be effective.

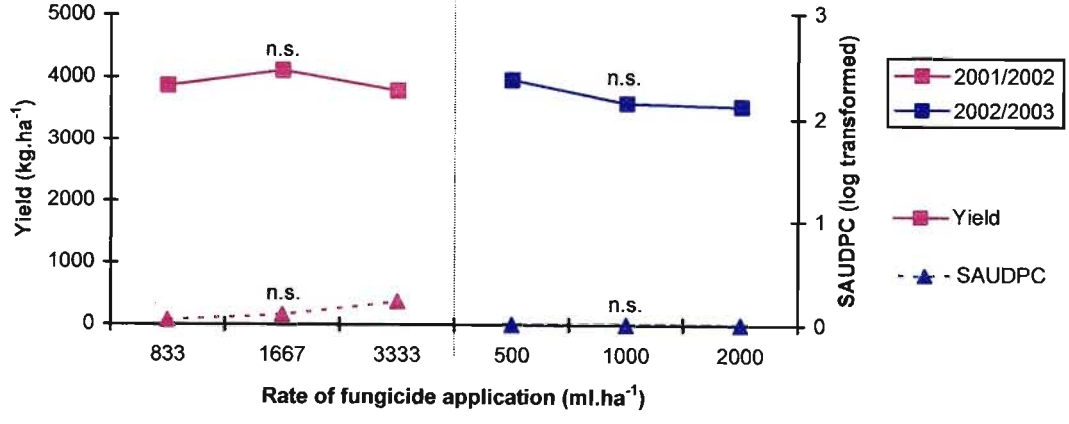

Figure 3.19 Relationship between yield and standardised area under disease progress curve (SAUDPC) for Impact in the control of soybean rust caused by *Phakopsora pachyrhizi* from 2001 – 2003

3.4 Discussion

The 2002/2003 season was dry for the entire growing period, with only 344mm rainfall compared with 612mm in 2003/2004. This drought resulted in low levels of soybean rust infection. The incidence of rust was also not uniform in distribution and this is evident in the high coefficients of variation obtained for disease ratings in 2002/2003. The drought also resulted in non-optimal growing conditions and soybean plants not achieving their full yield potential.

Fungicides resulted in similar responses, as observed and discussed in Chapter 2. Folicur and Fungicide X resulted in very low disease levels, while Amistar had much higher disease levels. Although disease incidence was high across all rates of Amistar, there was a significant increase in seed mass and a noticeable yield increase from applications of 300 to 400ml.ha^{-1}. Amistar at 400ml.ha^{-1} resulted in yield and seed mass that was as high as, or higher than, the target rates of Folicur, Fungicide X and Punch Xtra. These 2003/2004 results are supported by the 2001/2002 results, where applications of 333 and 500ml.ha^{-1} were made. At 333ml.ha^{-1}, Amistar resulted in yields that ranked third out of the five fungicides that were evaluated, whereas at 500ml.ha^{-1}, it ranked first. This indicates that Amistar is as effective as other fungicides in reducing yield loss when applied at higher dosage rates, despite higher disease levels in Amistar treatments.

Fungicide efficacy is not the only factor used to select which fungicide to use. The economics of spraying must always be considered. The chemical cost of Amistar at 300ml.ha^{-1} was R229.ha^{-1}. If it were to be registered at the optimal rate of 400ml.ha^{-1} this would increase to R305.ha^{-1}, compared with R128.ha^{-1} for Punch Xtra or R202.ha^{-1} for Folicur. At more than double the cost of Punch Xtra the use of Amistar would be difficult to justify.

Adjustment of dosage rates for SBR control is ongoing. Since there was very little prior research on SBR control with SBI and strobilurin fungicides, there was uncertainty about which rates would be optimal. In Zimbabwe, Punch Xtra is registered at a ground application rate of 325ml.ha^{-1}. Legislation in Zimbabwe for the registration of agrochemicals is different from that in SA in that it allows the lowest effective rate to be

registered. This rate, however, is not necessarily the optimum rate and furthermore, does not protect the fungicide from a resistance management perspective. In SA in 2001, an emergency registration was granted for the same formulation of Punch Xtra at 800ml.ha^{-1}. After the first set of SA trials conducted in 2001/2002, DuPont applied for, and was granted, a reduced application rate of 600ml.ha^{-1} for Punch Xtra. Similarly, in 2004 Bayer requested a reduction in rate from 750ml.ha^{-1} to 500ml.ha^{-1} for Folicur. Results have shown that these reduced rates are effective. The rate reduction will reduce chemical cost, making these fungicides more competitive against more expensive strobilurin-containing fungicides.

The long-term cost of fungicide resistance should be measured against the actual cost of fungicide use. Single site triazole and strobilurin fungicides, no matter the cost, should ideally not be used alone if the life of the fungicide is to be preserved. Reduced efficacy and resistance to triazoles (*Septoria tritici* on wheat) and strobilurins (cucurbit powdery & downy mildews; stem blight & black rot in cucurbits) have already been recorded (Anon., 2004b; McGrath, 2005). At present, Amistar is not registered in SA to control SBR. However, statistics reveal that 65% of soybeans sprayed for SBR in the 2003/2004 season were sprayed with Amistar, at an arguably non-optimal rate of 300ml.ha^{-1} (Broeksma, pers. comm.)[2]. This is disturbing from both an economic perspective, considering the cost of the product, as well as from a fungicide resistance management perspective. It is for this reason that mixtures of triazole and strobilurin fungicides are now being investigated for SBR control. In 2004 BASF applied for registration of Fungicide X, a triazole/strobilurin premix. It is hoped that there will be a move towards these triazole/strobilurin premixes and an increase in the already registered triazole/benzimidazole premixes, in order to reduce the development of fungicide resistance.

The effect of spray volume on the expression of phytotoxicity symptoms was well demonstrated with Folicur. In 2001/2002 and 2002/2003 spray volumes of 160ℓ.ha^{-1} were used, compared with 420ℓ.ha^{-1} in 2003/2004. Phytotoxicity was easily noticed when low spray volumes were used. Symptoms appeared approximately 5 – 7d after fungicide application and the effect was cumulative, with symptoms more obvious after

[2] Mr Andre Broeksma, Product Development Manager, Bayer Crop Science, P.O. Box 143, Isando, 1600, South Africa

each successive application. Such severe symptoms were not noticed with other fungicides and Bayer, the manufacturer of Folicur, was concerned about these symptoms. In 2003/2004, phytotoxicity was still apparent at the higher spray volume. It was, however, more difficult to notice and the degree of damage would have been considered acceptable by farmers' standards.

Discussion over the cause of these phytotoxicity symptoms arose, with factors such as spray volume, spraying conditions and cultivar being mentioned. Having had no other reports about phytotoxicity problems, Wimpie Roux of Bayer established his own trials to investigate the cause of the problem (pers. comm.)[3]. LS666, the same cultivar on which the scorch was originally noticed, was planted for comparative purposes. Treatments comprised four different spray volumes, each at the registered and double the registered application rates of Folicur. Findings were that scorch was apparent even on the treatments sprayed with a low spray volume.

Having corroborated the phytotoxicity findings, the next step was to investigate the effect of cultivar selection on the appearance of phytotoxicity symptoms. A simple observation trial was conducted at Cedara on 50 cultivars or breeding lines, with one application of Folicur at a spray volume of 420$\ell.ha^{-1}$. Phytotoxicity symptoms were evident on 14 cultivars (including LS 666), absent on 21 cultivars and marginal on 15 cultivars. The effect may therefore be genetically influenced. This trend is not uncommon. In potatoes the herbicide, bendioxide, is only registered for certain cultivars (Grobler *et al.*, 2000). Use in non-registered cultivars results in phytotoxic symptoms visible as scorching of the leaves (personal observation).

Since the research results given in Chapter 4 show that there is no significant difference between two or three fungicide applications and that, in reality, most farmers are only spraying twice for a cultivar of a medium growing length, this dosage rate study would have been more useful if it had been conducted with two sprays, where differences in rates may have been more clearly demonstrated. It is essential in a fungicide efficacy study that the crop is fully protected. However, in a dosage rate study, the trial should mimic farmer practices, rather than aim for complete protection by fungicides.

[3] Mr Wimpie Roux, Product Development Specialist, Bayer Crop Science, P.O. Box 11157, Dorpspruit, 3206, South Africa

Although these trials were conducted with 3 fungicide applications useful conclusions could be made from the research data. Yield or seed mass reduction when fungicides are used is a good indicator of dosage rate being too high. Although disease control may be excellent, when yield starts to decrease the rate needs to be decreased. None of the fungicides evaluated were registered at a rate where yields declined. Several fungicides need more research data in order to further optimise dosage rates. Amistar's proposed dosage rate for SBR control should be increased from 300 to 400ml.ha^{-1}.

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CHAPTER 4

Number of fungicide applications for control of soybean rust (*Phakopsora pachyrhizi* Syd.) on soybeans (Glycine max (L.) Merr.)

Abstract

An evaluation of the number of fungicide applications required to effectively control soybean rust (SBR) was conducted in several trials over three seasons, from 2001 – 2004, at Cedara (29°32'S, 30°16'E and alt. 1071m), 20km north-west of Pietermaritzburg. Two fungicides, Amistar and Punch C, were evaluated with one, two or three fungicide applications, commencing at flowering and with additional sprays at 21d intervals. In 2003/2004, evaluation of one fungicide application was omitted and the trial expanded to assess fourteen fungicides at two and three applications. Results showed that the number of fungicide applications impacted on final disease severity and standardised area under disease progress curve (SAUDPC), with disease decreasing as the number of fungicide applications increased. Differences in yield and seed mass results for number of applications were not significant. Treatments sprayed with Amistar had more disease than those sprayed with Punch C. Response to number of applications was distinctly linear for Amistar, but not for Punch C. Seed mass was significantly lower for Amistar treatments in 2003/2004 due to the high disease levels sustained. In 2003/2004, Amistar and Dithane M-45 treatments resulted in poor overall disease control and low yield and seed mass, whilst Early Impact, 'Impact with carbendazim', Folicur, Punch C and Punch Xtra resulted in good overall disease control, with high yields and seed mass. Oil content was not affected by fungicide sprays. Protein content was lower in fungicide sprayed treatments in 2001/2002. For all other seasons, protein content was not affected by spraying.

4.1 Introduction

Popularity of soybeans as a crop in South Africa (SA) grew in the late 1980s, when low production costs and good producer prices resulted in better gross margins than maize (Duxbury *et al.*, 1990). The climatic requirements of soybeans are essentially similar to those of maize and therefore the two crops are in direct competition with one another. Soybeans are commonly grown in rotation with maize, but maize is the principal crop of most KwaZulu-Natal (KZN) farmers. Despite the benefits of crop rotations including soybeans, maize will remain the principal crop in KZN, unless soybean cultivation can consistently maintain higher gross margins than maize. The appearance of *Phakopsora pachyrhizi* (Syd.) in SA in 2001 (Pretorius *et al.*, 2001) has resulted in increased production costs with fungicide sprays, which were not routine in soybean production prior to the arrival of soybean rust (SBR). This will further increase production costs of soybeans, compromising the competitiveness of soybean cultivation in KZN.

The occurrence of SBR in SA is mainly restricted to KZN, with epidemics occasionally reported outside the province in Mpumalanga. Seasonal incidence within KZN is usually first reported from production areas closer to the coast (bioclimatic group 3: mist-belt) and approximately 3 – 4wk later from the inland, north-western production fields (bioclimatic group 8: upland, drier type). Rust severity is usually higher in mist-belt production areas, compared with the upland, drier areas. Descriptions of bioclimatic groups for the two contrasting areas are provided by le Roux (1990). Altitudes in the mist-belt (bioclimatic group 3) range from 900 - 1400m. Annual rainfall varies from 800 - 1600mm. Mist is common and average annual temperatures are between 16 and 18°C. Climatic hazards include occasional dry spells of short duration in summer and excessive cloudiness in early summer. The upland, drier type bioclimatic group 8 ranges from 900 - 1000m in altitude. Annual rainfall, however, ranges from 600 - 800mm. Average annual temperatures also vary between 16 and 18°C. Climatic hazards include erratic rainfall and frequent periods of moisture stress.

SBR epidemics are most severe under climatic conditions with extended periods of leaf wetness (~ $10h.d^{-1}$) and moderate temperatures (18 - 26°C) (Casey, 1981). Extreme temperatures (> 30°C and/or < 15°C) and/or dry conditions retard the development of SBR. Environmental factors are critical in determining rust severity. High humidity and

long periods of leaf wetness are probably the main reasons for first reports of the growing season being made from mist-belt production areas. Although first reports of SBR in SA for a season have been made as early as the end of December, the disease usually only becomes epidemic during the month of February in the mist-belt. In north-western production areas, epidemics may only occur in March. Some northern areas of KZN escape SBR incidence altogether, probably due to drier conditions.

Apart from the effect of environment on SBR incidence, another factor which influences the start of new seasonal epidemics is the source of inoculum. *Phakopsora pachyrhizi* only survives on living hosts as an obligate parasite. Soybeans are frost-sensitive and therefore only cultivated in summer. Although *P. pachyrhizi* has more than 95 alternate host plants (Miles *et al.*, 2003), no studies have as yet been undertaken to determine exactly where the pathogen is overwintering in SA. It is suspected that the fungus may be overwintering on legume hosts cultivated year-round in coastal areas. Lupins, an alternate host, are however, also cultivated as a winter crop in the upland, drier type production areas. The fact that first reports are always made from the mist-belt, which is closer to the coast than the other production areas, supports the theory that the main inoculum source are legumes cultivated on the coast. The windborne urediospores would then be carried inland, causing new infections in the mist-belt areas and later, more spores would be blown further inland to start infections in the upland, drier type production areas.

Soybean cultivars in KZN are selected for adaptability to certain climatic areas. For each bioclimatic group, the choice of the optimum planting period is important, as it influences length of the growing season, plant height, total plant mass, bottom pod height, lodging, shattering, harvest date, seed yield and seed protein content (Birch *et al.*, 1990). Generally, late-maturing cultivars are planted earlier than medium- and early-maturing cultivars. This ensures that each cultivar makes sufficient vegetative growth prior to reaching the threshold photoperiod and temperature which triggers flowering. Differences in growing length of cultivars can affect the number of sprays required to protect the plant from SBR.

Cultivar selection, planting date and environmental conditions therefore noticeably impact SBR fungicide spray programmes. Since the application of fungicides increases

production costs, the soybean industry, in an effort to remain competitive, requires that gross margins are not decreased through the unnecessary application of fungicides. This study was conducted to determine the number of fungicide applications required to effectively control SBR.

4.2 Materials and methods

Trial site

Trials were conducted at Cedara agricultural research farm (29°32'S, 30°16'E and alt. 1071m), 20km north-west of Pietermaritzburg. Evaluations of different numbers of fungicide applications were conducted in 2001/2002, 2002/2003 and 2003/2004. The 2001/2002 and 2002/2003 trials were conducted on the same land, previously planted to potatoes. The 2003/2004 trial was planted on land that had previously been planted to maize.

Land preparation

Soil samples were taken of the topsoil (0-15cm) and fertilizer was supplied according to Fertrec recommendations from the Cedara Fertilizer Advisory Service (Farina and Channon, 1988). Phosphorus was band-applied in the rows at planting, to supply 21kg.ha^{-1} in 2001/2002 and 10.5kg.ha^{-1} in 2002/2003 (source: superphosphate (10.5%)). In 2003/2004, nitrogen, phosphorus and potassium (source: 2:3:4 (40)) were broadcast and disced in prior to planting, to supply 46kg.ha^{-1}, 69kg.ha^{-1} and 92kg.ha^{-1}, respectively.

Climatic data

Automatic weather stations based at Cedara were used to collect information on rainfall and temperature over the growing seasons. Long-term monthly rainfall and temperature averages at Cedara were supplied by the Institute for Soil, Climate and Water[1].

[1] Institute for Soil, Climate and Water, Agromet Section, Private Bag X79, Pretoria, 0001, South Africa

Trial design

In 2001/2002 and 2002/2003 a factorial design with randomised complete blocks and three replications was used to lay out the trials. Plots comprised four 5m rows spaced 45cm apart. The 2003/2004 trial was a split-plot design with fungicides as the whole-plot and number of applications as the sub-plot, replicated three times. Plots comprised six 5m rows spaced 45cm apart. The two central rows of each plot were used as data rows for disease evaluation and yield determination. The unsprayed outer rows of the plots are referred to as border rows.

Planting

Trials were hand-planted with the soybean cultivar LS666. Planting date, seeding rate and final plant population are shown in Table 4.1 Soybeans were inoculated with *Bradyrhizobium japonicum* ((Kirchner) Jordan) at planting, to enhance good nodule formation. Normal pest and weed control practices for the area were followed.

Table 4.1 Planting date, cultivar, seeding rate and plant population of soybean trials planted at Cedara in the 2001 - 2004 growing seasons

Season	Planting date	Cultivar & expected germination	Seeding rate (seeds.ha^{-1})	Plant population (plants.ha^{-1})
2001/2002	22 December 2001	LS666 (70-79% germination)	350 000	215 000 (61% germination)
2002/2003	12 November 2002	LS666 (>90% germination)	300 000	270 000 (90% germination)
2003/2004	12 November 2003	LS666 (>90% germination)	300 000	260 000 (87% germination)

Fungicide treatments

In 2001/2002 and 2002/2003 the effect of one, two and three fungicide applications was evaluated for two fungicides, Amistar and Punch C (Tables 4.2a and b) and compared against an unsprayed control. In 2001/2002 calibration of the spray equipment was based on a walking speed of 1m.s^{-1}. After the first two applications, however, it was realised that the actual walking speed in the canopied plants was slower, at 0.5m.s^{-1}. Effectively, this resulted in double the fungicide being applied for these two applications. The spray volume was recalculated on the slower walking speed and the corrected target application rates were applied for the third spray in 2001/2002 and all

applications in the proceeding seasons. In 2003/2004, the trial was reduced to evaluate the difference between two and three sprays, since results from the 2001/2002 and 2002/2003 trials showed that one spray was inadequate for sufficient control of the pathogen.

Table 4.2a Fungicides evaluated for the control of soybean rust caused by *Phakopsora pachyrhizi* in trials at Cedara from 2001 - 2004

Active ingredient (a.i.)	Concentration & formulation	Trade name	Manufacturer	Application rate (.ha^{-1})	
				ml	g a.i.
azoxystrobin	250 SC	Amistar	Syngenta	300	75
flusilazole & carbendazim	250 SC 125	Punch C	Du Pont	400	100 50

Table 4.2b Amount of fungicide active ingredient applied for the control of soybean rust caused by *Phakopsora pachyrhizi* in trials at Cedara from 2001 - 2004

Active ingredient (a.i.)	Application rate (.ha^{-1})		Total active ingredient applied					
			2001/2002 †			2002/2003 & 2003/2004		
	ml	g a.i.	One	Two	Three	One	Two	Three
azoxystrobin	300	75	150	300	375	75	150	225
flusilazole & carbendazim	400	100 50	200 100	400 200	500 250	100 50	200 100	300 150

† The first two applications received double the chemical dose. This error was corrected for the third application.

In 2003/2004 the effect of two and three sprays was extended to evaluate twelve additional fungicides (Table 4.3). An unsprayed treatment was included in each trial to check efficacy of the products.

All applications were applied during flowering and subsequent sprays were made at 21d intervals (Table 4.4). In 2001/2002 and 2002/2003 the application was made at early flowering, while in 2003/2004 the first application was made slightly later, at the late flowering stage.

Table 4.3 Fungicides evaluated and amount of fungicide active ingredient applied for the control of soybean rust caused by *Phakopsora pachyrhizi* in trials at Cedara in 2003/2004

Active ingredient (a.i.)	Concentration & formulation	Trade name	Manufacturer	Application rate (.ha^{-1})		Total a.i. applied	
				ml	g a.i.	2 applic.	3 applic.
azoxystrobin	250 SC	Amistar	Syngenta	300	75	150	225
carbendazim & flusilazole	250 SC 125	Punch Xtra	Du Pont	600	150 75	300 150	450 225
carbendazim & flutriafol	150 SC 94	Early Impact	Cheminova	1200	180 112.8	360 225.6	540 338.4
difenoconazole	250 EC	Score	Syngenta	325	81.25	162.5	243.75
flusilazole	250 EW	Capitan	Du Pont	400	100	200	300
flusilazole & carbendazim	250 SC 125	Punch C	Du Pont	400	100 50	200 100	300 150
flutriafol (& carbendazim) †	125 SC (117.5) (250)	'Impact with carbendazim'	Cheminova	1000	125 (117.5) (250)	242.5 (250)	367.5 (250)
mancozeb	800 WP	Dithane M-45	Algro-Chem	2kg	1600	3200	4800
tebuconazole	250 EW	Folicur	Bayer	750	187.5	375	562.5
triadimenol	250 DC	Bayfidan	Bayer	500	125	250	375
triadimenol	250 EC	Shavit	Makhteshim-Agan	500	125	250	375
triforine	190 EC	Denarin	BASF	1500	285	570	855
triazole & strobilurin	225 SC	Fungicide X	BASF	600	135	270	405
triazole & strobilurin	125 SC 200	Fungicide Y	Syngenta	375	46.875 75	93.75 150	140.625 225

† Due to the wrong product being supplied, the first application was made with flutriafol (117.5g) + carbendazim (250g) and the second and third applications were made with flutriafol (125g).

Table 4.4 Time (days after planting) of flowering of soybeans and fungicide applications for the control of soybean rust caused by *Phakopsora pachyrhizi* in trials at Cedara from 2001 - 2004

Season	First flower (DAP) †	1st spray (DAP)	2nd spray (DAP)	3rd spray (DAP)
2001/2002	61	65	86	107
2002/2003	71	71	92	114
2003/2004	71	82	103	124

† DAP = days after planting

From 2001 – 2003, full-cover fungicide sprays of 160ℓ.ha^{-1} at 200kPa pressure were applied to the central two rows of each plot, leaving the outer rows as border rows to reduce interplot interference. Fungicide spray solutions were applied using a CO_2-pressured back-pack sprayer with a horizontal spray-boom comprising two Spraying Systems TeeJet No8001 nozzles spaced 45cm apart. In 2003/2004, all fungicide spray solutions were applied with a CO_2-pressured backpack sprayer with a horizontal spray-boom and two Albuz ATR 80 hollow cone nozzles spaced 45cm apart. Full cover sprays of 420ℓ.ha^{-1} at 200kPa pressure were applied to the two central rows.

Artificial inoculation

In an effort to ensure even inoculum pressure throughout the trials, field plots were artificially inoculated with *P. pachyrhizi*. In 2001/2002, freshly collected *P. pachyrhizi* spores from early-infected trap plants were sprayed in a solution containing 0.01% household dishwashing liquid, using a knapsack sprayer, over the plots on 4 and 11 March at R3 growth stage (72 and 79d after planting (DAP), respectively). In 2002/2003, soybeans were planted in plastic bags (one plant per bag) and grown in tunnels, where favourable conditions allowed them to become naturally infected. On 19 February (99 DAP), these bags were placed in every second plot within the trial. In 2003/2004, five infected leaves were stapled singly, at approximately1m intervals, onto plants in the first row of each six-row plot, 98 DAP.

Disease assessment

Disease severity assessments were made regularly, at twice-weekly intervals on plants in the data rows, from flowering (R1) until physiological maturity (R7) of the crop. A plot rating was given after examining at least ten plants in the central two rows. Disease was assessed according to a rating scale developed at Cedara (see Table 2.4 of Chapter 2). The scale uses the position of rust pustules on the plant, pustule density, chlorosis and defoliation as parameters for determining disease severity. Data were used to calculate the area under disease progress curve (AUDPC), which summarises the disease epidemic. The AUDPC was calculated using a trapezoidal integration program (Berger, 1981) and standardised (SAUDPC) by dividing the AUDPC value by the duration of the epidemic. The SAUDPC allows for comparisons of disease from one season to another.

Harvesting

Leaving a 0.5m border on either end of each of the 5m rows, the central 4m of the central two rows in each plot, were hand-harvested. Grain yields were adjusted to a moisture content of 12.5% and expressed as $kg.ha^{-1}$. Seed mass (g) was determined for 100 seeds and corrected for moisture content. Protein and oil analyses were conducted on the seed using the Dumas combustion method (Dumas, 1831) and supercritical fluid extraction, respectively.

Statistical analyses

Statistical analyses of trial data (final disease severity, SAUDPC, yield, seed mass, protein and oil content) were conducted by analysis of variance (ANOVA), using Genstat 6.1. Mean separations were based on the least significant differences (LSD) or Duncan's multiple range test (DMRT) at the 5% level of probability. The LSD test is used when planned pair comparisons are being made and when the treatment number is not too large (less than six). DMRT is used when the treatment number exceeds five (Steel and Torrie, 1981). Linear and quadratic responses to the number of fungicide applications were analysed for 2001/2002 and 2002/2003 data. Due to one of the number of applications being omitted from the 2003/2004 trial, data could not be analysed for linear or quadratic responses.

4.3 Results

Flowering, first sign of disease and physiological maturity of the crop are recorded in Table 4.5.

Table 4.5 Time (days after planting) of flowering, first sign of disease and physiological maturity for soybean rust trials, caused by *Phakopsora pachyrhizi*, conducted on soybeans at Cedara from 2001 - 2004

Season	First flower (DAP) †	First sign of disease (DAP)	Physiological maturity (DAP)
2001/2002	61	83	122
2002/2003	71	97	139
2003/2004	71	106	141

† DAP = days after planting

Climatic data

Although rainfall was good early in the season, the 2001/2002 season was characterised by a mid-season drought which persisted from flowering (mid-February) until physiological maturity. The 2002/2003 season was warm, but very dry for the duration of the growing season. In contrast, the 2003/2004 season was warm with well-distributed rainfall throughout the growing season (Table 4.6).

Table 4.6 Rainfall and temperature data at Cedara for the 2001 - 2004 soybean growing seasons

	November	December	January	February	March	April
Rainfall (mm)						
2001/2002	126	158	153	38	59	61
2002/2003	52	84	47	66	95	44
2003/2004	104	103	162	154	89	4
Mean monthly †	111	132	135	123	111	52
Mean temperature (°C)						
2001/2002	18.5	18.7	20.7	18.6	19.5	17.6
2002/2003	16.7	19.1	19.8	20.8	19.7	17.5
2003/2004	17.6	18.8	19.7	19.3	18.5	17.3
Mean monthly†	18.0	19.5	20.2	20.2	19.3	16.7

† Long-term mean from 01/07/1914 to 30/06/2004

4.3.1 Final disease severity

A warm, wet 2003/2004 season resulted in more favourable conditions for disease development compared to 2001/2002 and 2002/2003. Together with an increased plot size (which both reduced interplot interference from fungicide drift and improved disease uniformity through larger unsprayed areas), as well as more effective inoculation techniques, there was an improvement in the coefficient of variation (c.v. %) compared to 2001/2002 and 2002/2003. Untransformed final disease severity is presented for all three seasons for comparative purposes, but square root transformations were used in 2001/2002 and 2002/2003 to normalise the data.

2001/2002 season

ANOVAs are recorded in Table 4.7a. Final disease severity reached 13.1% and 70.8% in the sprayed and unsprayed plots, respectively (Table 4.7b). Fungicide was not

Table 4.7a ANOVA table of final disease severity (percentage) for soybean rust caused by *Phakopsora pachyrhizi* when sprayed with Amistar and Punch C as affected by number of fungicide applications at Cedara from 2001 - 2004

Main effects and interaction	Degrees of freedom		Mean square			F-value			F-probability		
	2001 - 2003	2003/ 2004	2001/ 2002	2002/ 2003	2003/ 2004	2001/ 2002	2002/ 2003	2003/ 2004	2001/ 2002	2002/ 2003	2003/ 2004
Spray	1	1	8575.9	4927.5	11482.9	337.39	20.88	115.57	<0.001 **	<0.001 **	<0.001 **
Fungicide	1	1	91.1	1923.0	7151.6	3.58	8.15	71.98	0.083	0.015 *	0.001 **
Residual	-	4	-	-	99.4	-	-	2.42	-		
Frequency	2	1	336.1	3552.0	468.1	13.22	15.05	11.39	<0.001 **	<0.001 **	0.012 *
linear	1	-	567.2	6601.2	-	22.31	27.97	-	<0.001 **	<0.001 **	-
quadratic	1	-	105.1	502.9	-	4.13	2.13	-	0.065	0.17	-
Fungicide. Frequency	2	1	34.0	569.1	252.5	1.34	2.41	6.15	0.299	0.132	0.042 *
linear	1	-	63.0	1110.7	-	2.48	4.71	-	0.141	0.051	-
quadratic	1	-	5.1	27.5	-	0.20	0.12	-	0.663	0.739	-
Residual	12	7	25.4	236.0	41.1	-			-		
% c.v.	2001/2002		23.6		2002/2003		55.9		2003/2004		14.5
Level of significance	P = > 0.05 n.s.				P = ≤ 0.05 *				P = ≤ 0.01 **		

Table 4.7b Table of means of final disease severity (percentage) for soybean rust caused by *Phakopsora pachyrhizi* when sprayed with Amistar and Punch C as affected by number of fungicide applications at Cedara from 2001 - 2004

Main effects and interaction		2001/2002			2002/2003			2003/2004	
Spray †		Sprayed	Unsprayed		Sprayed	Unsprayed		Sprayed	Unsprayed
		13.1 a	70.8 b		21.2 a	65.0 b		26.4 a	80.0 b
LSD (0.05)		6.85			20.87			13.84	
Fungicide †		Amistar	Punch C		Amistar	Punch C		Amistar	Punch C
		15.3	10.8		31.6 b	10.9 a		50.8 b	2.0 a
LSD (0.05)		n.s.			15.78			15.98	
Frequency †		One	Two	Three	One	Two	Three	Two	Three
		21.7 b	9.7 a	7.9 a	48.4 b	13.8 a	1.5 a	32.7 b	20.2 a
LSD (0.05)		6.34			19.33			8.75	
Fungicide.Frequency †		One	Two	Three	One	Two	Three	Two	Three
	Amistar	25.8	12.7	7.5	67.5	25.8	1.3	61.7 b	40.0 a
	Punch C	17.5	6.7	8.3	29.3	1.7	1.7	3.7 a	0.3 a
LSD (0.05)		n.s.			n.s.			12.38	
† Means followed by a different letter in the same row of the same year are significantly different at P = 0.05, using Fisher's LSD									

significant. Number of applications was significant, with a linear response (Table 4.8a). One fungicide application resulted in a significantly higher final disease level than two or three applications (Fig. 4.1). Final disease levels decreased as number of applications increased (Table 4.8b). The interaction between fungicide and number of applications was not significant.

2002/2003 season

Final disease levels were 65% in the unsprayed plots (Table 4.7b). Coefficients of variation were reduced from 55.4 % to 34.1% by square root transformation (Tables 4.7a and 4.8a). There were significant differences in sprayed versus unsprayed plots, fungicide type and number of applications (Table 4.8a). The response to number of applications was linear, with a reduction in final disease level as number of fungicide applications increased (Table 4.8b and Fig. 4.1). The interaction between fungicide type and number of applications was not significant.

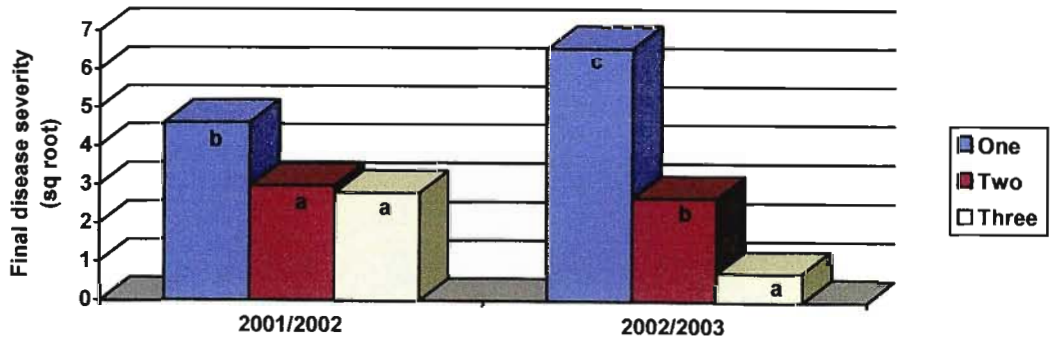


Figure 4.1 Effect of number of fungicide sprays on final disease severity (square root transformed) for soybean rust caused by *Phakopsora pachyrhizi* in 2001/2002 and 2002/2003

2003/2004 season

Amistar and Punch C only

All main effects and the interaction were significant (Table 4.7a). Punch C and Amistar treatments resulted in different responses to number of applications. Final disease severity for Punch C treatments was low and not significantly different between two

Table 4.8a ANOVA table of final disease severity (square root transformed) for soybean rust caused by *Phakopsora pachyrhizi* when sprayed with Amistar and Punch C as affected by number of fungicide applications at Cedara from 2001 - 2003

Main effects and interaction	Degrees of freedom	Mean square		F-value		F-probability	
	2001 - 2003	2001/2002	2002/2003	2001/2002	2002/2003	2001/2002	2002/2003
Spray	1	62.946	56.197	143.96	30.43	<0.001 **	<0.001 **
Fungicide	1	1.172	21.960	2.68	11.89	0.128	0.005 **
Frequency	2	6.083	52.459	13.91	28.41	<0.001 **	<0.001 **
linear	1	9.962	101.128	22.78	54.76	<0.001 **	<0.001 **
quadratic	1	2.203	3.790	5.04	2.05	0.044 *	0.178
Fungicide.Frequency	2	0.523	5.512	1.19	2.98	0.336	0.089
linear	1	0.872	8.117	1.99	4.40	0.183	0.058
quadratic	1	0.174	2.907	0.40	1.57	0.541	0.234
Residual	12	0.437	1.847	-		-	
% c.v.	**2001/2002**	15.9		**2002/2003**		34.1	
Level of significance	P = > 0.05 n.s.		P = ≤ 0.05 *			P = ≤ 0.01 **	

Table 4.8b Table of means of final disease severity (square root transformed) for soybean rust caused by *Phakopsora pachyrhizi* when sprayed with Amistar and Punch C as affected by number of fungicide applications at Cedara from 2001 - 2003

Main effects and interaction		2001/2002			2002/2003		
Spray †		**Sprayed**	**Unsprayed**		**Sprayed**	**Unsprayed**	
		3.46 a	8.41 b		3.32 a	8.00 b	
LSD (0.05)		0.898			1.846		
Fungicide †		**Amistar**	**Punch C**		**Amistar**	**Punch C**	
		3.71	3.20		4.43 b	2.22 a	
LSD (0.05)		n.s.			1.396		
Frequency †		**One**	**Two**	**Three**	**One**	**Two**	**Three**
		4.62 b	2.97 a	2.80 a	6.55 c	2.67 b	0.74 a
LSD (0.05)		0.832			1.710		
Fungicide.Frequency		**One**	**Two**	**Three**	**One**	**Two**	**Three**
	Amistar	5.07	3.36	2.71	8.19	4.35	0.74
	Punch C	4.16	2.57	2.88	4.91	1.00	0.75
LSD (0.05)		n.s.			n.s.		

† Means followed by a different letter in the same row of the same year are significantly different at P = 0.05, using Fisher's LSD

(3.7%) and three (0.3%) fungicide sprays. In contrast, final disease severity for Amistar treatments were high, with significant differences between two (61.7%) and three (40%) fungicide applications (Table 4.7b).

All fungicides

Coefficient of variation (c.v.) was higher in this trial (34.8%) due to the larger variation in fungicide activity. Percentage final disease severity data are presented in Tables 4.9a & b. Square root transformation reduced the c.v. to 22.1%. All main effects and the interaction were highly significant (Table 4.10a). Early Impact and 'Impact with carbendazim' treatments had zero final disease for two and three fungicide applications. The other twelve fungicides had significantly different final disease levels between two and three fungicide applications. Although the difference between two and three applications was significant, for Folicur, Punch C and Punch Xtra the differences were not substantial (Tables 4.9b & 4.10b). Two applications of Early Impact, 'Impact with carbendazim', Folicur, Punch C and Punch Xtra resulted in final disease levels of less than 10%. However, three applications of Bayfidan, Capitan, Fungicide X, Fungicide Y and Shavit also resulted in final disease levels of less than 10%. Final disease levels were always lower when three fungicide applications were administered.

Table 4.9a ANOVA table of final disease severity for soybean rust caused by *Phakopsora pachyrhizi* for all fungicides as affected by number of fungicide applications at Cedara in 2003/2004

Stratum	Main effects and interaction	Degrees of freedom	Mean square	F-value	F-probability
Whole-plot	Spray	1	23150.57	389.01	<0.001 **
	Fungicide	13	1418.07	23.81	<0.001 **
	Residual	28	59.51	1.23	-
Sub-plot	Frequency	1	4708.51	97.20	<0.001 **
	Fungicide.Frequency	13	163.67	3.38	0.003 **
	Residual	31	48.44	-	
% c.v.		34.8			
Level of significance		P = > 0.05 n.s.	P = ≤ 0.05 *	P = ≤ 0.01 **	

Table 4.9b Table of means of final disease severity for soybean rust caused by *Phakopsora pachyrhizi* for all fungicides as affected by number of fungicide applications at Cedara in 2003/2004

Spray †	Sprayed		Unsprayed	
	15.7	a	80.0	b
LSD (0.05)	6.678			

Fungicide ‡	Amistar	Bayfidan	Capitan	Denarin	Dithane M-45	Early Impact	Fungicide X
	50.8	24.2	7.8	25.0	40.0	0.0	9.5
	Fungicide Y	Folicur	'Impact'	Punch C	Punch Xtra	Score	Shavit
	13.3	2.5	0.0	2.0	5.8	20.8	18.0

Frequency †	Two		Three	
	23.2	b	8.2	a
LSD (0.05)	3.210			

Fungicide.Frequency §		Amistar	Bayfidan	Capitan	Denarin	Dithane M-45	Early Impact	Fungicide X
	Two	61.7 b	40.0 b	15.0 b	33.3 b	53.3 b	0.0 a	18.3 b
	Three	40.0 a	8.3 a	0.7 a	16.7 a	26.7 a	0.0 a	0.7 a
		Fungicide Y	Folicur	'Impact'	Punch C	Punch Xtra	Score	Shavit
	Two	21.7 b	5.0 a	0.0 a	3.7 a	9.3 a	31.7 b	31.7 b
	Three	5.0 a	0.0 a	0.0 a	0.4 a	2.3 a	10.0 a	4.3 a
LSD (0.05)		11.590						

† Means followed by a different letter in the same **row** are significantly different at P = 0.05, using Fisher's LSD

‡ DMRT not performed as fungicide.frequency interaction is significant

§ Means followed by a different letter in the same **column** are significantly different at P = 0.05, using Fisher's LSD

Table 4.10a ANOVA table of final disease severity (square root transformed) for soybean rust caused by *Phakopsora pachyrhizi* for all fungicides as affected by number of fungicide applications at Cedara in 2003/2004

Stratum	Main effects and interaction	Degrees of freedom	Mean square	F-value	F-probability
Whole-plot	Spray	1	193.004	227.95	<0.001 **
	Fungicide	13	28.090	33.18	<0.001 **
	Residual	28	0.847	1.46	-
Sub-plot	Frequency	1	91.758	157.69	<0.001 **
	Fungicide.Frequency	13	2.115	3.64	0.002 **
	Residual	31	0.582	-	
% c.v.		22.1			
Level of significance		P = > 0.05 n.s.	P = ≤ 0.05 *	P = ≤ 0.01 **	

Table 4.10b Table of means of final disease severity (square root transformed) for soybean rust caused by *Phakopsora pachyrhizi* for all fungicides as affected by number of fungicide applications at Cedara in 2003/2004

Spray †		Sprayed			Unsprayed			
		3.07	a		8.94	b		
LSD (0.05)		0.797						
Fungicide ‡		Amistar	Bayfidan	Capitan	Denarin	Dithane M-45	Early Impact	Fungicide X
		7.03	4.41	2.23	4.83	6.17	0.00	2.44
		Fungicide Y	Folicur	'Impact'	Punch C	Punch Xtra	Score	Shavit
		3.32	1.12	0.00	1.12	2.11	3.32	3.83
Frequency †		Two			Three			
		4.11	b		2.02	a		
LSD (0.05)		0.340						
Fungicide.Frequency §		Amistar	Bayfidan	Capitan	Denarin	Dithane M-45	Early Impact	Fungicide X
	Two	7.83 b	6.33 b	3.73 b	5.76 b	7.20 b	0.00 a	4.21 b
	Three	6.23 a	2.49 a	0.74 a	3.90 a	5.14 a	0.00 a	0.67 a
		Fungicide Y	Folicur	'Impact'	Punch C	Punch Xtra	Score	Shavit
	Two	4.40 b	2.24 b	0.00 a	1.82 b	2.81 b	5.62 b	5.60 b
	Three	2.24 a	0.00 a	0.00 a	0.41 a	1.41 a	2.98 a	2.07 a
LSD (0.05)		1.2703						

† Means followed by a different letter in the same **row** are significantly different at P = 0.05, using Fisher's LSD

‡ DMRT not performed as fungicide.frequency interaction is significant

§ Means followed by a different letter in the same **column** are significantly different at P = 0.05, using Fisher's LSD

4.3.2 Standardised area under disease progress curve (SAUDPC)

All SAUDPC data were log transformed in an attempt to normalise the data. As with final disease severity, coefficients of variation were higher in the first two seasons (25.0 and 40.4% in 2001/2002 and 2002/2003, respectively) and acceptable in 2003/2004 at 10.8%.

2001/2002 season

All main effects were significant, but the interaction was not (Table 4.11a). A significant linear response was observed for number of fungicide applications, with treatments receiving only one fungicide spray having significantly higher SAUDPC values. Although treatments receiving three fungicide sprays had lower SAUDPC values, they were not significantly different from those which received two fungicide sprays (Table 4.11b). Even though the interaction between fungicide and number of sprays was not significant, there was a significantly different response between the two fungicides (Table 4.11a). Treatments which were sprayed with Amistar had a linear response to number of sprays. Treatments sprayed with Punch C had more of a quadratic response to number of sprays, although the differences between number of applications was not significant (Table 4.11b).

2002/2003 season

All main effects, linear responses and interaction were significant (Table 4.11a). Amistar treatments resulted in different responses to number of applications than Punch C treatments (Table 4.11b). The response to number of sprays was linear for Amistar, with lower SAUDPC values as number of applications increased. SAUDPC values were more quadratic in response to number of applications for Punch C treatments. Two Punch C applications resulted in the lowest SAUDPC, which was significantly different from one Punch C application. Punch C treatments which received three fungicide sprays resulted in SAUDPC values that were not significantly different from two sprays (Table 4.11b).

Table 4.11a ANOVA table of log transformed standardised area under disease progress curve (SAUDPC) for soybean rust caused by *Phakopsora pachyrhizi* when sprayed with Amistar and Punch C as affected by number of fungicide applications at Cedara from 2001 - 2004

Main effects and interaction	Degrees of freedom		Mean square			F-value			F-probability		
	2001 - 2003	2003/ 2004	2001/ 2002	2002/ 2003	2003/ 2004	2001/ 2002	2002/ 2003	2003/ 2004	2001/ 2002	2002/ 2003	2003/ 2004
Spray	1	1	11.989	7.891	14.460	79.41	31.95	73.60	<0.001 **	<0.001 **	0.001 **
Fungicide	1	1	1.834	2.805	15.408	12.15	11.36	78.43	0.005 **	0.006 **	<0.001 **
Residual	-	4	-	-	0.196	-	-	4.42	-		
Frequency	2	1	1.233	4.702	0.040	8.16	19.04	0.91	0.006 **	<0.001 **	0.372
linear	1	-	1.940	8.879	-	12.85	35.95	-	0.004 **	<0.001 **	-
quadratic	1	-	0.526	0.524	-	3.48	2.12	-	0.087	0.171	-
Fungicide. Frequency	2	1	0.419	1.080	0.020	2.78	4.37	0.45	0.102	0.037 *	0.523
linear	1	-	0.839	2.003	-	5.56	8.11	-	0.036 *	0.015 *	-
quadratic	1	-	0.000	0.156	-	0.00	0.63	-	0.987	0.442	-
Residual	12	7	0.151	0.247	0.044	-			-		
% c.v.	2001/2002	25.0	2002/2003	40.4	2003/2004	10.8					
Level of significance	P = > 0.05 n.s.	P = ≤ 0.05 *	P = ≤ 0.01 **								

Table 4.11b Table of means of log transformed standardised area under disease progress curve (SAUDPC) for soybean rust caused by *Phakopsora pachyrhizi* when sprayed with Amistar and Punch C as affected by number of fungicide applications at Cedara from 2001 - 2004

Main effects and interaction		2001/2002			2002/2003			2003/2004	
Spray †		Sprayed	Unsprayed		Sprayed	Unsprayed		Sprayed	Unsprayed
		1.24 a	3.40 b		0.98 a	2.73 b		1.32 a	3.22 b
LSD (0.05)		0.528			0.675			0.615	
Fungicide †		Amistar	Punch C		Amistar	Punch C		Amistar	Punch C
		1.56 b	0.92 a		1.38 b	0.59 a		2.45 b	0.18 a
LSD (0.05)		0.399			0.510			0.711	
Frequency †		One	Two	Three	One	Two	Three	Two	Three
		1.77 b	1.00 a	0.96 a	1.96 b	0.74 a	0.24 a	1.38	1.26
LSD (0.05)		0.489			0.625			n.s.	
Fungicide.Frequency †		One	Two	Three	One	Two	Three	Two	Three
	Amistar	2.35	1.32	1.02	2.70 c	1.27 b	0.16 a	2.47	2.43
	Punch C	1.18	0.68	0.91	1.22 b	0.21 a	0.32 a	0.28	0.09
LSD (0.05)		n.s.			0.884			n.s.	

† Means followed by a different letter in the same row of the same year are significantly different at P = 0.05, using Fisher's LSD

2003/2004 season

Amistar and Punch C only

Fungicides significantly reduced SAUDPC values. Punch C treatments resulted in significantly lower SAUDPC values than Amistar treatments. Number of fungicide applications and the interaction between fungicide and number of applications were not significant (Table 4.11a).

All fungicides

All main effects and the interaction were significant (Table 4.12a). Bayfidan, Capitan, Fungicide X, Fungicide Y, Score and Shavit treatments resulted in significantly lower SAUDPC values through application of a third fungicide spray. Early Impact, Folicur, 'Impact with carbendazim', Punch C and Punch Xtra treatments resulted in low SAUDPC values and no significant difference between number of applications. Amistar, Denarin and Dithane M-45 had the highest SAUDPC values of the fungicide treatments, with no significant reduction in SAUDPC through a third fungicide application (Table 4.12b and Fig. 4.2).

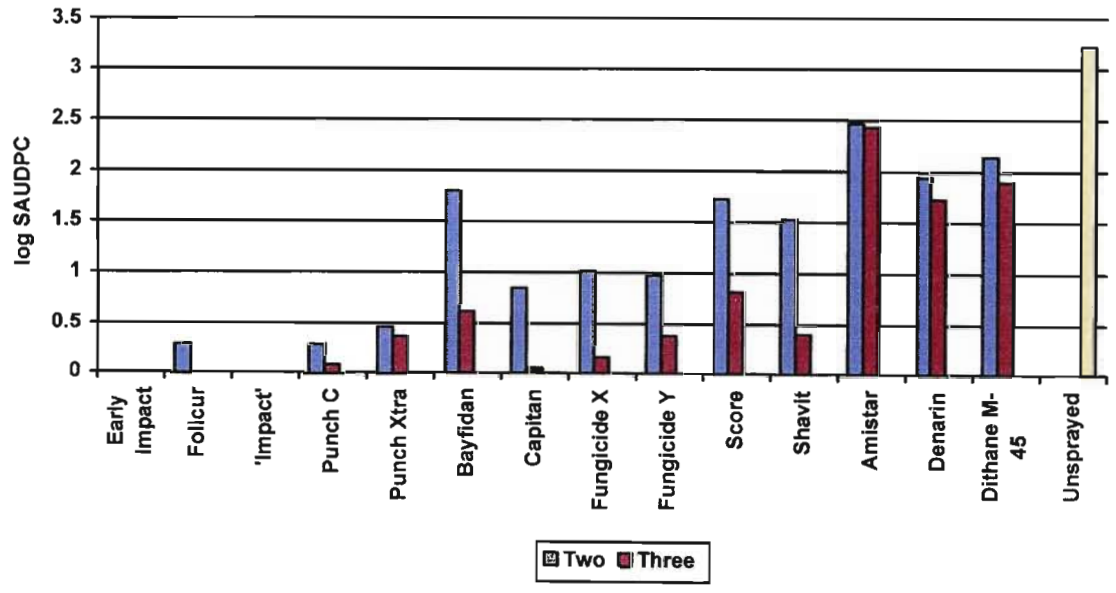


Figure 4.2 Effect of number of fungicide sprays on log transformed standardised area under disease progress curve (SAUDPC) for soybean rust caused by *Phakopsora pachyrhizi* in 2003/2004

Table 4.12a ANOVA table of log transformed standardised area under disease progress curve (SAUDPC) for soybean rust caused by *Phakopsora pachyrhizi* for all fungicides as affected by number of fungicide applications at Cedara in 2003/2004

Stratum	Main effects and interaction	Degrees of freedom	Mean square	F-value	F-probability
Whole-plot	Spray	1	30.813	216.62	<0.001 **
	Fungicide	13	3.727	26.20	<0.001 **
	Residual	28	0.142	1.42	-
Sub-plot	Frequency	1	4.582	45.79	<0.001 **
	Fungicide.Frequency	13	0.275	2.74	0.010 **
	Residual	31	0.100	-	
% c.v.		30.7			
Level of significance		P = > 0.05 n.s.	P = ≤ 0.05 *	P = ≤ 0.01 **	

Table 4.12b Table of means of log transformed standardised area under disease progress curve (SAUDPC) for soybean rust caused by *Phakopsora pachyrhizi* for all fungicides as affected by number of fungicide applications at Cedara in 2003/2004

Spray †	Sprayed			Unsprayed			
	0.87 a			3.22 b			
LSD (0.05)	0.327						
Fungicide ‡	Amistar	Bayfidan	Capitan	Denarin	Dithane M-45	Early Impact	Fungicide X
	2.45	1.21	0.45	1.84	2.03	0.00	0.59
	Fungicide Y	Folicur	'Impact'	Punch C	Punch Xtra	Score	Shavit
	0.68	0.14	0.00	0.18	0.41	1.28	0.97
Frequency †	Two			Three			
	1.11 b			0.64 a			
LSD (0.05)	0.141						
Fungicide.Frequency §	Amistar	Bayfidan	Capitan	Denarin	Dithane M-45	Early Impact	Fungicide X
Two	2.47 a	1.80 b	0.85 b	1.95 a	2.15 a	0.00 a	1.02 b
Three	2.43 a	0.63 a	0.050 a	1.73 a	1.90 a	0.00 a	0.16 a
	Fungicide Y	Folicur	'Impact'	Punch C	Punch Xtra	Score	Shavit
Two	0.98 b	0.29 a	0.00 a	0.28 a	0.46 a	1.73 b	1.53 b
Three	0.38 a	0.00 a	0.00 a	0.09 a	0.36 a	0.82 a	0.40 a
LSD (0.05)	0.527						

† Means followed by a different letter in the same **row** are significantly different at P = 0.05, using Fisher's LSD

‡ DMRT not performed as fungicide.frequency interaction is significant

§ Means followed by a different letter in the same **column** are significantly different at P = 0.05, using Fisher's LSD

4.3.3 Yield

ANOVAs are recorded in Table 4.13a. Yields varied across the three seasons, with the lowest yields realised for the 2001/2002 season due to a late planting date and the highest yields for the 2003/2004 season (Table 4.13b). Due to the dry conditions in 2002/2003 the crop did not realise its full yield potential.

2001/2002 season

Number of fungicide applications and fungicide type were not significant (Table 4.13a). The only effect that was significant was sprayed versus unsprayed, in which 917kg.ha^{-1} gain in yield was obtained through the application of fungicides (Table 4.13b).

2002/2003 season

No effects or interactions were significant during this dry season (Table 4.13a). Although 341kg.ha^{-1} was gained through spraying, the difference was not statistically significant (Table 4.13b).

2003/2004 season

Amistar and Punch C only

Similar to 2001/2002, the only significant effect was that of spraying (Table 4.13a). A yield gain of 849kg.ha^{-1} was obtained from fungicide application (Table 4.13b). Fungicide type was almost significant (P = 0.062), with Punch C treatments resulting in higher yields than Amistar treatments. Number of sprays and the interaction between fungicide type and number of sprays were not significant (Table 4.13a).

All fungicides

Spray and fungicide type effects were significant (Table 4.14a). The yield gain from fungicide application was 1155kg.ha^{-1} (Table 4.14b). 'Impact with carbendazim' resulted in the highest yield, but was not significantly different from Early Impact, Punch Xtra, Fungicide X, Capitan, Fungicide Y, Folicur, Shavit and Punch C. Amistar treatments resulted in the lowest yield, but was not significantly different from Dithane M-45, Bayfidan, Denarin, Score, Punch C, Shavit and Folicur (Figure 4.3). Number of

Table 4.13a ANOVA table of yield ($kg.ha^{-1}$) for soybean rust caused by *Phakopsora pachyrhizi* when sprayed with Amistar and Punch C as affected by number of fungicide applications at Cedara from 2001 - 2004

Main effects and interaction	Degrees of freedom		Mean square			F-value			F-probability		
	2001 - 2003	2003/ 2004	2001/ 2002	2002/ 2003	2003/ 2004	2001/ 2002	2002/ 2003	2003/ 2004	2001/ 2002	2002/ 2003	2003/ 2004
Spray	1	1	2161391	298131	2882931	10.08	1.73	27.42	0.008 **	0.213	0.006 **
Fungicide	1	1	111021	109855	690173	0.52	0.64	6.57	0.486	0.441	0.062
Residual	-	4	-	-	105122	-	-	1.27	-		
Frequency	2	1	214244	270661	27529	1.00	1.57	0.33	0.397	0.248	0.583
linear	1	-	384118	387107	-	1.79	2.24	-	0.206	0.160	-
quadratic	1	-	44370	154215	-	0.21	0.89	-	0.657	0.363	-
Fungicide. Frequency	2	1	81945	10855	25530	0.38	0.06	0.31	0.690	0.939	0.596
linear	1	-	80994	21206	-	0.38	0.12	-	0.550	0.732	-
quadratic	1	-	82895	504	-	0.39	0.00	-	0.546	0.958	-
Residual	12	7	214369	172696	82962	-			-		
% c.v.	2001/2002		13.3	2002/2003		10.7	2003/2004		7.5		
Level of significance	P = > 0.05 n.s.			P = ≤ 0.05 *			P = ≤ 0.01 **				

Table 4.13b Table of means of yield ($kg.ha^{-1}$) for soybean rust caused by *Phakopsora pachyrhizi* when sprayed with Amistar and Punch C as affected by number of fungicide applications at Cedara from 2001 - 2004

Main effects and interaction		2001/2002			2002/2003			2003/2004	
Spray †		Sprayed	Unsprayed		Sprayed	Unsprayed		Sprayed	Unsprayed
		3600 a	2683 b		3915	3574		4122 a	3273 b
LSD (0.05)		629.1			n.s.			450.1	
Fungicide		Amistar	Punch C		Amistar	Punch C		Amistar	Punch C
		3679	3521		3837	3993		3882	4361
LSD (0.05)		n.s.			n.s.			n.s.	
Frequency		One	Two	Three	One	Two	Three	Two	Three
		3386	3670	3744	3670	4046	4029	4074	4169
LSD (0.05)		n.s.			n.s.			n.s.	
Fungicide.Frequency		One	Two	Three	One	Two	Three	Two	Three
	Amistar	3499	3845	3692	3553	3960	3997	3880	3884
	Punch C	3273	3496	3795	3786	4131	4061	4267	4455
LSD (0.05)		n.s.			n.s.			n.s.	

† Means followed by a different letter in the same row of the same year are significantly different at P = 0.05, using Fisher's LSD

Table 4.14a ANOVA table of yield (kg.ha^{-1}) for soybean rust caused by *Phakopsora pachyrhizi* for all fungicides as affected by number of fungicide applications at Cedara in 2003/2004

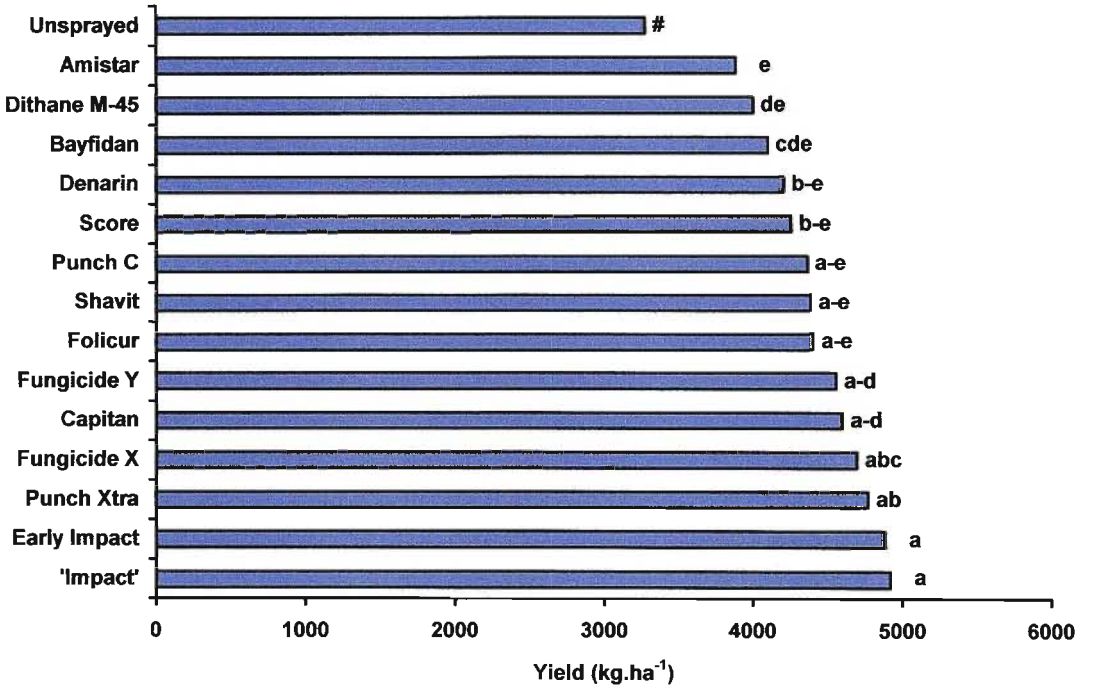
Stratum	Main effects and interaction	Degrees of freedom	Mean square	F-value	F-probability
Whole-plot	Spray	1	7480758	36.82	<0.001 **
	Fungicide	13	623693	3.07	0.006 **
	Residual	28	203178	2.18	-
Sub-plot	Frequency	1	274205	2.95	0.096
	Fungicide.Frequency	13	94561	1.02	0.460
	Residual	31	92997	-	
% c.v.		7.0			
Level of significance		P = > 0.05 n.s.	P = ≤ 0.05 *	P = ≤ 0.01 **	

Table 4.14b Table of means of yield (kg.ha^{-1}) for soybean rust caused by *Phakopsora pachyrhizi* for all fungicides as affected by number of fungicide applications at Cedara in 2003/2004

Spray †	Sprayed				Unsprayed		
	4428	a			3273	b	
LSD (0.05)	390.2						
Fungicide †	**Amistar**	**Bayfidan**	**Capitan**	**Denarin**	**Dithane M-45**	**Early Impact**	**Fungicide X**
	3882	4099	4597	4204	3999	4881	4693
DMRT (0.05)	e	cde	a-d	b-e	de	a	abc
	Fungicide Y	**Folicur**	**'Impact'**	**Punch C**	**Punch Xtra**	**Score**	**Shavit**
	4556	4404	4915	4361	4768	4253	4386
DMRT (0.05)	a-d	a-e	a	a-e	ab	b-e	a-e
Frequency	**Two**				**Three**		
	4371				4486		
LSD (0.05)	n.s.						
Fungicide.Frequency	**Amistar**	**Bayfidan**	**Capitan**	**Denarin**	**Dithane M-45**	**Early Impact**	**Fungicide X**
Two	3880	4325	4516	3961	3909	4907	4649
Three	3884	3873	4677	4447	4088	4854	4737
	Fungicide Y	**Folicur**	**'Impact'**	**Punch C**	**Punch Xtra**	**Score**	**Shavit**
Two	4639	4264	4857	4267	4691	4224	4107
Three	4472	4544	4973	4455	4845	4283	4665
LSD (0.05)	n.s.						

† Means followed by a different letter in the same row are significantly different at P = 0.05

applications was almost significant (P = 0.096), with three fungicide applications resulting in higher yields than two (Table 4.14b). The interaction between fungicide and number of applications was not significant. However, there is a significant difference in yield between two and three sprays for the fungicide Shavit when an orthogonal contrast was performed.



 # Determination of fungicide treatment differences did not include the unsprayed control

Figure 4.3 Soybean yield (kg.ha^{-1}) response to fungicides evaluated for the control of soybean rust caused by *Phakopsora pachyrhizi* at Cedara in 2003/2004

4.3.4 Seed mass

ANOVAs are recorded in Table 4.15a. Seed mass varied across the three seasons. Lowest seed mass was obtained in the 2001/2002 season due to a late planting date and the highest seed mass in 2003/2004, similar to the trends seen in the yield results (Table 4.15b).

2001/2002 season

Significant effects were seen only for sprayed compared to unsprayed treatments and a linear response to number of fungicide applications. Fungicide, number of applications and their interaction were not significant (Table 4.15a). Fungicide-sprayed soybeans resulted in 1.35g increased mass per 100 seeds compared with unsprayed soybeans (Table 4.15b). Although the difference between number of fungicide applications was not significant, seed mass increased as number of applications increased with a significant linear trend (Fig. 4.4).

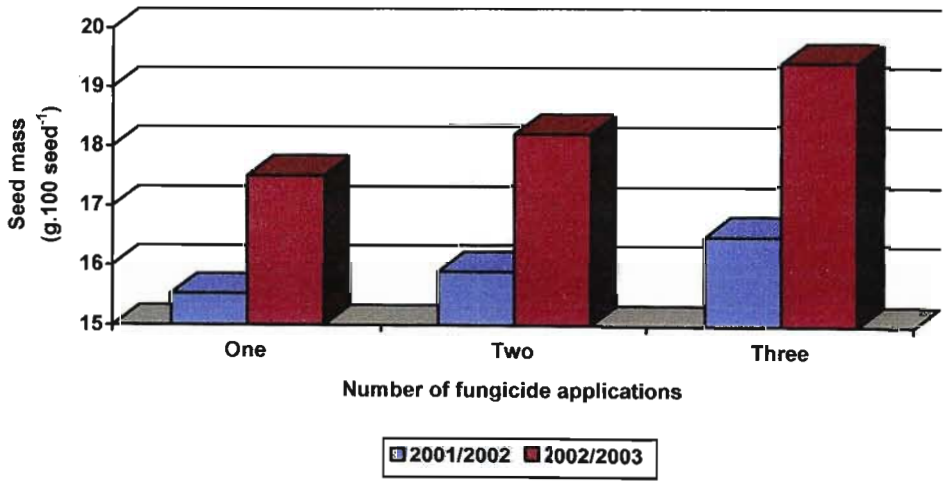


Figure 4.4 Linear relationship between number of fungicide sprays for the control of soybean rust caused by *Phakopsora pachyrhizi* and soybean seed mass (g.100 seed^{-1}) at Cedara in 2001/2002 and 2002/2003

2002/2003 season

The only significant effect was that of the linear trend to number of applications (Table 4.15a). Number of applications was nearly significant (P = 0.056), but there was a significant linear increase in seed mass as number of fungicide applications increased

Table 4.15a ANOVA table of seed mass (g.100 seed^{-1}) for soybean rust caused by *Phakopsora pachyrhizi* when sprayed with Amistar and Punch C as affected by number of fungicide applications at Cedara from 2001 - 2004

Main effects and interaction	Degrees of freedom		Mean square			F-value			F-probability		
	2001 - 2003	2003/ 2004	2001/ 2002	2002/ 2003	2003/ 2004	2001/ 2002	2002/ 2003	2003/ 2004	2001/ 2002	2002/ 2003	2003/ 2004
Spray	1	1	4.645	3.010	57.737	9.14	2.04	61.42	0.011 *	0.179	0.001 **
Fungicide	1	1	0.069	0.004	15.783	0.14	0.00	16.79	0.718	0.957	0.015 *
Residual	-	4	-	-	0.940	-	-	1.19	-		
Frequency	2	1	1.375	5.468	0.184	2.70	3.71	0.23	0.107	0.056	0.644
linear	1	-	2.708	10.674	-	5.33	7.24	-	0.040 *	0.020 *	-
quadratic	1	-	0.042	0.261	-	0.08	0.18	-	0.780	0.681	-
Fungicide.Frequency	2	1	0.344	3.469	0.889	0.68	2.35	1.12	0.526	0.137	0.324
linear	1	-	0.620	3.855	-	1.22	2.62	-	0.291	0.132	-
quadratic	1	-	0.068	3.084	-	0.13	2.09	-	0.720	0.174	-
Residual	12	7	0.508	1.474	0.791	-			-		
% c.v.	**2001/2002**	4.5	**2002/2003**	6.7		**2003/2004**	4.8				
Level of significance	P = > 0.05 n.s.		P = ≤ 0.05 *			P = ≤ 0.01 **					

Table 4.15b Table of means of seed mass (g.100 seed^{-1}) for soybean rust caused by *Phakopsora pachyrhizi* when sprayed with Amistar and Punch C as affected by number of fungicide applications at Cedara from 2001 - 2004

Main effects and interaction		2001/2002			2002/2003			2003/2004	
Spray †		**Sprayed**	**Unsprayed**		**Sprayed**	**Unsprayed**		**Sprayed**	**Unsprayed**
		15.96 a	14.61 b		18.35	17.27		19.80 a	16.00 b
LSD (0.05)		0.969			n.s.			1.346	
Fungicide †		**Amistar**	**Punch C**		**Amistar**	**Punch C**		**Amistar**	**Punch C**
		16.02	15.90		18.37	18.34		18.66 b	20.95 a
LSD (0.05)		n.s.			n.s.			1.554	
Frequency		**One**	**Two**	**Three**	**One**	**Two**	**Three**	**Two**	**Three**
		15.52	15.89	16.47	17.50	18.18	19.38	19.68	19.93
LSD (0.05)		n.s			n.s.			n.s.	
Fungicide.Frequency		**One**	**Two**	**Three**	**One**	**Two**	**Three**	**Two**	**Three**
	Amistar	15.85	15.86	16.35	17.24	17.61	20.26	18.26	19.05
	Punch C	15.18	15.92	16.59	17.75	18.75	18.51	21.10	20.80
LSD (0.05)		n.s.			n.s.			n.s.	

† Means followed by a different letter in the same row of the same year are significantly different at P = 0.05, using Fisher's LSD

(Table 4.15b and Fig. 4.4). Although seed mass was 1.08g higher in sprayed soybeans, the difference was not significant (Table 4.15a and b).

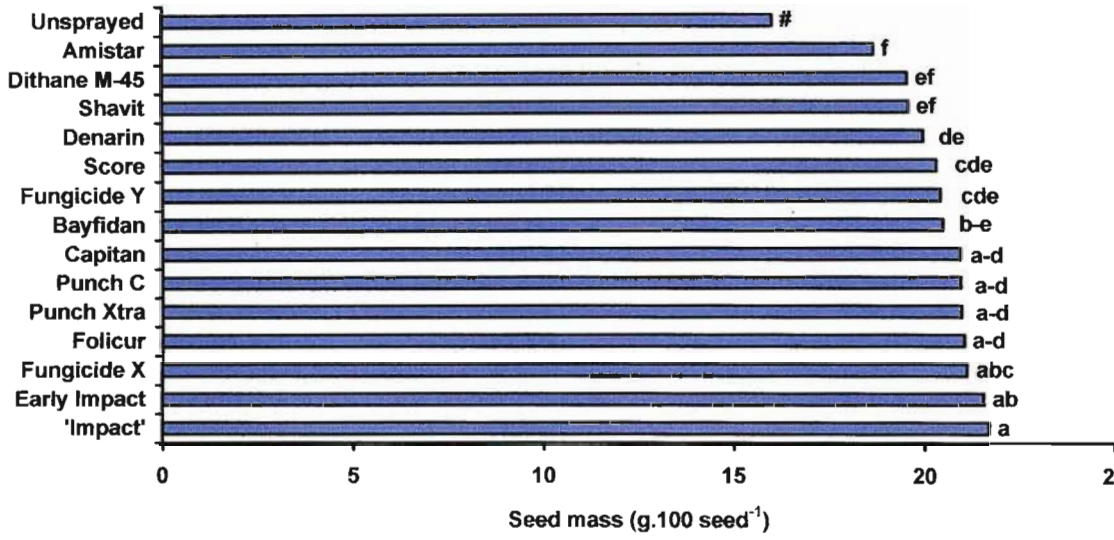
2003/2004 season

Amistar and Punch C only

Sprayed compared with unsprayed soybeans and fungicide type were significant, but there were no significant differences for number of fungicide applications and the interaction between fungicide and number of applications (Table 4.15a). Fungicide sprays resulted in a 3.8g increase in seed mass per 100 seeds (Table 4.15b). Punch C treatments resulted in a significantly higher seed mass than Amistar treatments (Table 4.15b).

All fungicides

Once again, only sprayed compared to unsprayed soybeans and fungicide type were significant (Table 4.16a). Number of fungicide applications resulted in a seed mass that was not significantly different (Table 4.16a). The interaction was also not significant. Sprayed treatments resulted in a seed mass that was 4.51g greater than the unsprayed treatment (Table 4.16b). 'Impact with carbendazim' resulted in the highest seed mass which was not significantly different from Early Impact, Fungicide X, Folicur, Punch Xtra, Punch C and Capitan. Amistar resulted in the lowest seed mass, which was not significantly different from Dithane M-45 and Shavit (Fig. 4.5).



Determination of fungicide treatment differences did not include the unsprayed control

Figure 4.5 Effect of fungicide used for the control of soybean rust caused by *Phakopsora pachyrhizi* on soybean seed mass (g.100 seed^{-1}) at Cedara in 2003/2004

Table 4.16a ANOVA table of soybean seed mass (g.100 seed^{-1}) for soybean rust caused by *Phakopsora pachyrhizi* for all fungicides as affected by number of fungicide applications at Cedara in 2003/2004

Stratum	Main effects and interaction	Degrees of freedom	Mean square	F-value	F-probability
Whole-plot	Spray	1	114.031	162.69	<0.001 **
	Fungicide	13	4.315	6.16	<0.001 **
	Residual	28	0.653	0.93	-
Sub-plot	Frequency	1	1.252	1.79	0.191
	Fungicide.Frequency	13	0.564	0.80	0.652
	Residual	31	0.701	-	
% c.v.		4.1			
Level of significance		P = > 0.05 n.s.	P = ≤ 0.05 *	P = ≤ 0.01 **	

Table 4.16b Table of means of soybean seed mass (g.100 seed^{-1}) for soybean rust caused by *Phakopsora pachyrhizi* for all fungicides as affected by number of fungicide applications at Cedara in 2003/2004

Spray †	Sprayed			Unsprayed			
	20.52	a		16.00	b		
LSD (0.05)	0.699						
Fungicide †	**Amistar**	**Bayfidan**	**Capitan**	**Denarin**	**Dithane M-45**	**Early Impact**	**Fungicide X**
DMRT (0.05)	18.66 f	20.48 b-e	20.94 a-d	19.96 de	19.54 ef	21.56 ab	21.13 abc
	Fungicide Y	**Folicur**	**'Impact'**	**Punch C**	**Punch Xtra**	**Score**	**Shavit**
DMRT (0.05)	20.42 cde	21.04 a-d	21.69 a	20.95 a-d	20.97 a-d	20.30 cde	19.58 ef
Frequency	**Two**			**Three**			
	20.39			20.64			
LSD (0.05)	n.s.						
Fungicide.Frequency	**Amistar**	**Bayfidan**	**Capitan**	**Denarin**	**Dithane M-45**	**Early Impact**	**Fungicide X**
Two	18.26	20.51	21.20	19.72	19.12	22.13	21.01
Three	19.05	20.46	20.67	20.20	19.96	20.99	21.25
	Fungicide Y	**Folicur**	**'Impact'**	**Punch C**	**Punch Xtra**	**Score**	**Shavit**
Two	20.16	21.10	21.32	21.10	20.63	20.18	19.07
Three	20.68	20.97	22.06	20.80	21.31	20.43	20.09
LSD (0.05)	n.s.						

† Means followed by a different letter in the same **row** are significantly different at P = 0.05

4.3.5 Protein content

Protein content was lower in 2001/2002 than in other seasons.

2001/2002 season

Only fungicide type was not significant; all other effects and the interaction were significant (Table 4.17a). Sprayed treatments had significantly lower protein content than unsprayed (Table 4.17b). The relationship between fungicide and number of applications was different for each fungicide (Table 4.17a). A significant linear response to number of fungicide applications was seen for Amistar treatments. Protein content increased as number of applications increased. For Punch C, however, the relationship was quadratic in nature, with the highest protein content realised for two applications (Fig. 4.6).

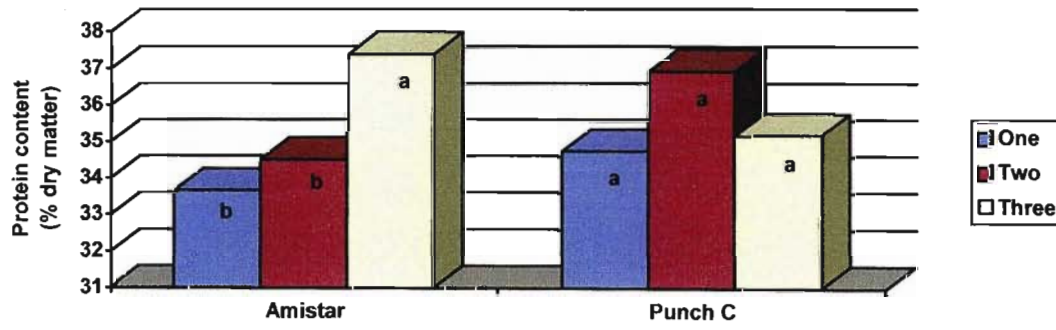


Figure 4.6 Soybean protein content (percentage dry matter) response to number of fungicide applications of Amistar and Punch C in the control of soybean rust caused by *Phakopsora pachyrhizi* at Cedara in 2001/2002

2002/2003 season

There were no significant effects for protein content (Table 4.17a). Although the interaction was not significant (P = 0.121), a difference in how the two fungicides responded to number of applications was apparent. One application of Amistar resulted in the highest protein content whereas, in contrast, three applications of Punch C resulted in the highest protein content (Table 4.17b).

Table 4.17a ANOVA table of protein content (percentage) for soybean rust caused by *Phakopsora pachyrhizi* when sprayed with Amistar and Punch C as affected by number of fungicide applications at Cedara from 2001 - 2004

Main effects and interaction	Degrees of freedom		Mean square			F-value			F-probability		
	2001 – 2003	2003/ 2004	2001/ 2002	2002/ 2003	2003/ 2004	2001/ 2002	2002/ 2003	2003/ 2004	2001/ 2002	2002/ 2003	2003/ 2004
Spray	1	1	9.772	0.010	0.136	6.03	0.00	0.08	0.030 *	0.945	0.795
Fungicide	1	1	0.858	0.845	0.452	0.53	0.43	0.26	0.481	0.524	0.640
Residual	-	4	-	-	1.768	-	-	3.80	-		
Frequency	2	1	6.899	1.736	0.085	4.26	0.89	0.18	0.040 *	0.438	0.682
linear	1	-	12.917	0.526	-	7.97	0.27	-	0.015 *	0.614	-
quadratic	1	-	0.880	2.947	-	0.54	1.50	-	0.475	0.244	-
Fungicide.Frequency	2	1	8.534	2.746	0.040	5.27	1.40	0.09	0.023 *	0.284	0.779
linear	1	-	8.217	5.475	-	5.07	2.79	-	0.044 *	0.121	-
quadratic	1	-	8.851	0.017	-	5.46	0.01	-	0.038 *	0.928	-
Residual	12	7	1.621	1.961	0.465	-			-		
% c.v.	**2001/2002**		3.6	**2002/2003**		3.7	**2003/2004**		1.8		
Level of significance	P = > 0.05 n.s.			P = ≤ 0.05 *			P = ≤ 0.01 **				

Table 4.17b Table of means of protein content (percentage) for soybean rust caused by *Phakopsora pachyrhizi* when sprayed with Amistar and Punch C as affected by number of fungicide applications at Cedara from 2001 - 2004

Main effects and interaction		2001/2002			2002/2003			2003/2004	
Spray †		**Sprayed**	**Unsprayed**		**Sprayed**	**Unsprayed**		**Sprayed**	**Unsprayed**
		35.40 b	37.35 a		38.17	38.11		38.57	38.75
LSD (0.05)		1.730			n.s.			n.s.	
Fungicide †		**Amistar**	**Punch C**		**Amistar**	**Punch C**		**Amistar**	**Punch C**
		35.19	35.62		38.39	37.96		38.37	38.76
LSD (0.05)		n.s.			n.s.			n.s.	
Frequency		**One**	**Two**	**Three**	**One**	**Two**	**Three**	**Two**	**Three**
		34.21 b	35.72 ab	36.29 a	38.67	37.60	38.25	38.65	38.48
LSD (0.05)		1.602			n.s.			n.s.	
Fungicide.Frequency		**One**	**Two**	**Three**	**One**	**Two**	**Three**	**Two**	**Three**
	Amistar	33.66 b	34.51 b	37.39 a	39.58	37.77	37.81	38.52	38.23
	Punch C	34.76 a	36.93 a	35.18 a	37.75	37.43	38.69	38.79	38.74
LSD (0.05)		2.265			n.s.			n.s.	

† Means followed by a different letter in the same row of the same year are significantly different at P = 0.05, using Fisher's LSD

2003/2004 season

Amistar and Punch C only

Again, as for 2002/2003, there were no significant effects for protein content (Table 4.17a)

All fungicides

No significant effects or interaction were obtained in this trial (Tables 4.18a and b).

Table 4.18a ANOVA table of soybean protein content (percentage) for soybean rust caused by *Phakopsora pachyrhizi* for all fungicides as affected by number of fungicide applications at Cedara in 2003/2004

Stratum	Main effects and interaction	Degrees of freedom	Mean square	F-value	F-probability
Whole-plot	Spray	1	1.073	0.61	0.441
	Fungicide	13	0.796	0.45	0.932
	Residual	28	1.752	3.13	-
Sub-plot	Frequency	1	0.220	0.39	0.535
	Fungicide.Frequency	13	0.748	1.34	0.244
	Residual	31	0.559	-	
% c.v.		2.0			
Level of significance		P = > 0.05 n.s.	P = ≤ 0.05 *	P = ≤ 0.01 **	

Table 4.18b Table of means of soybean protein content (percentage) for soybean rust caused by *Phakopsora pachyrhizi* for all fungicides as affected by number of fungicide applications at Cedara in 2003/2004

Spray		Sprayed			Unsprayed			
		38.32			38.75			
LSD (0.05)		n.s.						
Fungicide		Amistar	Bayfidan	Capitan	Denarin	Dithane M-45	Early Impact	Fungicide X
		38.38	37.65	38.33	38.90	38.31	38.36	38.57
		Fungicide Y	Folicur	'Impact'	Punch C	Punch Xtra	Score	Shavit
		38.69	37.87	38.09	38.76	37.94	38.03	38.54
DMRT (0.05)		n.s.						
Frequency		Two			Three			
		38.37			38.27			
LSD (0.05)		n.s.						
Fungicide.Frequency		Amistar	Bayfidan	Capitan	Denarin	Dithane M-45	Early Impact	Fungicide X
	Two	38.52	37.40	38.55	39.53	38.56	38.29	38.51
	Three	38.23	37.89	38.11	38.27	38.05	38.44	38.62
		Fungicide Y	Folicur	'Impact'	Punch C	Punch Xtra	Score	Shavit
	Two	39.12	38.33	37.84	38.79	37.78	37.28	38.64
	Three	38.26	37.41	38.35	38.74	38.11	38.78	38.44
LSD (0.05)		n.s.						

4.3.6 Oil content

ANOVAs are recorded in Table 4.19a. Oil content was much lower in 2003/2004 than in other seasons (Table 4.19b). Although the spray effect was not significant, oil content was always higher in treatments that received applications of fungicides.

2001/2002 season

There were no significant effects for oil content (Table 4.19a). Fungicide at P = 0.09 was nearly significant. Oil content was higher in treatments which received applications of Punch C.

2002/2003 season

No effects or interactions were significant (Table 4.19a). Although number of fungicide applications was not significant, there was an almost significant (P = 0.08) linear response to number of applications, with an increase in oil content from one to two fungicide applications (Table 4.19b).

2003/2004 season

Amistar and Punch C only

There was no significant response to any of the treatment effects and interaction (Table 4.19a).

All fungicides

The only significant effect was that of spray (Table 4.20a). Fungicide-treated soybeans resulted in an oil content that was 0.81% higher than untreated soybeans (Table 4.20b).

Table 4.19a ANOVA table of soybean oil content (percentage) for soybean rust caused by *Phakopsora pachyrhizi* when sprayed with Amistar and Punch C as affected by number of fungicide applications at Cedara from 2001 - 2004

Main effects and interaction	Degrees of freedom		Mean square			F-value			F-probability		
	2001 – 2003	2003/ 2004	2001/ 2002	2002/ 2003	2003/ 2004	2001/ 2002	2002/ 2003	2003/ 2004	2001/ 2002	2002/ 2003	2003/ 2004
Spray	1	1	1.214	0.312	2.050	0.58	0.54	3.96	0.460	0.478	0.117
Fungicide	1	1	7.106	0.245	0.460	3.41	0.42	0.89	0.090	0.529	0.399
Residual	-	4	-	-	0.518	-	-	1.43		-	
Frequency	2	1	0.270	1.418	0.033	0.13	2.43	0.09	0.880	0.130	0.772
linear	1	-	0.533	2.133	-	0.26	3.66	-	0.622	0.080	-
quadratic	1	-	0.006	0.703	-	0.00	1.21	-	0.958	0.294	-
Fungicide.Frequency	2	1	1.669	0.437	0.460	0.80	0.75	1.27	0.472	0.493	0.298
linear	1	-	3.131	0.688	-	0.10	1.18	-	0.244	0.298	-
quadratic	1	-	0.207	0.186	-	0.10	0.32	-	0.758	0.582	-
Residual	12	7	2.086	0.582	0.363		-			-	
% c.v.	**2001/2002**		7.1	**2002/2003**		4.0	**2003/2004**		3.8		
Level of significance	P = > 0.05 n.s.			P = ≤ 0.05 *			P = ≤ 0.01 **				

Table 4.19b Table of means of soybean oil content (percentage) for soybean rust caused by *Phakopsora pachyrhizi* when sprayed with Amistar and Punch C as affected by number of fungicide applications at Cedara from 2001 - 2004

Main effects and interaction		2001/2002			2002/2003			2003/2004	
Spray		**Sprayed**	**Unsprayed**		**Sprayed**	**Unsprayed**		**Sprayed**	**Unsprayed**
		20.41	19.72		19.07	18.72		16.12	15.40
LSD (0.05)		n.s.			n.s.			n.s.	
Fungicide		**Amistar**	**Punch C**		**Amistar**	**Punch C**		**Amistar**	**Punch C**
		19.78	21.04		19.19	18.95		15.92	16.31
LSD (0.05)		n.s.			n.s.			n.s.	
Frequency		**One**	**Two**	**Three**	**One**	**Two**	**Three**	**Two**	**Three**
		20.19	20.44	20.61	18.51	19.35	19.35	16.17	16.07
LSD (0.05)		n.s.			n.s.			n.s.	
Fungicide.Frequency		**One**	**Two**	**Three**	**One**	**Two**	**Three**	**Two**	**Three**
	Amistar	19.99	19.96	19.39	18.46	19.32	19.78	16.17	15.67
	Punch C	20.38	20.91	21.82	18.56	19.38	18.93	16.17	16.46
LSD (0.05)		n.s.			n.s.			n.s.	

Table 4.20a ANOVA table of soybean oil content (percentage) for soybean rust caused by *Phakopsora pachyrhizi* for all fungicides as affected by number of fungicide applications at Cedara in 2003/2004

Stratum	Main effects and interaction	Degrees of freedom	Mean square	F-value	F-probability
Whole-plot	Spray	1	3.672	6.34	0.018 *
	Fungicide	13	0.292	0.50	0.904
	Residual	28	0.580	1.22	-
Sub-plot	Frequency	1	0.620	1.30	0.262
	Fungicide.Frequency	13	0.403	0.85	0.612
	Residual	31	0.476	-	
% c.v.		4.3			
Level of significance		P = > 0.05 n.s.	P = ≤ 0.05 *	P = ≤ 0.01 **	

Table 4.20b Table of means of soybean oil content (percentage) for soybean rust caused by *Phakopsora pachyrhizi* for all fungicides as affected by number of fungicide applications at Cedara in 2003/2004

Spray †		Sprayed			Unsprayed			
		16.21	a		15.40	b		
LSD (0.05)		0.659						
Fungicide		Amistar	Bayfidan	Capitan	Denarin	Dithane M-45	Early Impact	Fungicide X
		15.92	16.55	16.15	16.03	16.26	16.16	16.30
		Fungicide Y	Folicur	'Impact'	Punch C	Punch Xtra	Score	Shavit
		15.69	16.40	16.27	16.31	16.25	16.46	16.22
DMRT (0.05)		n.s.						
Frequency		Two			Three			
		16.30			16.13			
LSD (0.05)		n.s.						
Fungicide.Frequency		Amistar	Bayfidan	Capitan	Denarin	Dithane M-45	Early Impact	Fungicide X
	Two	16.17	16.89	16.18	16.05	16.52	16.37	16.05
	Three	15.67	16.21	16.11	16.00	15.99	15.95	16.54
		Fungicide Y	Folicur	'Impact'	Punch C	Punch Xtra	Score	Shavit
	Two	15.20	16.81	16.39	16.17	16.12	16.80	16.43
	Three	16.18	15.98	16.15	16.46	16.39	16.11	16.00
LSD (0.05)		n.s.						

† Means followed by a different letter in the same row are significantly different at P = 0.05, using Fisher's LSD

4.4 Discussion

Final disease levels in unsprayed plots of 71%, 65% and 80% for 2001/2002, 2002/2003 and 2003/2004, respectively, reflect the influence environment has on disease development of SBR. Fungicide-sprayed treatments had final disease levels of 13%, 21% and 26% in 2001/2002, 2002/2003 and 2003/2004, respectively. In 2003/2004 treatments which were not significantly different from the best treatments ('Impact with carbendazim' and Early Impact) had mean final disease severity levels of 2.0 – 7.8%. The increase in final disease severity for fungicide-sprayed treatments from 13 – 26% may be attributed to the change in efficacy of Amistar. This is explained by the combination of decrease in total amount of active ingredient applied and favourable environmental conditions for disease development. In 2001/2002, nearly double the amount of active ingredient was applied. This enhanced the impact of the fungicide, as is demonstrated by the fact that 2001/2002 was the only season in which there was no significant difference between Amistar and Punch C. In fact, 2001/2002 was the only season for which yields were higher in Amistar than in Punch C treatments. When Amistar was applied at normal rates in 2003/2004, a season which was favourable for disease, it was unable to effectively control SBR, resulting in much lower yields than plots treated with Punch C.

There was a significant response in disease levels to the number of fungicide applications. Final disease severity decreased with an increase in the number of applications. This is expected, as one fungicide application made at flowering will cease to be active long before crop maturity. Similarly, two fungicide applications, although protecting the crop for longer than one application, will cease to be effective before physiological maturity of the crop. When three fungicide applications are made, the crop is protected for most of the growing period, so that very little infection occurs and disease development remains low until maturity. However, for the best treatments ('Impact with carbendazim', Early Impact, Folicur, Punch C and Punch Xtra) the third fungicide application was of no or little value.

The SAUDPC decreased as number of fungicide applications increased. In all seasons, analysis of the trials involving only Amistar and Punch C showed no significant difference between two and three sprays. However, when number of fungicides

evaluated increased in 2003/2004 the difference between two and three sprays was significant. This was due to the fact that disease pressure was higher and also more fungicides were evaluated with greater variation in their ability to control SBR.

Statistical analysis of data showed that although yield and seed mass increased as the number of fungicide applications increased, the relationship was not significant. The effect was, however, significantly linear in response for seed mass. The reduction in yield and seed mass for decreased number of fungicide applications is related to fungicide protection during the critical R4.5 – R5.5 growth stage (McWilliams *et al.*, 2004). One application resulted in the crop being unprotected during the stage when seed mass is determined, compared with three fungicide applications where the crop is protected until maturity. The real relevance for number of fungicide applications would need to be further expanded to include a cost-benefit analysis to determine whether or not there is a significant economic benefit to increasing the number of fungicide applications.

In the 2003/2004 trial containing all fungicides, no response to number of fungicide applications was seen in SAUDPC for fungicides which did not effectively control the disease. The fungicides which resulted in high SAUDPCs were Amistar, Denarin and Dithane M-45. Similarly, there was no response to number of applications for fungicides which were most effective in disease control. Most of these fungicides resulted in such low levels of disease with two sprays, that even though a third application reduced SAUDPC, the difference was not significant. These fungicides were Early Impact, Folicur, 'Impact with carbendazim', Punch C and Punch Xtra. These fungicides, with the exception of Folicur, were fungicide mixtures of triazole compounds and carbendazim. Three fungicide applications of Capitan, Fungicide X, Shavit, Fungicide Y and Bayfidan resulted in SAUDPCs as low as those of the five best fungicide treatments applied twice.

Fungicides significantly increased yield in the wetter 2001/2002 and 2003/2004 seasons. However, in the dry 2002/2003 season there was no significant yield benefit from spraying. The reason for this could be due to environmental conditions limiting both soybean yield potential and disease development. This suggests that the application of fungicide in a dry season is not necessary. This would have to be addressed

economically, to determine whether the slight yield gain would result in a significant economic benefit. Another factor to consider is the difference between trial data and on-farm realities. For example, the unsprayed plots in the fungicide trials have lower inoculum pressure than the unsprayed fields of farmers. This is because the large number of fungicide-sprayed plots surrounding the unsprayed trial plots resulted in lower inoculum pressure.

The only season in which significant effects were obtained for protein content was 2001/2002. More fungicide active ingredient was applied in this first season and it is possible that the increased fungicide was responsible for lower than normal protein content. Planting date is also reported to affect seed protein content (Birch *et al.*, 1990). The 2001/2002 trial was planted very late (22 December), compared with the 2002/2003 and 2003/2004 trials, which were planted on 12 November. The shorter vegetative growing length may have contributed to decreased protein content for the 2001/2002 season.

Oil contents were not affected by SBR, as there were no significant differences between sprayed and unsprayed soybeans. Oil contents were, however, much lower in the 2003/2004 season. Hurburgh *et al.* (1987) found oil concentration to be higher in hot, dry years. Since rainfall was very good throughout the 2003/2004 season, this may be the reason oil content was much lower than during other seasons which experienced warmer, drier weather.

This research was conducted on medium-growing soybean cultivars in the mist-belt. In northern KZN, some farmers' early-planted, early-maturing soybean crops escape rust. Due to drier conditions in northern KZN, farmers can in certain seasons successfully grow late-maturing cultivars with only one fungicide spray, whereas in the mist-belt, farmers would have to make up to three fungicide applications to protect a late-maturing cultivar. So although differences are noticeable between the number of applications according to fungicide type, factors such as climate and maturity group of soybean planted may also need to be considered when deciding how many fungicide applications are necessary to protect a soybean crop. In conclusion, trial results using a medium-season cultivar grown in the mist-belt, under high disease pressure, show that two

fungicide sprays generally provide adequate protection against SBR but fungicide selection also plays a factor.

4.5 References

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CHAPTER 5

Timing of initial fungicide application for soybean rust (*Phakopsora pachyrhizi* Syd.) control on soybeans (*Glycine max* (L.) Merr.)

Abstract

Timing of initial application trials for the control of soybean rust (*Phakopsora pachyrhizi* Syd.) on soybeans (*Glycine max* (L.) Merr.) were conducted over two seasons from 2001 – 2003 at Cedara (29°32'S, 30°16'E and alt. 1071m), 20km north-west of Pietermaritzburg. Four different times were evaluated for initial fungicide application: before flowering, during flowering, at first sign of disease and at the R5 growth stage. All treatments received a second fungicide spray 21d after the initial application. Two fungicides, Amistar and Punch C, with different modes of action, were assessed in their response to timing of application. Treatments sprayed with Amistar showed better response to timing of application than those sprayed with Punch C. The curative activity of Punch C caused a significant cessation in disease development for R5 treatments, whereas Amistar did not. This impacted on yield, resulting in R5 Amistar treatments having significantly lower yields compared to those where applications were made at other times. Generally, lower levels of disease and the highest yields were obtained from spraying during flowering. Protein and oil content were not affected by spraying.

5.1 Introduction

Soybean rust (SBR) on soybeans (*Glycine max* (L.) Merr.) caused by the fungus *Phakopsora pachyrhizi* (Syd.), usually only occurs on field crops once they have reached the reproductive growth stage. Exceptions, however, have been noted when soybeans are growing close to a host where the fungus has already reached epidemic status. Yorinori (2004) reported that 25 – 30d old soybeans, still in their vegetative growth stage, became infected from neighbouring kudzu vine (*Pueraria lobata* (Willd.) Ohwi) which was heavily infected with *P. pachyrhizi*. The relevance of alternate hosts for the establishment of the pathogen early in the season must be considered in the management of this disease. If inoculum levels are high early in the season, infection in the vegetative stage is possible. Early-planted indicator crops are useful as an early warning system to assist in determining when to spray the first fungicide application. As *P. pachyrhizi* establishes in SA, its presence on alternate hosts, especially early in the season, will become more important. The first report of *P. pachyrhizi* on dry beans in SA was made in April 2004 (du Preez *et al.*, 2005), but an unconfirmed report at the start of the 2003/2004 season was made from soybeans growing adjacent to dry beans, which may have served as the inoculum source.

SBR infections in the vegetative growth stage appear to be linked to the available inoculum. Such infections are seen in late or second plantings where soybeans, growing close to infected mature soybeans or alternate hosts, become infected due to high inoculum levels. Due to weather limitations (frost risk) the practice of second plantings in a single season is not common in South Africa (SA). The reason SBR infections only develop once plants are in their reproductive stage in SA, is either related to plant physiology or a lack of inoculum early in the season. If there were more early plantings of alternate hosts, the risk of infection in the vegetative growth stage may be greater. With the invasive kudzu vine serving as a year-round host, SBR epidemics in Brazil and the USA can be expected to be quite different from SA epidemics.

The time of disease onset and the intensity of disease at particular growth stages determine the severity of loss and the particular components of yield affected (Bromfield, 1984). Shanmugasundaram *et al.* (2004) recorded losses of up to 91%. Rust infection disrupts plant functioning and hence decreases yield in several ways. Severe

infection results in leaf abscission and this premature defoliation decreases the pod-filling period. The production of photosynthate is reduced and its distribution within the plant is altered (Livne and Daly, 1966). Most of the photosynthate remains in the leaves where it was produced and the amount available to the plant is reduced. Rust infection also contributes to yield loss by disrupting the internal water balance of plants. Shaw and Laing (1966) found the greatest reduction in seed yield when water stress occurred during the last week of pod development and during pod-filling.

McWilliams *et al.* (2004) recorded expected yield loss from damage occurring at different soybean growth stages. Fifty percent defoliation of the plant at V6 and at R2 (full bloom) will reduce yield by 3% and 6%, respectively. At R3 (beginning pod development) very favourable conditions will result in greater pod number per plant. Late pod formation at R4.5 to early seed fill at R5.5 is the most critical period for seed yield. Any stress from R4 – R6 causes more yield reduction than at any other time. Leaf loss of 100% at R5 (beginning seed development) will reduce yields by 80%. Stress at R7 (beginning maturity) or later has almost no effect on yield unless pods drop to the ground or seeds are shattered from the pods.

Casey (1981) reported that SBR epidemics are most severe in environments with extended periods of leaf wetness (~ $10h.d^{-1}$) and moderate temperatures (18 - 26°C). Extreme temperatures (>30°C and/or <15°C) and/or dry conditions retarded the development of the rust. Even when leaf-surface wetness was theoretically adequate, prolonged temperatures above 27°C appeared to inhibit *P. pachyrhizi*. Under favourable conditions, the time from infection to the production of urediospores, when first symptoms are noticed, is 11d. Real-time PCR (Frederick *et al.*, 2002) can be used prior to symptom expression to determine whether infection has occurred but this technology is not always cost-effective, accessible or practical for use by farmers.

Symptoms of SBR are often only noticed by farmers after the appearance of uredia containing urediospores, once plants have become chlorotic. By the time the farmer notices the infection and arranges to make a fungicide application, substantial crop damage, and consequently yield loss, has already occurred. Yorinori (2004) stated that, despite numerous talks and training courses, farmers and field agronomists still have difficulties in recognising the early symptoms and signs of SBR. He believes that the

skill to identify rust symptoms at the earliest stage is the key to successful and timely application of fungicide.

SBR infection can result in severe yield loss, particularly when plants are left unprotected at certain critical growth stages. This study was designed to determine the optimum time for initial fungicide applications to obtain good disease control with optimal yield benefits.

5.2 Materials and methods

Trial site

Trials were conducted at Cedara agricultural research farm (29°32'S, 30°16'E and alt. 1071m), 20km north-west of Pietermaritzburg. Evaluations of different timing of initial fungicide applications were conducted in 2001/2002 and 2002/2003 on the same land, previously planted to potatoes.

Land preparation

Soil samples were taken of the topsoil (0-15cm) and fertilizer was supplied according to Fertrec recommendations from the Cedara Fertilizer Advisory Service (Farina and Channon, 1988). Phosphorus was band-applied in the rows at planting to supply 21kg.ha^{-1} in 2001/2002 and 10.5kg.ha^{-1} in 2002/2003 (source: superphosphate (10.5%)).

Trial design

A factorial design with randomised complete blocks and three replications was used. Plots comprised four 5m rows spaced 45cm apart. The two central rows of each plot were used as data rows for disease evaluation and yield determination. The unsprayed outer rows of the plots are referred to as border rows.

Climatic data

Automatic weather stations based at Cedara were used to collect information on rainfall and temperature over the growing seasons. Long-term monthly rainfall and temperature averages at Cedara were supplied by the Institute for Soil, Climate and Water[1].

Planting

Trials were hand-planted with the soybean cultivar LS666. Planting date, seeding rate and final plant population are shown in Table 5.1 Soybeans were inoculated with *Bradyrhizobium japonicum* ((Kirchner) Jordan) at planting to enhance good nodule formation. Normal pest and weed control practices for the area were followed.

Table 5.1 Planting date, cultivar, seeding rate and plant population of soybean trials planted at Cedara in the 2001 - 2003 growing seasons

Season	Planting date	Cultivar & expected germination	Seeding rate (seeds.ha^{-1})	Plant population (plants.ha^{-1})
2001/2002	22 December 2001	LS666 (70-79% germination)	350 000	215 000 (61% germination)
2002/2003	12 November 2002	LS666 (>90% germination)	300 000	270 000 (90% germination)

Fungicide treatments

Four different times of initial fungicide applications were evaluated (Table 5.2) using two fungicides, Amistar and Punch C (Table 5.3). A second spray was applied 21d after the first application. An unsprayed treatment was included as a control to check fungicide efficacy.

Calibration of the spray equipment was based on a walking speed of 1m.s^{-1}. After the first two applications in 2001/2002, it was realised that the actual walking speed in the canopied plants was slower, at 0.5m.s^{-1}. Effectively, this resulted in double the fungicide being applied for these two applications. The spray volume was recalculated on the slower walking speed and the corrected target application rates were applied for the third spray in 2001/2002.

[1] Institute for Soil, Climate and Water, Agromet Section, Private Bag X79, Pretoria, 0001, South Africa

Table 5.2 Timing of fungicide applications made at Cedara for the control of soybean rust caused by *Phakopsora pachyrhizi* in 2001 - 2003 trials

Time of application	2001/2002			2002/2003		
	Date	DAP †	Growth stage	Date	DAP	Growth stage
Before flower	18 February	58	V12	8 January	57	V9
Flower	25 February	65	R2	22 January	71	R2
First signs	18 March	86	R4	12 February	92	R3
R5	26 March	94	R5	27 February	107	R5

† DAP = days after plantings

Table 5.3 Fungicides evaluated and amount of fungicide active ingredient applied in trials conducted at Cedara from 2001 - 2003 for the control of soybean rust caused by *Phakopsora pachyrhizi*

Active ingredient	Concen-tration & formu-lation	Trade name	Manu-facturer	Application rate (.ha^{-1})		Total active ingredient applied			
						Before flower & Flower		First signs & R5	
				ml	g a.i.	2001/2002†	2002/2003	2001/2002‡	2002/2003
azoxystrobin	250 SC	Amistar	Syngenta	300	75	300	150	225	150
flusilazole & carbendazim	250 SC 125	Punch C	Du Pont	400	100 50	400 200	200 100	300 150	200 100

† The first two applications received double the chemical dose.

‡ The first application received double the chemical dose. This error was corrected for the second application.

Full-cover fungicide sprays of 160ℓ.ha^{-1} at 200kPa pressure were applied to the central two rows of each plot, leaving the outer rows as border rows to reduce interplot interference. Fungicides were applied using a CO_2-pressured back-pack sprayer with a horizontal spray-boom comprising two Spraying Systems TeeJet No8001 nozzles spaced 45cm apart.

Artificial inoculation

To ensure infection, freshly collected *P. pachyrhizi* spores from early-infected trap plants were sprayed in a solution containing 0.01% household dishwashing liquid, using a knapsack sprayer, over the plots on 4 March 2002 at the R3 growth stage (72 DAP). As environmental conditions were not favourable following inoculation, a second artificial inoculation was made on 11 March. In 2002/2003, soybeans were planted in plastic bags (one plant per bag) and grown in tunnels, where favourable conditions

allowed them to become naturally infected. On 19 February (99 DAP), these bags were placed in every second plot within the trial.

Disease assessment

Disease severity assessments were made regularly, at twice-weekly intervals on plants in the data rows, from flowering (R1) until physiological maturity (R7) of the crop. A plot rating was given after examining at least ten plants in the central two rows. Disease was assessed according to a rating scale developed at Cedara (see Table 2.4 of Chapter 2). The scale uses the position of rust pustules on the plant, pustule density, chlorosis and defoliation as parameters for determining disease severity. Data was used to calculate the area under disease progress curve (AUDPC), which summarises the disease epidemic. The AUDPC was calculated using a trapezoidal integration program (Berger, 1981) and standardised (SAUDPC) by dividing the AUDPC value by the duration of the epidemic. The SAUDPC allows for comparisons of disease from one season to another.

Harvesting

Leaving a 0.5m border on either end of each of the 5m rows, the central 4m of the central two rows in each plot, were hand-harvested. Grain yields were adjusted to a moisture content of 12.5% and expressed as $kg.ha^{-1}$. Seed mass (g) was determined for 100 seeds. Protein and oil analyses were conducted on the seed using the Dumas combustion method (Dumas, 1831) and supercritical fluid extraction, respectively.

Statistical analyses

Statistical analyses of trial data (final disease severity, SAUDPC, yield, seed mass, protein and oil content) were conducted by analysis of variance (ANOVA) using Genstat 6.1. Mean separations were based on least significant differences (LSD) at the 5% level of probability.

5.3 Results

Climatic data

Although rainfall was good early in the season, the 2001/2002 season was characterised by a mid-season drought which persisted from flowering (mid-February) until physiological maturity of the soybean crop. The 2002/2003 season was warm, but very dry, throughout the duration of the trial (Table 5.4).

Table 5.4 Rainfall and temperature data at Cedara for the 2001 – 2003 soybean growing seasons

	November	December	January	February	March	April
Rainfall (mm)						
2001/2002	126	158	153	38	59	61
2002/2003	52	84	47	66	95	44
Mean monthly†	111	131	134	122	110	51
Mean temperature (°C)						
2001/2002	18.5	18.7	20.7	18.6	19.5	17.6
2002/2003	16.7	19.1	19.8	20.8	19.7	17.5
Mean monthly†	18.0	19.5	20.2	20.2	19.3	16.7

† Long-term mean from 01/07/1914 to 30/06/2004

Flowering, first signs of disease and physiological maturity of the crop are presented in Table 5.5.

Table 5.5 Dates of various soybean growth stages in trials conducted at Cedara in 2001/2002 and 2002/2003 for the control of soybean rust caused by *Phakopsora pachyrhizi*

Event	2001/2002		2002/2003	
	Date	DAP †	Date	DAP
Planting date	22 December	0	12 November	0
Flowering	21 February	61	22 January	71
First sign of disease	15 March	83	17 February	97
Physiological maturity	23 April	122	31 March	139

† DAP = days after planting

Percentage disease severity at the application of each spray is summarised in Table 5.6.

Table 5.6 Percentage soybean rust disease severity recorded at various times of fungicide application in trials conducted at Cedara from 2001 – 2003 for the control of soybean rust caused by *Phakopsora pachyrhizi*

Time of application	2001/2002			2002/2003		
	DAP †	Disease (%)		DAP	Disease (%)	
		Amistar	Punch C		Amistar	Punch C
Before flower	58	0	0	57	0	0
	79	0	0	78	0	0
Flower	65	0	0	71	0	0
	86	0.2	0	92	0	0
First signs	86	1.3	1.5	92	0.5	0.1
	107	15.8	14.2	114	15.8	0.7
R5	94	10.8	10.8	107	2.7	1.0
	115	26.7	21.7	132	24.3	7.7

† DAP = days after plantings

Assuming a 21d persistence period (based on recommended spray intervals) for both fungicides, the length of time that different treatments would have been left unprotected is presented in Table 5.7. These figures are based on the date of the first fungicide application (Table 5.6) and physiological maturity of the crop (Table 5.5).

Table 5.7 Length of protection provided by fungicide sprays against soybean rust, caused by *Phakopsora pachyrhizi*, in trials conducted at Cedara during 2001 – 2003 growing seasons

Time of application	Fungicide applications (DAP) †	Fungicide wears off (DAP)	Days unprotected	
			Before first application	After last application
2001/2002				
Before flower	58 & 79	100	0	22
Flower	65 & 86	107	0	15
First signs	86 & 107	128	3	0
R5	94 & 115	136	11	0
2002/2003				
Before flower	57 & 78	99	0	40
Flower	71 & 92	113	0	26
First signs	92 &114	135	0	4
R5	107 & 132	153	10	0

† DAP = days after flowering

5.3.1 Final disease severity

Means of fungicide sprayed treatments were significantly lower than unsprayed for both seasons (Tables 5.8a and b). Final disease levels were higher in 2001/2002 (Table 5.8b).

2001/2002 season

In this trial only time of application was significant (Table 5.8a). For both fungicides, applications made before flowering and at flowering resulted in lower final disease levels than applications made at first sign of disease and at the R5 growth stage (Table 5.8b and Fig. 5.1). Although Amistar treatments resulted in higher final disease levels, they were not significantly different from Punch C.

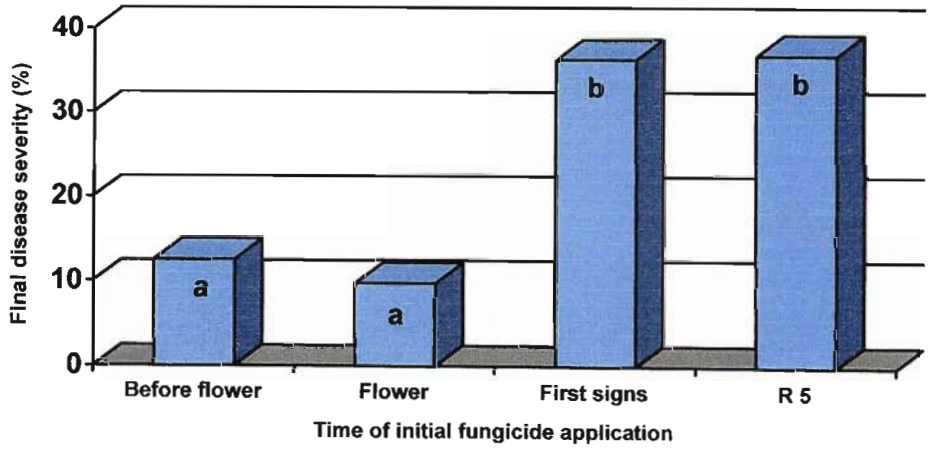

Figure 5.1 Effect of timing of initial fungicide application on final soybean rust disease severity, caused by *Phakopsora pachyrhizi*, on soybeans at trials conducted at Cedara in 2001/2002

2002/2003 season

Amistar treatments resulted in significantly higher final disease severity than Punch C treatments (Table 5.8b). Timing of initial application was not significant as a main effect or as an interaction between fungicide used and timing. The lowest final disease severity however was from treatments sprayed at flowering.

Table 5.8a ANOVA table of final disease severity (square root transformed) as affected by the timing of Amistar and Punch C applications for the control of soybean rust caused by *Phakopsora pachyrhizi* in trials conducted at Cedara from 2001 - 2003

Main effects and interaction	Degrees of freedom	Mean square		F-value		F-probability	
	2001/2002 & 2002/2003	2001/2002	2002/2003	2001/2002	2002/2003	2001/2002	2002/2003
Spray	1	41.412	50.058	16.30	12.85	<0.001 **	0.002 **
Fungicide	1	7.049	66.480	2.78	17.07	0.115	<0.001 **
Time	3	13.591	8.718	5.35	2.24	0.010 **	0.123
Fungicide.Time	3	0.271	0.372	0.11	0.10	0.955	0.961
Residual	16	2.540	3.896	-		-	
% c.v.	2001/2002	32.5		2002/2003		47.6	
Level of significance	P = > 0.05 n.s.		P = ≤ 0.05 *		P = ≤ 0.01 **		

Table 5.8b Table of means of final disease severity (square root transformed) as affected by the timing of Amistar and Punch C applications for the control of soybean rust caused by *Phakopsora pachyrhizi* in trials conducted at Cedara from 2001 - 2003

Main effects and interaction		2001/2002				2002/2003			
Spray †		Sprayed		Unsprayed		Sprayed		Unsprayed	
		4.47	a	8.41	b	3.66	a	8.00	b
LSD (0.05)		2.069				2.562			
Fungicide †		Amistar		Punch C		Amistar		Punch C	
		5.01		3.92		5.33	b	2.00	a
LSD (0.05)		n.s.				1.708			
Time †		Before flower	Flower	First sign	R5	Before flower	Flower	First sign	R5
		3.41 a	2.97 a	5.47 b	6.02 b	5.38	2.67	3.01	3.59
LSD (0.05)		1.951				n.s.			
Fungicide.Time		Before flower	Flower	First sign	R5	Before flower	Flower	First sign	R5
	Amistar	4.25	3.36	5.85	6.57	6.90	4.35	5.02	5.04
	Punch C	2.57	2.57	5.09	5.47	3.86	1.00	1.00	2.14
LSD (0.05)		n.s.				n.s.			

† Means followed by a different letter in the same row of the same year are significantly different at P = 0.05, using Fisher's LSD

5.3.2 Standardised area under disease progress curve (SAUDPC)

Significant differences in SAUDPC were seen in both seasons for fungicides, as well as sprayed compared with unsprayed treatments (Table 5.9a). Punch C treatments resulted in the lowest SAUDPC (Table 5.9b and Fig. 5.2).

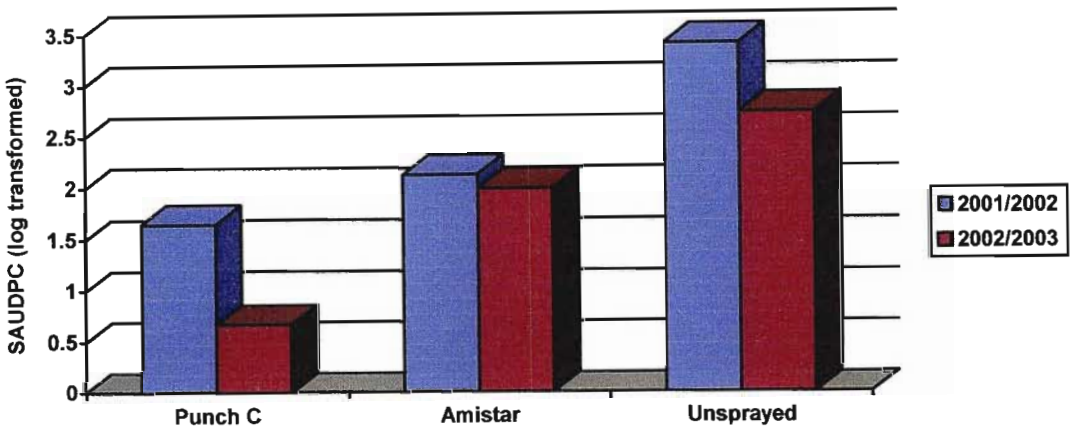

Figure 5.2 Log transformed standardised area under disease progress curve (SAUDPC) for fungicide treatments used in the control of soybean rust, caused by *Phakopsora pachyrhizi*, at Cedara from 2001-2003

2001/2002 season

Timing of the initial fungicide application was significant (Table 5.9a). Similar to final disease severity, fungicides applied before and at flowering resulted in lower SAUDPC values than fungicides applied at first signs of disease and the R5 growth stage. Amistar and Punch C applications did not respond significantly differently to timing of application with trends, indicating that SAUDPC values were lowest at flowering and highest at the R5 growth stage.

2002/2003 season

Time of initial fungicide application was not significant (P = 0.255)). However, lowest SAUDPC values resulted from fungicide applications made during flowering.

Table 5.9a ANOVA table of log transformed standardised area under disease progress curve (SAUDPC) as affected by the timing of Amistar and Punch C applications for the control of soybean rust caused by *Phakopsora pachyrhizi* in trials conducted at Cedara from 2001 - 2003

Main effects and interaction	Degrees of freedom	Mean square		F-value		F-probability	
	2001 - 2003	2001/2002	2002/2003	2001/2002	2002/2003	2001/2002	2002/2003
Spray	1	6.137	5.264	30.91	7.82	<0.001 **	0.013 *
Fungicide	1	1.357	10.480	6.84	15.57	0.019 *	0.001 **
Time	3	5.136	1.004	25.87	1.49	<0.001 **	0.255
Fungicide.Time	3	0.153	0.128	0.77	0.19	0.528	0.901
Residual	16	0.199	0.673	-		-	
% c.v.	2001/2002	21.7		2002/2003		55.3	
Level of significance	P = > 0.05 n.s.		P = ≤ 0.05 *			P = ≤ 0.01 **	

Table 5.9b Table of means of log transformed standardised area under disease progress curve (SAUDPC) as affected by the timing of Amistar and Punch C applications for the control of soybean rust caused by *Phakopsora pachyrhizi* in trials conducted at Cedara from 2001 - 2003

Main effects and interaction		2001/2002				2002/2003			
Spray †		Sprayed		Unsprayed		Sprayed		Unsprayed	
		1.89	a	3.40	b	1.33	a	2.73	b
LSD (0.05)		0.578				1.065			
Fungicide †		Amistar		Punch C		Amistar		Punch C	
		2.12	b	1.65	a	1.99	b	0.67	a
LSD (0.05)		0.386				0.710			
Time †		Before flower	Flower	First signs	R5	Before flower	Flower	First signs	R5
		1.19 a	1.00 a	2.53 b	2.83 b	1.57	0.74	1.37	1.64
LSD (0.05)		0.545				n.s.			
Fungicide.Time		Before flower	Flower	First signs	R5	Before flower	Flower	First signs	R5
	Amistar	1.61	1.32	2.63	2.93	2.11	1.27	2.18	2.40
	Punch C	0.77	0.68	2.42	2.72	1.02	0.21	0.55	0.88
LSD (0.05)		n.s.				n.s.			

† Means followed by a different letter in the same row of the same year are significantly different at P = 0.05, using Fisher's LSD

5.3.3 Yield

The difference in yield was not as great as in disease assessments between sprayed and unsprayed treatments. Differences in treatments were also more difficult to detect in the yield analyses compared to the diseases.

2001/2002 season

Fungicide sprayed treatments resulted in significantly higher yields (Tables 5.10a and b). Yield gain from spraying was 856kg.ha^{-1}. Main effects of fungicide and timing of application were not significant. The interaction, however, was significant (Table 5.10a & Fig. 5.3). Amistar and Punch C resulted in different yield responses to timing of application. Punch C showed no response to timing of application (Table 5.10b). Amistar showed that applications made at the R5 growth stage yielded significantly less (>800kg.ha^{-1}) than applications made earlier in soybean development. Amistar applied at R5 resulted in yields (2820kg.ha^{-1}) similar to unsprayed soybeans (2683kg.ha^{-1}).

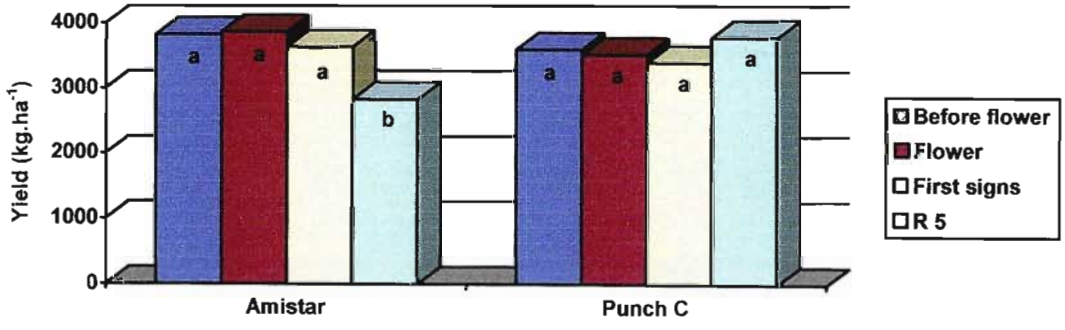

Figure 5.3 Effect of timing of initial fungicide application, using Amistar and Punch C, for the control of soybean rust, caused by *Phakopsora pachyrhizi*, on soybean yield (kg.ha^{-1}) in trials conducted at Cedara during 2001/2002

2002/2003 season

In this hot and dry season there were no significant results (Table 5.10a). However, fungicide sprayed treatments yielded more than unsprayed and Punch C treatments resulted in higher yields than Amistar treatments. Applications made at the R5 growth stage also resulted in the lowest yields (Table 5.10b).

Table 5.10a ANOVA table of soybean yield ($kg.ha^{-1}$) as affected by the timing of Amistar and Punch C applications for the control of soybean rust caused by *Phakopsora pachyrhizi* in trials conducted at Cedara from 2001 - 2003

Main effects and interactions	Degrees of freedom	Mean square		F-value		F-probability	
	2001-2003	2001/2002	2002/2003	2001/2002	2002/2003	2001/2002	2002/2003
Spray	1	1951393	334820	14.97	1.09	0.001 **	0.311
Fungicide	1	5935	143096	0.05	0.47	0.834	0.504
Time	3	202947	112727	1.56	0.37	0.239	0.777
Fungicide.Time	3	564495	614	4.33	0.00	0.020 *	1.000
Residual	16	130354	306279	-		-	
% c.v.	2001/2002	10.5		2002/2003		14.2	
Level of significance	P = > 0.05 n.s.		P = ≤ 0.05 *			P = ≤ 0.01 **	

Table 5.10b Table of means of soybean yield ($kg.ha^{-1}$) as affected by the timing of Amistar and Punch C applications for the control of soybean rust caused by *Phakopsora pachyrhizi* in trials conducted at Cedara from 2001 - 2003

Main effects and interaction		2001/2002				2002/2003			
Spray †		Sprayed		Unsprayed		Sprayed		Unsprayed	
		3539	a	2683	b	3929		3574	
LSD (0.05)		468.7				n.s.			
Fungicide		Amistar		Punch C		Amistar		Punch C	
		3523		3554		3851		4006	
LSD (0.05)		n.s.				n.s.			
Time		Before flower	Flower	First signs	R5	Before flower	Flower	First signs	R5
		3690	3670	3499	3295	3893	4046	4026	3750
LSD (0.05)		n.s.				n.s.			
Fungicide.Time †		Before flower	Flower	First signs	R5	Before flower	Flower	First signs	R5
	Amistar	3807 a	3845 a	3619 a	2820 b	3827	3960	3954	3664
	Punch C	3573 a	3496 a	3380 a	3769 a	3959	4131	4098	3836
LSD (0.05)		624.9				n.s.			

† Means followed by a different letter in the same row of the same year are significantly different at P = 0.05, using Fisher's LSD

5.3.4 Seed mass

Significant differences for seed mass between sprayed and unsprayed soybeans were obtained in 2001/2002, but not in 2002/2003 (Table 5.11a). Lower seed masses were obtained in 2001/2002 than 2002/2003 (Table 5.11b).

2001/2002 season

Fungicide sprayed soybeans had a significantly higher seed mass than unsprayed controls (Table 5.11b). Although timing of fungicide application was not significant seed mass decreased the later the fungicide was applied (Table 5.11a).

2002/2003 season

Fungicide was the only significant effect (Table 5.11a). Application of Punch C resulted in greater seed masses than Amistar (Table 5.11b). The differences between sprayed and unsprayed seed mass and timing of fungicide application was not significant in this low disease pressure season.

Table 5.11a ANOVA table of soybean seed mass (g.100 seed^{-1}) as affected by the timing of Amistar and Punch C applications for the control of soybean rust caused by *Phakopsora pachyrhizi* in trials conducted at Cedara from 2001 - 2003

Main effects and interaction	Degrees of freedom	Mean square		F-value		F-probability	
	2001-2003	2001/2002	2002/2003	2001/2002	2002/2003	2001/2002	2002/2003
Spray	1	2.854	0.889	8.76	0.39	0.009 **	0.543
Fungicide	1	0.740	11.971	2.27	5.21	0.151	0.037 *
Time	3	0.893	1.009	2.74	0.44	0.077	0.728
Fungicide.Time	3	0.659	1.753	2.02	0.76	0.151	0.532
Residual	16	0.326	2.299	-		-	
% c.v.	2001/2002	3.7		2002/2003		8.5	
Level of significance	P = > 0.05 n.s.		P = ≤ 0.05 *			P = ≤ 0.01 **	

Table 5.11b Table of means of soybean seed mass (g.100 seed^{-1}) as affected by the timing of Amistar and Punch C applications for the control of soybean rust caused by *Phakopsora pachyrhizi* in trials conducted at Cedara from 2001 - 2003

Main effects and interaction		2001/2002				2002/2003			
Spray †		Sprayed		Unsprayed		Sprayed		Unsprayed	
		15.65	a	14.61	b	17.85		17.27	
LSD (0.05)		0.741				n.s.			
Fungicide †		Amistar		Punch C		Amistar		Punch C	
		15.47		15.82		17.14	b	18.56	a
LSD (0.05)		n.s.				1.312			
Time		Before flower	Flower	First signs	R5	Before flower	Flower	First signs	R5
		16.02	15.89	15.51	15.17	17.89	18.18	17.26	18.06
LSD (0.05)		n.s.				n.s.			
Fungicide.Time		Before flower	Flower	First signs	R5	Before flower	Flower	First signs	R5
	Amistar	15.98	15.86	15.55	14.50	16.54	17.61	17.21	17.21
	Punch C	16.07	15.92	15.48	15.84	19.24	18.75	17.32	18.91
LSD (0.05)		n.s.				n.s.			
† Means followed by a different letter in the same row of the same year are significantly different at P = 0.05, using Fisher's LSD									

5.3.5 Protein content

There were no significant responses of fungicide spraying to seed protein content in either season (Table 5.12a). Protein content, similar to seed mass, was lower in 2001/2002 (Table 5.12b).

2001/2002 season

No significant effects were noticed for protein content in soybean seed between sprayed and unsprayed, Amistar and Punch C, or time of application (Table 5.12a). Protein levels appeared lower for fungicide sprayed soybeans (Table 5.12b).

2002/2003 season

Protein content in sprayed and unsprayed soybean seed was not significantly different, nor for Amistar and Punch C (Table 5.12b).

Table 5.12a ANOVA table of soybean protein content (percentage) as affected by the timing of Amistar and Punch C applications for the control of soybean rust caused by *Phakopsora pachyrhizi* in trials conducted at Cedara from 2001 - 2003

Main effects and interaction	Degrees of freedom	Mean square		F-value		F-probability	
	2001-2003	2001/2002	2002/2003	2001/2002	2002/2003	2001/2002	2002/2003
Spray	1	8.027	0.005	1.29	0.00	0.272	0.956
Fungicide	1	1.363	0.101	0.22	0.07	0.646	0.796
Time	3	4.446	1.644	0.72	1.12	0.557	0.369
Fungicide.Time	3	13.217	0.810	2.13	0.55	0.137	0.653
Residual	16	6.206	1.462	-		-	
% c.v.	2001/2002	7.0		2002/2003		3.2	
Level of significance	P = > 0.05 n.s.		P = ≤ 0.05 *			P = ≤ 0.01 **	

Table 5.12b Table of means of soybean protein content (percentage) as affected by the timing of Amistar and Punch C applications for the control of soybean rust caused by *Phakopsora pachyrhizi* in trials conducted at Cedara from 2001 - 2003

Main effects and interaction		2001/2002				2002/2003			
Spray		Sprayed		Unsprayed		Sprayed		Unsprayed	
		35.62		37.35		38.15		38.11	
LSD (0.05)		n.s.				n.s.			
Fungicide		Amistar		Punch C		Amistar		Punch C	
		35.86		35.38		38.09		38.22	
LSD (0.05)		n.s.				n.s.			
Time		Before flower	Flower	First signs	R5	Before flower	Flower	First signs	R5
		34.40	35.72	36.41	35.94	38.01	37.60	38.14	38.86
LSD (0.05)		n.s.				n.s.			
Fungicide.Time		Before flower	Flower	First signs	R5	Before flower	Flower	First signs	R5
	Amistar	35.21	34.51	35.71	37.99	38.07	37.77	37.53	38.98
	Punch C	33.59	36.93	37.12	33.88	37.95	37.43	38.75	38.74
LSD (0.05)		n.s.				n.s.			

5.3.6 Oil content

As with protein content, there were no significant responses in seed oil content to fungicide spraying in either season (Table 5.13a). Oil content was lower for the unsprayed treatments in 2002/2003 (Table 5.13b).

2001/2002 season

No significant effects were obtained in oil content of soybeans (Table 5.13a). Oil content of sprayed and unsprayed soybean seed was similar. Fungicide was not significant (P = 0.066), with Amistar treatments resulting in higher oil content than Punch C (Table 5.13b). Time of initial fungicide application was not significant.

2002/2003 season

Although not significant, oil content was noticeably different between sprayed and unsprayed treatments, with unsprayed soybeans having less oil (Table 5.13b). Fungicide, time of application and their interaction were not significant (Table 5.13a).

Table 5.13a ANOVA table of soybean oil content (percentage) as affected by the timing of Amistar and Punch C applications for the control of soybean rust caused by *Phakopsora pachyrhizi* in trials conducted at Cedara from 2001 - 2003

Main effects and interaction	Degrees of freedom	Mean square		F-value		F-probability	
	2001-2003	2001/2002	2002/2003	2001/2002	2002/2003	2001/2002	2002/2003
Spray	1	0.016	1.132	0.01	2.39	0.908	0.141
Fungicide	1	4.550	0.031	3.90	0.06	0.066	0.802
Time	3	1.110	0.496	0.95	1.05	0.439	0.398
Fungicide.Time	3	2.509	0.488	2.15	1.03	0.134	0.406
Residual	16	1.167	0.473	-		-	
% c.v.	2001/2002	5.5		2002/2003		3.6	
Level of significance	P = > 0.05 n.s.		P = ≤ 0.05 *			P = ≤ 0.01 **	

Table 5.13b Table of means of soybean oil content (percentage) as affected by the timing of Amistar and Punch C applications for the control of soybean rust caused by *Phakopsora pachyrhizi* in trials conducted at Cedara from 2001 - 2003

Main effects and interaction		2001/2002				2002/2003			
Spray		Sprayed		Unsprayed		Sprayed		Unsprayed	
		19.80		19.72		19.37		18.72	
LSD (0.05)		n.s.				n.s.			
Fungicide		Amistar		Punch C		Amistar		Punch C	
		20.24		19.37		19.41		19.34	
LSD (0.05)		n.s.				n.s.			
Time		Before flower	Flower	First signs	R5	Before flower	Flower	First signs	R5
		19.51	20.44	19.57	19.69	19.71	19.35	19.42	19.01
LSD (0.05)		n.s.				n.s.			
Fungicide.Time		Before flower	Flower	First signs	R5	Before flower	Flower	First signs	R5
	Amistar	20.50	19.96	20.34	20.15	19.47	19.32	19.85	19.00
	Punch C	18.52	20.91	18.80	19.23	19.96	19.38	18.99	19.03
LSD (0.05)		n.s.				n.s.			

5.4 Discussion

In 2001/2002 final disease reached 70.8% and 23.8% for unsprayed and sprayed treatments, respectively. In the hot, dry 2002/2003 season final disease for unsprayed and fungicide sprayed treatments was 65% and 21.3%. Although there were significant differences in disease levels for different treatments there was no difference in yield or seed mass between sprayed and unsprayed treatments in the 2002/2003 trials. This is probably due to environmental conditions not being optimal for soybeans to reach their full yield potential. Consequently, unless otherwise specified, only results from 2001/2002 are discussed.

Timing of initial fungicide application for optimum yield is dependent on the type of fungicide applied. Sterol biosynthesis inhibiting fungicides (SBIs) penetrate the leaf cuticle and are therefore highly effective in curative applications after infection has already taken place (Agrios, 1997). For the R5 time of application disease levels were 11% in the plots at time of first fungicide application. When the second fungicide application was made disease levels had increased in plots for both treatments with 22% disease in Punch C plots and 27% disease in Amistar plots. The final disease rating in Punch C plots was 30%, whereas final disease in Amistar plots increased to 43%. With Punch C there was no yield response to time of initial application. Although disease levels were high (>10%) when the R5 application of Punch C was made, the curative activity of the SBI/benzimidazole fungicide ensured that yield was not significantly affected. For Amistar, an application at the R5 growth stage resulted in a significantly lower yield than earlier applications. Difference in yield response for the two fungicides is linked to mode of action. Amistar, unlike Punch C, is not curative in action. Amistar applications made at first sign of disease and at R5 were not preventative sprays since infection was present at the time of application. Disease levels were low (1.3%) for the application at first sign of disease, but much higher (>10%) when sprayed at the R5 growth stage. Symptoms were present in the crop for 11d before Amistar was applied in the R5 treatment. Stress at the R4 – R6 growth stage causes more yield reduction than at any other time (McWilliams *et al.*, 2004). Since Amistar was unable to halt disease development at this critical growth stage, noticeable yield reductions resulted from Amistar applications at the R5 growth stage.

Although time of initial application did not significantly affect seed mass, Amistar applications made at the R5 growth stage, like yield, resulted in lower seed mass than applications made earlier. Amistar applications made at first sign of disease were made at the R4 growth stage, just before the critical R4.5 – R5.5 stage, and therefore seed mass was not as noticeably affected as seed mass for Amistar applications made at the R5 growth stage.

Timing of fungicide application was significant for final disease severity and SAUDPC. Applications made before and during flowering resulted in less disease than applications made at first sign of disease and R5. Polycyclic diseases, such as SBR, are characterised by sigmoidal curves of disease progress (Agrios, 1997). Early fungicide applications were made before visible symptoms of disease were present, while inoculum levels were still low, and hence slowed the build-up of inoculum, keeping the rate of disease development low for a longer period. Late fungicide applications were made when disease was already present and inoculum levels were high, resulting in higher rates of disease development and hence higher levels of disease for late applications of fungicide. Another confounding factor and uncontrolled variable which makes comparisons between different times of fungicide applications difficult is environment. Weather patterns are different at each time of application and this can influence disease development in relation to the fungicide treatment.

Short daylength and warm temperatures control soybean flowering (McWilliams *et al.*, 2004). Planting date therefore, also affects flowering (Fehr and Caviness, 1977). Due to a difference in planting dates in the two seasons (22 December 2001 and 12 November 2002) the before-flowering fungicide application was made earlier in 2002/2003 than 2001/2002. This resulted in a much longer period where the before-flowering treatments were left unprotected at the end of the growing season (40d in 2002/2003 vs 22d in 2001/2002). As a result of this, final disease levels were highest for the before flowering treatment in 2002/2003, whereas in 2001/2002 R5 treatments resulted in the highest final disease severity.

Several researchers have emphasised the impact of stress between R4 – R6 growth stages on seed yield (Fehr and Caviness, 1977 and McWilliams *et al.*, 2004). Comparison of different times of initial fungicide application illustrates this well. The

before flowering applications were made 58 and 79 DAP. Assuming a 21d fungicide persistence period, the crop would have been protected until 100 DAP. At this stage the crop had already reached the critical R5.5 growth stage. If the fungicide persistence wore off, then the crop was left unprotected for 22d until physiological maturity. Similarly, applications made at flowering left the crop unprotected for the last 15d, but protected during the most critical stage. Applications made at first sign of disease were applied when the crop was at the R4 (full pod) growth stage and the second application resulted in protection until maturity. The first R5 spray was applied 94 DAP, 11d after first signs of disease were recorded and when the crop was at a critical stage for determining seed yield. Disease levels (final disease severity and SAUDPC) were significantly lower when fungicide applications were made before and at flowering than at first sign of disease and R5. Although not significant, yield and seed mass were noticeably lower in applications that were made at first sign of disease and at the R5 growth stage. Since time to uredial development and sporulation varies from 9-18 days (Table 1.6, Chapter 1), weather and the presence of spores for approximately two weeks prior to first sign of disease may influence the amount of latent infection at the time of the initial fungicide application. The effect of latent infection in treatments sprayed, particularly at the first sign of disease, may be quite important in influencing fungicide efficacy for different times of fungicide application.

Fehr and Caviness (1977) stated that a soybean plant's response to the conditions it encounters depends on its stage of development. Benefits and financial returns from application of chemicals can be influenced by stage of plant development when the material is applied. Results from these trials emphasise the need to protect the crop during the R4 – R6 growth stages.

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CHAPTER 6

Thesis overview

6.1 Inoculum pressure

One of the biggest challenges to this research was obtaining even levels of inoculum throughout plots within trials. Pathogens such as *Sclerotinia sclerotiorum* (Lib.) de Bary can be cultured. Sclerotia produced in culture may be spread evenly around trial plots to create an even inoculum pressure. *Cercospora zeae-maydis* Tehon and E.Y. Daniels, the causal organism of grey leaf spot in maize, is able to survive in maize debris (Ward *et al.*, 1999). At the end of the season, infected maize leaves may be collected and kept in cold storage (4°C) for use as an inoculum source the following season. Infected leaves may be spread throughout trial plots, supplying a more or less even inoculum pressure for the start of new epidemics. However, as an obligate parasite, *Phakopsora pachyrhizi* cannot be artificially grown in culture nor survive on leaf debris.

Depending solely on natural infection of trial plots by wind-dispersed urediospores was deemed to be risky since the disease develops in focal areas, with resulting severe infection in some plots and very little infection in other plots. Although trial design and replications are used to help eliminate some of these effects, statistical analysis is based on homogeneity of variance (Steel and Torrie, 1981). Efforts were therefore made to create even inoculum pressure throughout trials.

In 2001/2002, *P. pachyrhizi* infected soybean leaves were collected from early planted soybean trap crops. Leaves were rinsed in water for collection of urediospores and then sprayed over trial plots. Very hot weather following inoculation resulted in a second attempt a week later. Even though supplementary irrigation was provided to supply the required leaf-wetness conditions, extended periods of high temperatures (>28°C) resulted in this inoculation method not succeeding.

In 2002/2003, soybeans were planted singly into bags and allowed to become naturally infected. These plants were then placed throughout the trials. It was hoped that since the

spores could survive for longer on these living hosts, that chances of successful infection would be greater using this technique. A hot, dry season resulted in very unfavourable conditions for disease development. Although the technique was sound, environmental factors meant that it too, did not succeed. This method may have had a better chance of succeeding in 2001/2002 or in 2003/2004 but perhaps would have promoted the development of focal points.

In 2003/2004, environmental conditions were extremely favourable for disease development. Any of the techniques implemented previously may have had a good chance of success. However, not knowing at the start of the season what weather conditions the trials would be exposed to, decisions were made to help ensure higher inoculum levels. One of these techniques was to increase plot size, without increasing the fungicide treated areas. This resulted in a larger unsprayed area to provide more inoculum. Conversations with Zimbabwean researchers led to the technique of stapling infected leaves onto plants within the plots. These two techniques, in combination with favourable environmental conditions, resulted in good disease development and reduced coefficients of variation.

Of the approaches to developing even inoculum, the six row plots and infected leaf approach of 2003/2004 was the most manageable, and will be adopted for future research trials.

6.2 Fungicide persistence

Determination of length of fungicide persistence is helpful in determining spray intervals. At present the recommended spray interval is 21d, although some fungicide labels vary from 14 – 28d, specifying weather conditions as a factor to consider in determining the spray interval. Sterol biosynthesis inhibiting fungicides (SBIs) are thought to have longer persistence than strobilurin fungicides (Miles *et al.*, 2004). Once fungicide persistence is known, trials with different spray intervals could be conducted. This information could help tailor more cost-effective spray programs.

6.3 Adjuvants

Adjuvants are used in combination with fungicides to enhance their performance. Their use in combination with application method (tractor or aeroplane) could be very important future research. When systemic fungicides are sprayed, penetrants can be included to improve fungicide penetration into the host plant. Stickers and spreaders can be applied with protectant fungicides to assist the fungicide adhering to the target area and providing some degree of rain-fastness as well as improving the coverage of the fungicide on the plant. (Witt, 1998; Petroff, 2005)

6.4 Alternation of fungicides

All trials were conducted with the use of the same fungicide in subsequent applications. Continued use of the same fungicide or fungicides from the same chemical group is a major contributing factor to fungicide resistance development (Delp, 1988). To minimise the risk of this happening, spray programs which incorporate alternations of fungicides from different chemical groups should be investigated.

6.5 Evaluation of rates with only two sprays

The rates trials presented in this research were conducted with three sprays from flowering. Research indicates that the third spray does not significantly increase yield and that two sprays are adequate for use on a cultivar with medium maturity under favourable environmental conditions. The determination of optimum rate should be conducted under conditions in which the fungicide would be used in practice.

6.6 Assessing the curative activity of fungicides

The SBI fungicides are curative in activity (Miles *et al.*, 2004). This means that they can be sprayed after infection has occurred and cure the infection by halting fungal

development. Other fungicides which are not curative in activity act by preventing further infections from occurring, but are unable to cure existing infections. The use of curative fungicides provides the advantage of spraying after symptoms have been noticed, rather than prophylactically. Determination of the degree of curative ability could be assessed and used to estimate the grace period a farmer has left before fungicides can no longer cure the infection. This is useful to know when dependent on availability of aircraft for fungicide applications or if weather is not conducive for spraying operations.

6.7 Aerial spraying

Although some farmers use tractors for fungicide applications, many use aeroplanes. Aerial applications are made in greatly reduced spray volumes of $40P.ha^{-1}$. Ground applications are recommended at between 300 – $500P.ha^{-1}$ (Nel *et al.*, 2003). Given the problem of phytotoxicity at reduced spray volumes, this effect would need to be checked for aerial spraying. Efficacy and the use of adjuvants to enhance fungicide performance should also be compared with ground spraying.

6.8 Economics of spraying (cost-benefit analysis)

The cost of production is a factor which determines gross profit margins. Increased production costs can erode profits. Sometimes, when the risk of yield reduction is real, it becomes more economical to increase production costs and provide the necessary protection than to do nothing and harvest reduced yields. *Phakopsora pachyrhizi* is known to be capable of causing serious yield reductions (Hartman *et al.*, 1999). Studies need to be conducted to determine the economics of fungicide control of soybean rust. Included in this study should be a comparison of costs across favourable and unfavourable SBR seasons. They should also incorporate comparisons of number of fungicide applications, as well as differences in costs for the different fungicides available for SBR control.

The damage function of SBR could also be modelled to calculate economic loss. This would be done by estimating the maximum yield and calculating yield multiplied by the price of the crop as a function of SBR disease severity. This could be extended to include variables such as application regimes and fungicide types.

6.9 Trials at other locations

Due to differences in climate, alternate hosts and other factors, incidence of SBR may vary at different locations (Bromfield, 1984). A SBR management program for one area may not be effective in another area. Research should be undertaken at different locations in SA to determine optimum management strategies for SBR in specific areas.

6.10 Cultivars from different maturity groups with different numbers of fungicide applications

As the length of the growing season differs for cultivars from different maturity groups, spray programs should consider the growing length when determining the optimum number of fungicide applications required to protect against SBR (Caldwell and McLaren, 2004). As soybean yield is most affected from the R4 - R6 growth stages this is the time that it needs to be protected. As early-maturing cultivars generally progress through their growth stages more quickly than late-maturing cultivars, an early-maturing cultivar may need only one critically timed fungicide spray. A late-maturing cultivar however may require three fungicide applications for protection from SBR.

6.11 Critical periods for fungicide protection

The most critical period for seed yield is the R4.5 – R5.5 growth stage (McWilliams *et al.*, 2004). Any stress from R4 – R6 can negatively affect yield. If fungicide applications are applied to provide protection until the end of the R6 growth stage, is this sufficient to prevent yield loss or does the plant need to be protected until physiological maturity? One of the most commonly asked questions by farmers is when

to stop spraying. If this critical period can be precisely determined it will be of great use to the soybean producers.

6.12 Research conducted, but not yet reported

Trials that have been conducted at Cedara, but not yet reported, include:

- length of fungicide persistence
- use of various adjuvants in combination with fungicides at different fungicide rates
- alternating fungicides with different modes of action and varying spray intervals
- cost-benefit analysis of fungicide use, applied to the results of all fungicide trials

6.13 Future research

Other than areas mentioned above for further fungicide trials, there are several other aspects where research needs to be conducted in order to better manage SBR in SA:

- Collecting rust isolates and identifying races
- Screening cultivars and breeding lines for tolerance
- Identifying overwintering hosts and better understanding the biology of *P. pachyrhizi*
- Mapping disease incidence
- Modelling SBR for predicting disease occurrence
- Breeding for horizontal resistance

6.14 References

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