The Epidemiology and Control of

Leptosphaeria maculans

Cause of Crucifer Blackleg, in KwaZulu-Natal

by

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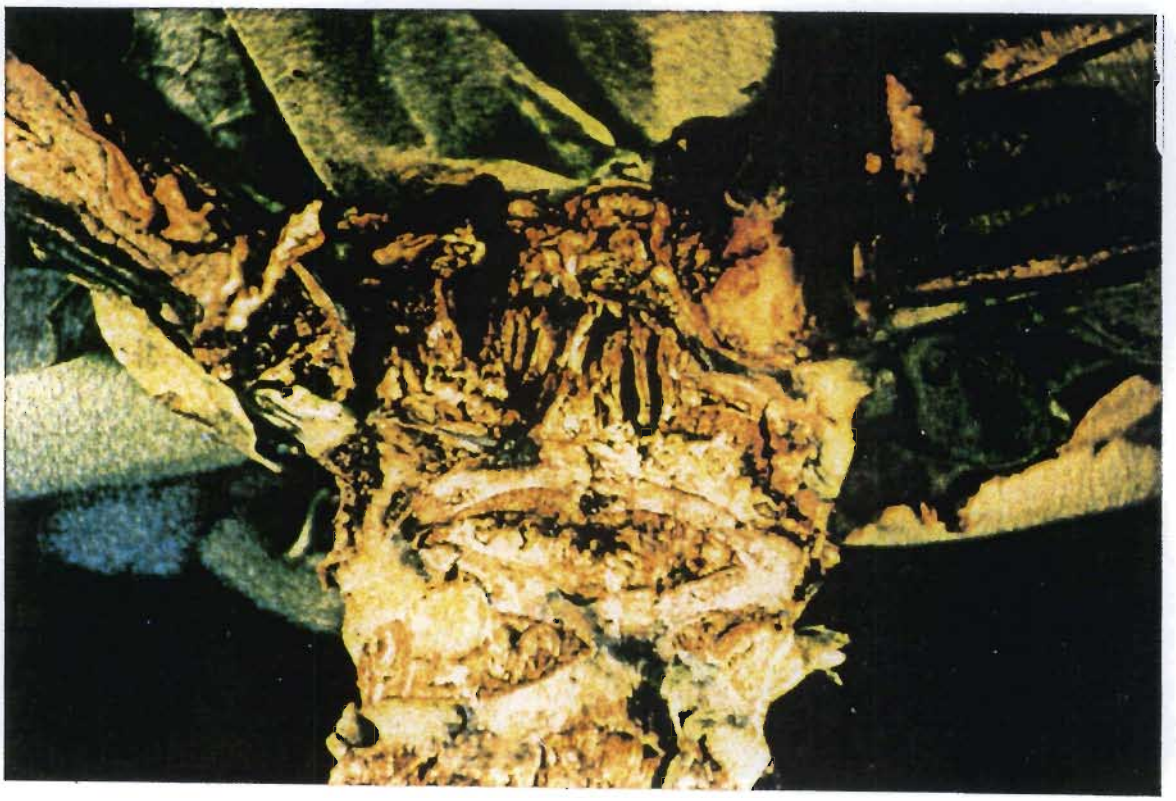
Cabbage production in KwaZulu Natal, South Africa. Wilted cabbages in the foreground have blackleg.



A Cabbage Stem Severely Infected by *Leptosphaeria maculans*

Thesis Abstract

The perfect stage of *Leptosphaeria maculans* is reported for the first time in South Africa. Viable pseudothecia and pycnidia were found on dead, weathered tissue, sometimes in close association, whereas only pycnidia were found on live tissue.

Some seedlots of imported cabbage seed were found to be internally infected with *L. maculans* at low levels and *Alternaria brassicicola* at higher levels. Fungicides iprodione (dicarboximide), triforine and propiconazole (sterol-biosynthesis inhibitor) eliminated both pathogens from infected seed.

In a field trial of eight cabbage and two cauliflower cultivars, incidence of stem infection by *L. maculans* ranged from 16-80%. Two seedlots of the cabbage cultivar Gloria Osena differed in blackleg stem susceptibility. No correlation was found between stem lesion incidence and foliar infection counts of each cultivar, or stem lesion incidence and each cultivar's average days-to-harvest. In a second trial, incidence of stem infection ranged from 50% (Rotan) to 95% (Dynasty) in cabbage, and 64.2 to 96.6% in cauliflower cultivars. All Brussels sprouts and broccoli cultivars tested were highly susceptible. The cultivars of turnip and tyfon tested were observed to be immune to blackleg, whereas the swedes, Japanese radish, chou moullier and red cabbage cultivars tested were highly susceptible. No correlation was found between stem length and incidence of stem infection. Different seedlots within several cabbage and cauliflower cultivars differed in their blackleg susceptibility. A third cultivar trial

with 10 replicates of four seedlots of one cabbage cultivar confirmed that different seedlots of a single cultivar may vary significantly in their susceptibility to blackleg.

Benomyl was applied to cabbage at the seedling stage only, or at the seedling stage followed by field applications every 14 d. Relative to an untreated control, multiple applications of benomyl resulted in a 33% reduction in stem infection, a ten-fold reduction in plants killed and a 50% reduction in the proportion of non-harvestable heads, relative to an untreated control. Seedling treatment resulted in a lower infection level, a lower mortality rate and a greater mean head mass than those of the untreated control. However, none of these differences were statistically significant.

In a debris degradation trial, more than 90% of buried debris (cabbage stems infected by *L. maculans*) had decomposed after 2.5 yr, whereas 80% of surface debris had decomposed over the same period. The susceptibilities of seedbed transplants (SBT) and container-grown seedlings (CGS) were compared using different forms of *L. maculans* inoculum. "Dunk" inoculation of SBT into a pycnidiosporial suspension resulted in a stem infection level of 50% greater than an uninoculated control. Contamination of seedbeds resulted in an infection level of 46%. "Dunk" inoculation of CGS resulted in infection level of 22%. When CGS were grown in contaminated trays an infection level of 33.4% resulted. Interplot interference in the form of inoculum dispersal over a 1 m border was low (1.8 and 2.7% for SBT and CGS, respectively).

In a further trial examining the relationship of inoculum level and blackleg, a strong interaction was found between inoculation technique and inoculum level. Inoculation of field plots with infected debris was a more efficient technique than dipping seedlings into a pycnidiospore suspension prior to transplanting.

Twenty nine blackleg epidemics were surveyed over 11 yr. Seedbed transplants (SBT) had been used in 83% of cases. Two cases (7%) had involved direct drilled seedlings (DDS). However, excess seedlings had been transplanted, making DDS epidemiologically equivalent to SBT. Three cases (10%) had involved container-grown seedlings (CGS) grown on monocropped cabbage lands. Disease occurred in two patterns: in crops grown from SBT and DDS, blackleg occurred down the lines. In all CGS cases, disease occurrence was randomly patterned. In all cases, diseased debris was found in seedbeds and production fields. Disease spread in the field was limited to the two plants on either side of the initially infected plant, 1.3 m or less, suggesting that infection had resulted from splash dispersed pycnidiospores. The disease cycle was mono- or oligocyclic but not polycyclic.

Over a period of 6 yr, cabbage fields of 26 farms were each examined once for cruciferous weeds infected with *L. maculans*. No viable blackleg lesions were discovered on cruciferous weeds, suggesting that weeds play no role in the local crucifer blackleg pathosystem.

A theory is proposed that windows of disease susceptibility open and shut during the different phenological stages of a crucifer's life, and that the susceptibility of different plant organs vary with the phenological state of the plant. It is also postulated that blackleg is a "low sugar disease". Disease incidence was lower in well fertilized cabbage plants than minimally fertilized plants. Organoleptic tests of cabbage cultivars correlated superior flavour and texture in cabbage with a high susceptibility to blackleg.

An integrated management strategy is proposed, based on seed treatment with fungicides, the use of container-grown seedlings rather than seedbed transplants, a 3 yr rotation of crucifer lands with non-cruciferous crops, implementation of either deep-ploughing or accelerated biodegradation to eliminate debris, the development of higher levels of horizontal resistance to *L. maculans* in cruciferous vegetables, application of field fungicides in high risk areas (benzimidazoles or triazoles, or combinations), and the minimization of stress and optimization of host nutrition.

- Declaration -

I declare that this research is the result of my own investigation.

Mark D. Laing

How hard and uncertaine it is to describe in words the true proportion of Plants.
They best do know who have deepliest waded in this sea of Simples.

John Gerard, 1636

We shall find the real epidemic muddy and uncomfortable. And the explicit and logical analysis of our scattered observations is not only less comfortable than the statement of generalities, it also puts our cards on the table for all to see. Nevertheless, we must put or shut up, striving constantly to use to the uttermost the knowledge accumulated with such pain.

P.E. Waggoner, 1962

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The richness of a nation does not depend on the number of its inhabitants but on the quantity of food at their disposition.

HAN FEI-TSE (third century)

The history of man from the beginning has been the history of his struggle for his daily bread.

José de Castro

Foreword

This thesis has evolved over several years. It has several parts which can be read independently. Chapter 1 is a review of the literature concerning crucifers and crucifer blackleg. Chapter 2 covers the mycology of *L. maculans* in KwaZulu-Natal. Chapter 3 concerns the construction of an ethograph describing the crucifer blackleg pathosystem in KwaZulu-Natal. Chapters 4-10 report the experimental research conducted on seed pathology and treatment, genetic resistance, field fungicides, the role of debris, disease spread, the role of cruciferous weeds, and host phenological and physiological effects on susceptibility. In Chapter 11 the experimental results are reviewed and synthesized into a practical disease management programme.

The chapters are each written as a discrete paper, in the "Dutch" style of thesis. This results in some duplication of references between chapters, and between the chapter abstract and the overall thesis abstract, but provides for a more accessible thesis overall.

Local constraints and imperatives shaped the structure and form of the thesis. When the investigations started, blackleg was a serious disease causing some farmers considerable losses. Early funding was therefore from farmers, and later from Government, with the expressed directive that obtaining practical solutions, applicable in the real farming environment, was to be the priority objective of the project. Considerable effort was therefore put into understanding the management of local cabbage farmers, their cropping practices and the disease. From the beginning, the goal was to produce a comprehensive ethograph from which to build a practical management programme which farmers would adopt.

Another element of the thesis which needs to be understood is that the multiple trials on seed treatments, genetic resistance and the role of debris were conducted in sequence. The first trial of each series produced results which defined the design of the subsequent trial, etc.

CHAPTER 1. CRUCIFERS AND CRUCIFER BLACKLEG

1.1 General Introduction

"If you lived on cabbage, you would not be obliged to flatter the powerful."

"If you flattered the powerful, you would not be obliged to live on cabbage."

Diogenes

quoted from Root, 1980

"The original victualling station at the Cape, and eventually the entire agricultural industry of South Africa, owe their existence to the fact that the country is so well suited to the cultivation of vegetables. It all started with the shipwreck of the Haarlem off the Cape in 1647. The crew salvaged some vegetable seeds from the ship and set about planting them immediately. The vegetables grew so well that two of the officials, Jansz and Proot, in their report of 1649 to the Dutch East India Company, recommended that a halfway victualling station be established at the Cape to provide the Dutch merchantmen with fresh water, meat and vegetables. The company accepted the recommendation and the victualling station was established in 1652" (Anon., 1989a).

Jan Van Riebeeck commanded the expedition, which was under specific orders from the Assembly of Seventeen of the Dutch East India Company. One of the specific instructions was "to make a garden which was to yield an adequate supply of vegetables not only for the inhabitants of the station but also for the crews of the Company's ships calling there. In fact, it was largely for the purpose of growing vegetables for supply to the passing ships in order to counteract the ravages of scurvy that the Company had resolved to occupy the Cape" (De Kock, 1924).

Cabbages were a significant component of the vegetables grown for the ships: "the origin of Cape spitz cabbage, which is unique to South Africa, can be traced to the early period" (Anon., 1989a). De Kock (1924) commented, "the vegetable gardens responded favourably to the laborious efforts of the gardeners, especially in the case of cabbage, turnip and carrots". He commented further, "the ships were provided with (1) water, (2) vegetables and fruit, such as cabbages, radishes, onion, cress, sorrel, beetroot, watermelons, etc., and (3) fresh meat, mainly mutton and beef. Fresh and green vegetables were needed to counteract the ravages of scurvy on board ship and so reduce the death-rate". Thus, the colonization of southern Africa was initiated by the need for vegetables, meat and water, some 250 yr ago.

A crucial step in the economic history of this country was the 1913 Land Act. It was initially set in motion by white vegetable farmers who were unable to compete with black farmers in the production of maize and vegetables (and cabbages, in particular), for purchase by the diamond and gold mines to feed their labour forces (Keegan, 1986). In one step, 87% of South Africa was declared "white land" and all blacks were confined to the remaining 13% of the land (Wickens, 1983) (See Fig 1.1.A-B).It was perhaps the single step which assured political strife and violence in this country. It provided the structural basis for apartheid, instituted some 35 yr later by legally alienating the majority of the country from its fundamental resources, and making them foreigners in the land of their forefathers (Natrass, 1981). Given that the indigenous religions of southern Africa are all based on ancestor-worship, it was an act guaranteed to evoke profound opposition, and bound to erode indigenous cultures. So in South Africa, the humble cabbage has a known historic and political status.

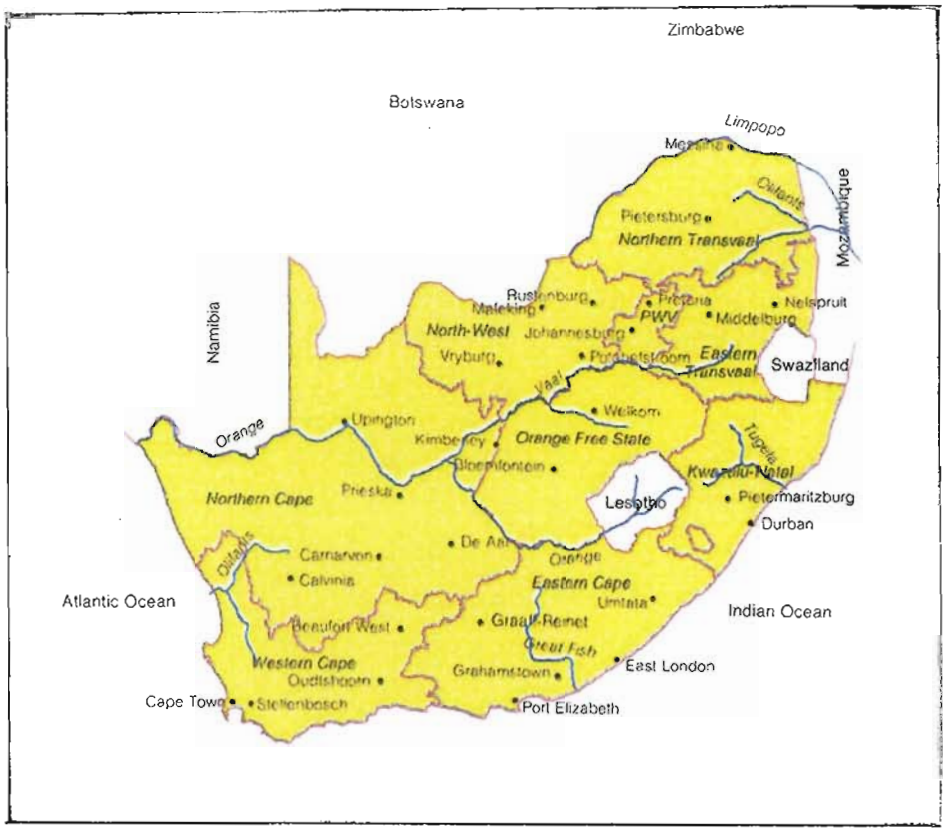

Fig. 1.1.A A General Orientation Map of Post-Apartheid South Africa

(Anon., 1996a)

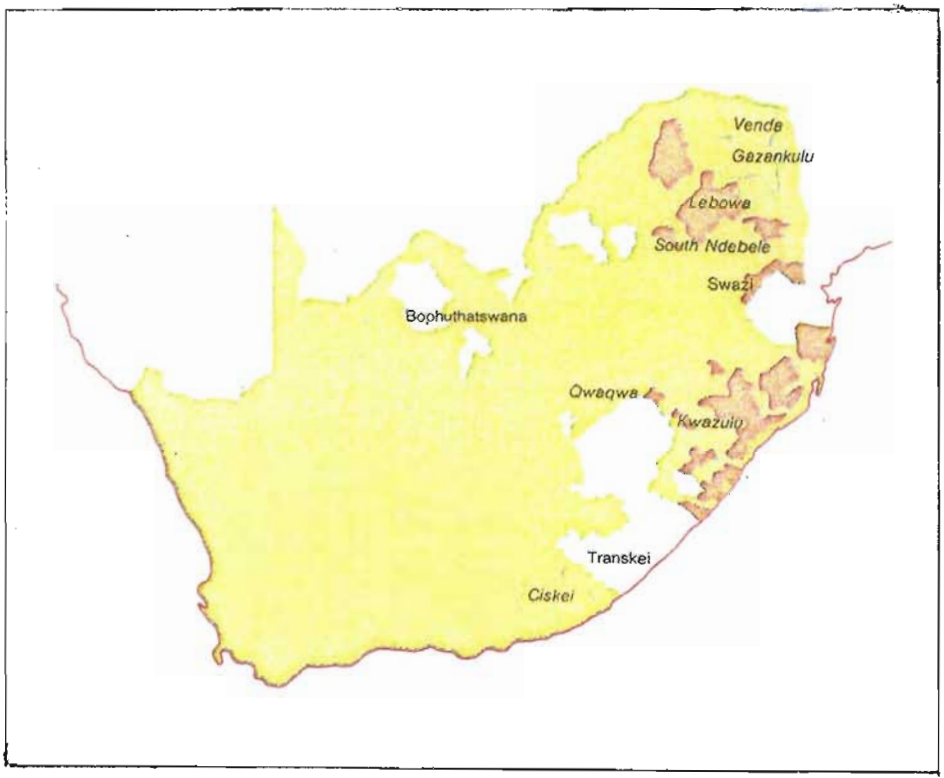

Fig. 1.1.B Black Homeland "States" in Apartheid South Africa

(Anon., 1989b)

KwaZulu-Natal is a province on the eastern seaboard of South Africa. It stretches from Mozambique in the North-East to about a third of the way down the coast towards Cape Town in the south, sweeping around in an arc bounded by the Drakensberg mountains, Swaziland, and Mozambique in the north. It has a wide diversity of biogeographic zones, from fully tropical in the North-East to alpine in the Drakensberg. Fig 1.1.C-F illustrate the mean annual precipitation in South Africa, the annual rainfall patterns, national mean thermoclines and the biogeographic zones of South Africa. They shows that KwaZulu-Natal is by far the wettest region of the country, with summer rainfall, a reasonably equable temperature and is a biologically productive region. The province, together with the neighbouring areas of Transkei and Ciskei ("homeland states" of the apartheid era, now incorporated into the Eastern Cape Province), contains most of South Africa's black population.

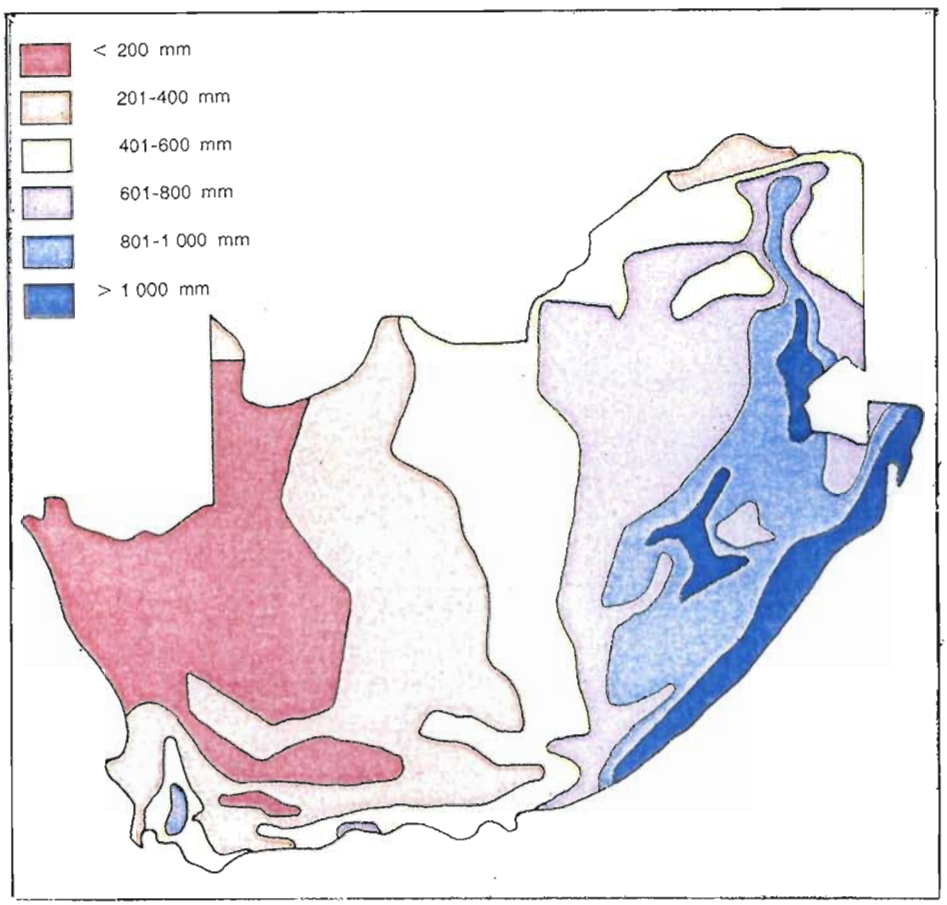

Fig. 1.1.C Simplified Distribution Map of Mean Annual Precipitation in South Africa (Anon., 1996a)

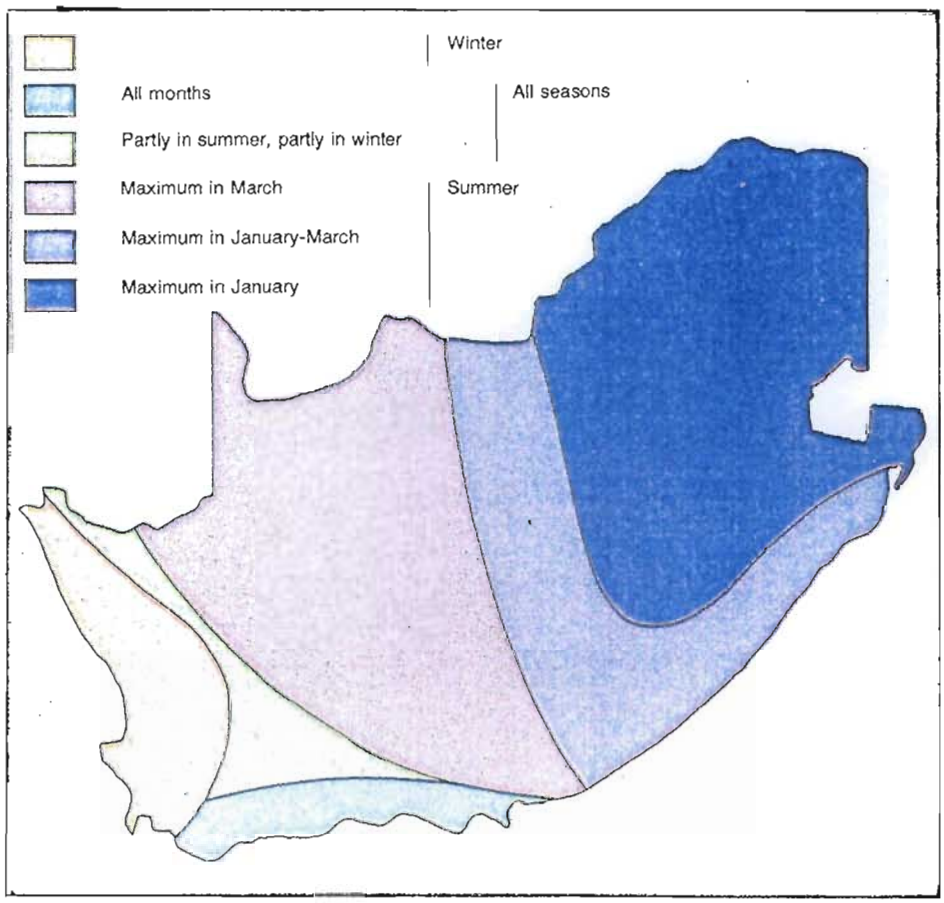

Fig. 1.1.D Seasonal Rainfall Regions of South Africa (Anon., 1996a)

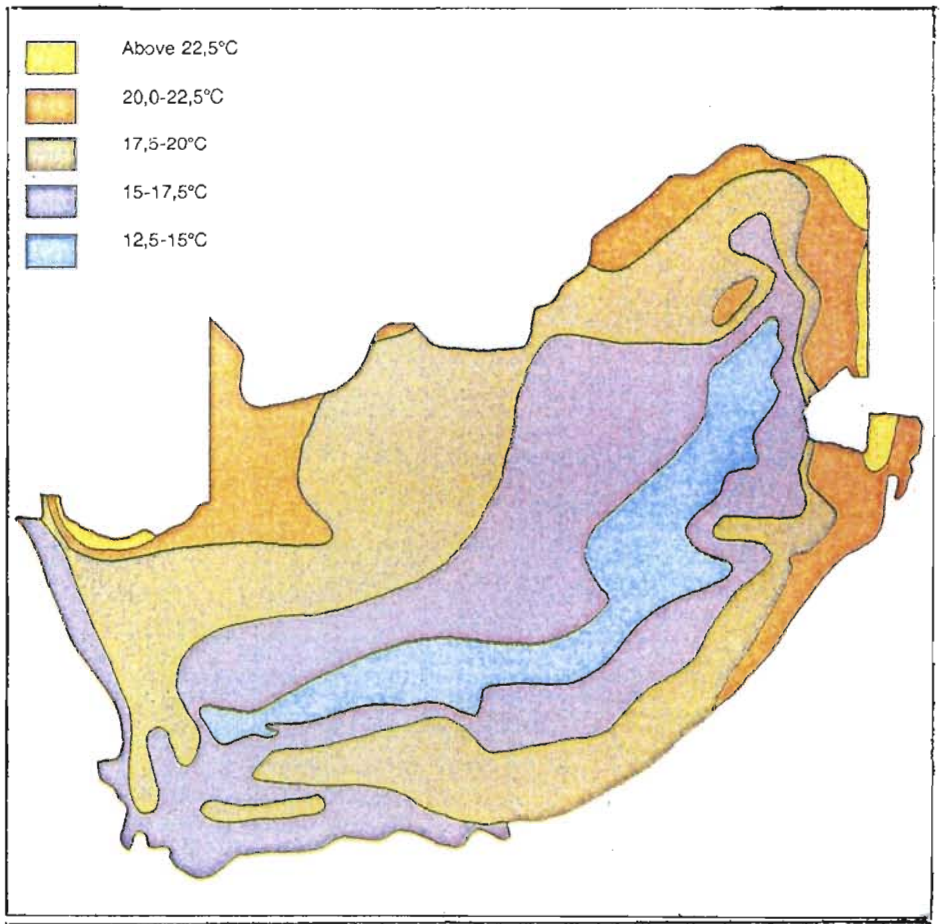

Fig. 1.1.E Mean Annual Surface Temperatures in South Africa
(Anon., 1996a)

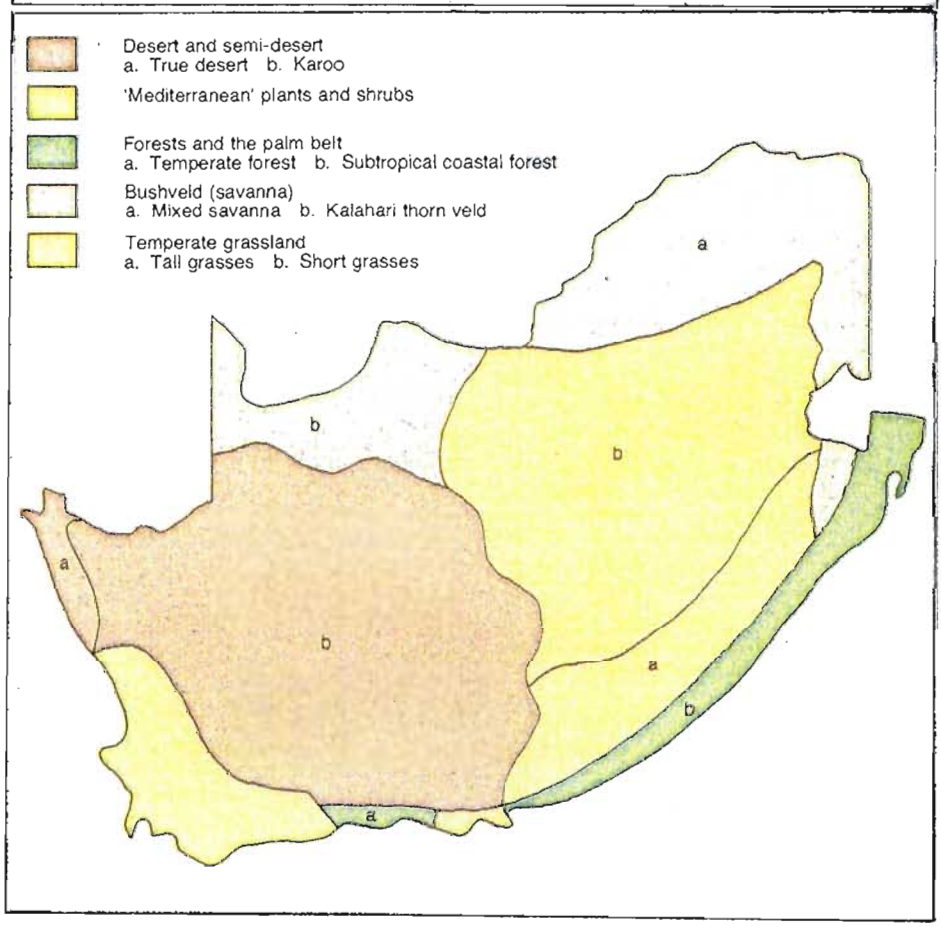

Fig. 1.1.F The Major Natural Vegetation Regions of South Africa
(Anon., 1996a)

Table 1.1.A Some Facts and Figures About Agriculture In KwaZulu-Natal

* KwaZulu-Natal has a total land area of 8.65 million ha. 7 million ha is farmland (81%), 1.2 million ha of which is potentially arable (17%). 4.09 million ha are designated for commercial agriculture (58.4%).
* In commercial farming areas, 1.2% of the population own land. In the former KwaZulu 68% of the population "own" land. However, 55% own 1 ha or less, and only 8.5% own more than 2 ha.
* There are about 6 500 white commercial farmers, 3 000 white and Indian smallholders, 64 000 black farmers and 233 000 black "micro cultivators".
* Of KwaZulu-Natal's estimated population of 8.5 million people, about 5.3 million live in rural areas. In commercial farming areas the population density is about 45 / km^2, whereas in the former homeland it is about 148 / km^2.
* Excluding farmworkers in commercial agriculture, between 68% and 74% of rural people live in poverty.

Anon., 1995a

In 1990, Durban had a total metropolitan population of 3.4 million, of which 2.3 million were black, and Pietermaritzburg had a black population of over 400 000. As is typical of most Third World countries, these cities' populations have more than trebled in the last 20 yr (Anon., 1989b; 1990). The population increase is largely the result of urban drift, whereby rural people leave impoverished, rural areas for urban areas, gambling on finding a better life there. Clearly there is a growing need for the provision of fresh green vegetables for this mushrooming black population which has

establishment of home gardens. They also constitute the poorest urban community and have no access to facilities such as refrigeration for the storage of fresh food.

To supply the demand for cabbage there are many farmers in the KwaZulu-Natal Midlands, producing millions of cabbages annually (See Sections 1.2.3.2 and 1.2.3.3 for details). For many years their primary problems were American bollworm (*Heliothis armigera* Hübn), bacterial black rot (*Xanthomonas campestris* pv *campestris* Pamm. Dowson), theft and fluctuating cabbage prices.

In the late 1970s and early 1980s, plant pathologists in the Department of Microbiology and Plant Pathology received reports of a new disease causing severe losses on several cabbage farms. On investigation, the disease was identified as crucifer blackleg, caused by *Leptosphaeria maculans* (Des.) Ces. et de Not.

This disease has been a persistent and ubiquitous problem in cabbage production, as the pathogen probably co-evolved with crucifers (Zadoks and Schein, 1979). The first reference in the literature to the disease was the naming of the imperfect stage of fungus as *Sphaeria lingam* in 1791 (Tode, 1791).

Given the importance of the crop to the affected farmers, and the need to find pragmatic solutions to their problem, a study of the disease was undertaken in the local pathosystem, with the objective of establishing an effective disease control programme.

The crucifer blackleg pathosystem examined is the sum of the interactions of the contributory biotic systems and the local environments, both natural and arising from farming practices. The thesis is therefore structured into chapters discussing hosts, the pathogen and the disease that results from their relationship. Further chapters deal with the systems interaction (epidemiology) and control strategies. Finally, a crucifer cropping pattern to minimize incidence of blackleg is proposed for the province of KwaZulu-Natal.

1.2 Crucifers in the World and in KwaZulu-Natal

Cabbage is delicious when shredded, drained to the last drop of water in which it was cooked, and then simmered gently in butter or lard with diced apples and spices - a wonderful accompaniment to all pork dishes, goose and game.

Robert Carrier, 1965

The family Cruciferae is a remarkable group of versatile, valuable and universally exploited plants. It contains the *Brassica oleracea* L. group of vegetables, the *B. napus* L. and *B. campestris L.* (synonym *B.rapa* L.) group of fodder crops and oilseeds, the mustards and radishes, and numerous weed genera. It constitutes a major taxonomic group in vegetable and winter fodder production, whilst canola (also called oilseed rape, particularly in older literature) contributed 22% to world oil crop production in 1994 (FAO, 1995).

1.2.1 The History and Origin of Cultivated Crucifers

Crucifers are some of the oldest cultivated plants known to man, cabbage having been cultivated for at least 3000 yr as a food crop (Swaider *et al.*, 1992). Theophrastis (372-286) wrote that three different kinds of cole crops were routinely eaten in his day. The Romans ate both cooked and raw cabbage, the latter as a cure for "hang-overs" (Nonnecke, 1992). Root (1980) had the following, florid comments to make:
"It is often difficult to determine the origin of man's oldest foods, but in the case of the cabbage, we are fortunate enough to have been provided with detailed documentation. According to the ancient Greeks, Lycurgus, king of the Thracian Edonians was caught by Dionysos in the act of pulling up grapevines. The god of wine tied him to a grape stalk to await punishment, in anticipation of which Lycurgus wept profusely, and with reason, for Dionysos, who was not known as "the raging god" for

nothing, had him first blinded and then torn limb from limb. Meanwhile, Lycurgus's tears falling to the ground, had engendered cabbages.

Modern botanists have for some reason been disinclined to accept this explanation and have sought clues elsewhere in the attempt to find out where the cabbage came from. We first hear it in the Mediterranean basin, but it is obviously not a plant of that warm region for it grows best in cool moist climates. It has been suggested that the cabbage originated in cool northern and central Europe, particularly along the coast, where it benefited from damp air, the area where it is most widely grown and consumed today, in the British Isles, Scandinavia, northern coastal and mountainous (hence cooler) France, central Europe from the Baltic to the southern slopes of the Alps, and the Slavic countries.

The probability that the original home of the cabbage was along the coasts of temperate northern Europe provides ammunition for those who maintain that the ancestor of the cabbage still exists in sea kale *(Crambe maritima)* which does indeed grow in a native state in this area. It resembles certain forms of loose-leaved cabbage and is popularly called "wild cabbage" by the French.

The theory that the cabbage originated in northern Europe is borne out by the direction in which it seems to have spread, apparently southward and eastward into the Mediterranean basin. If it had arrived through the Middle East, as some authorities have suggested, it should have been known to the Hebrews; but it is not mentioned in the Bible. This might also explain the fact that the ancient Greeks, farthest from its home base, accepted it with more reluctance than the Romans, one step closer. The Greeks ate a good deal of it, because it was filling and easy to come by, but they regarded it more as nourishment than a treat".

Cruciferous crops spread throughout Europe and the Middle East during the Middle Ages, and then with European trade and colonization of the world, became significant crops in all countries of the world (Nonnecke, 1992).

In KwaZulu-Natal, most crucifer production is of cabbages for the large rural population and especially the burgeoning populations of the urban and peri-urban areas of Durban and Pietermaritzburg. Substantial quantities are sold to the neighbouring countries of Lesotho and Swaziland.

Cabbage is the only green vegetable which does not lose its turgidity on storage without refrigeration. It is commonly transported great distances stored in *"cabbage bags"* (open-weave plastic bags). Apart from onions and potatoes, no other common vegetable can tolerate such treatment and still remain an edible, desirable food. Cabbage will probably continue to play a significant role in the diet of the majority of South Africans for the foreseeable future.

1.2.2 Nomenclature

The family name, *Cruciferae*, is derived from the simple cross formed by the four petals of all cruciferous flowers, the Latin meaning "cross-bearer". Within the family, there is a singular lack of clarity in the nomenclature of cultivated crucifers. First, there are commonly several names for the same crop. Second, it is common to use subspecies and, or, variety names to separate closely related crops of the same species. For example, two rape species are *B. napus* and *B. campestris*. These two species each have three subspecies, a root fodder, a foliar fodder and an oilseed. These are subdivided further into annual and biennial varieties, and then into cultivars. Discussing the taxonomy of canola, Holmes (1980) comments, "nomenclature is not fully standardized and there are many synonyms. The reasons for a rather confused situation are twofold. In part it arises from varying local terminology and mixed cropping and in part from progressive reassessment of the specific relationships involved; the *Brassica napus* varieties grown in India were formerly described as *Brassica campestris* and are so described in much of the recent literature".

Tables 1.2.2.A - 1.2.2.C list some of the various names of the common crucifer crops.

Table 1.2.2.A: A List of Common Cruciferous Vegetables and Salads

Latin Name		Common Name	Author
Armoracia rusticana		horseradish	Ware and McCollum, 1975
Armoracia laptifolia		"	Knott, 1957
Cochlearia armoracia		"	Crockett, 1972
Barbarea praecox		upland cress	Ware and McCollum, 1975
Barbarea verna		"	Ware and McCollum, 1975
Barbarea vulgaris		"	I.E.Smith, unpubl.
Brassica alba		white mustard	I.E.Smith, unpubl.
Brassica insularis			Mithen and Lewis, 1988
Brassica alboglabra		chinese kale	Plunknett and Beemer, 1981
Brassica campestris	N = 10	Turnip group	
Brassica campestris		Irdrape	Plunknett and Beemer, 1981
var *rapa*		turnip	Knott, 1957
var *chinensis*		bok choy, pak choi	Nonnecke, 1992
var *nipposinica*		Pak-choi	Bassett, 1986
var *oleifera*		turnip rape	Bassett, 1986
var *parachinensis*		Choy sum	Bassett, 1986
var *pekinensis*		Pe-tsai, chinese cabbage	Bassett, 1986, Nonnecke, 1992
var *rapifera*		turnip	Bassett, 1986
var *ruvo*		broccoli	I.E.Smith, unpubl.
var *trilocularis*		sarson	Bassett, 1986
Brassica carinata		Ethiopian mustard	Bassett, 1986
Brassica cernua		Chinese salad	Harlan, 1992
Brassica chinensis		Chinese cabbage (Pak Choi)	Ware and McCollum, 1975
subv *parachinensis*		flowering white cabbage	Plunknett and Beemer, 1981
		Choi Sum	I.E.Smith, unpubl.
var *rosularis*		flat cabbage	Plunknett and Beemer, 1981
Brassica caulorapa		kohlrabi	I.E.Smith, unpubl.
Brassica rapa		kohlrabi	Plunknett and Beemer, 1981
		turnip	Harlan, 1992
var *septiceps*		Seven Top turnip	Ware and McCollum, 1975
var *pekinensis*		Pe-tsai	De Wolf *et al.*, 1987
var *chinensis*		Pak-choi, Bok-choi	De Wolf *et al.*, 1987
var *perviridis*		tendergreen mustard	De Wolf *et al.*, 1987
var *rapifera*		turnip	De Wolf *et al.*, 1987
Brassica juncea		leaf/spinach mustard, sarson	Harlan, 1992
		mustard greens	De Wolf *et al.*, 1987
var *crispifolia*		curled mustard	Knott, 1957
Brassica carinata		mustard	Sjodin and Glimelius, 1988
Brassica napus	N = 19		
		rapeseed, canola	Harlan, 1992
var *rapifera*		turnip	Nonnecke, 1992
Brassica napus		rape fodder	De Wolf *et al.*, 1987
var *pabularia*		Hanover salad	De Wolf *et al.*, 1987
var *napobrassica*		rutubaga, swede	De Wolf *et al.*, 1987; Nonnecke, 1992
Brassica oleracae L.	N = 9	cole crops	
var *acephela laciniata* L.		curly kale, chou moullier	Nonnecke, 1992
		collards	
Brassica oleracae			
var *acephela plana*		smooth kale	Nonnecke, 1992
var *acephela millecapitata*		thousand-head kale	Nonnecke, 1992
var *acephela palmifolia*		tree kale	Nonnecke, 1992
var *acephela medullosa*		marrow-stem kale	Nonnecke, 1992
var *acephela*		ornamental cabbages	De Wolf *et al.* 1987
var *alboglabra*		Chinese broccoli	I.E.Smith, unpubl.
var *bullata*		Savoy cabbage	Hyam, 1982
var *botrytis* L.		cauliflower, broccoli	De Wolf *et al*, 1987
var *botrytis cauliflora*		cauliflower	Nonnecke, 1992

continued on next page

Table 1.2.2.A–*Continued*

Latin Name	Common Name	Author
Brassica oleracae		
var *botrytis cymosa*	calabrese, sprouting broccoli	Nonnecke, 1992,
	winter broccoli	Crockett, 1972
	spear cauliflower	Root, 1980
var *capitata*	cabbage	Brouk, 1975
var *capitata alba*	white cabbage	Nonnecke, 1992
var *capitata rubra*	red cabbage	Nonnecke, 1992
var *capitata sabauda*	Savoy cabbage	Nonnecke, 1992
var *gemmifera*	Brussels sprout	Brouk, 1975, Nonnecke, 1992
var *gongylodes*	kohlrabi	Ware and McCollum, 1975
var *gongylodes* L. *caulo-rapa* Pasq.		
var *italica*	sprouting broccoli	De Wolf *et al.*, 1987
var *ramosa*	marrow stem kale, chou moullier	Smith, 1956
var *sabellica*	kale	Crockett, 1972
var *viridis*	borecole, collard, kale	Ware and McCollum, 1975
Brassica parachinensis	Choi Sum	I.E.Smith, unpubl.
Brassica pekinensis	Chinese cabbage (Pe tsai)	Crockett, 1972
var *cephalata*	celery cabbage	Plunknett and Beemer, 1981
var *cylindrica*	celery cabbage	Plunknett and Beemer, 1981
var *chinensis*	Chinese white cabbage	Plunknett and Beemer, 1981
Lepidium meyenii	maca (Andes root vegetable)	Harlan, 1992

Table 1.2.2.B: A List of Common Cruciferous Salad Herbs

Latin Name	Common Name	Author
Brassica nigra (L.) W. Koch	black mustard	De Wolf *et al.*, 1987; Harlan, 1992
Capsella bursa-pastoralis (L.) Medic.	shepherd's purse	De Wolf *et al.*, 1987
Crambe maritima L.	seakale	Ware and McCollum, 1975
Crambe abyssinica	?	Mihail *et al.*, 1991
Eruca sativa	roquettte	I.E.Smith, unpubl.
Hesperis matronalis	sweet rocket	De Wolf *et al.*, 1987
Lepidium sativum	garden cress	Harlan, 1992
var *crispum*	curled cress	De Wolf *et al.*, 1987
Lepidium virginecum	pepper cress	I.E.Smith, unpubl.
Nasturtium officinale	watercress	De Wolf *et al.*, 1987
Rorippa nasturtium-aquaticum	watercress	I.E.Smith, unpubl.
Sinapsis alba	mustard	Crockett, 1972

Table 1.2.2.C: A List of Common Radishes

Latin Name	Common Name	Author
Raphanus	radish genus, (enlarged tap root)	Brouk, 1975
sativus L.	cultivated radishes	Brouk, 1975; Harlan, 1992
var *esculenta*	elongated edible radish	Brouk, 1975
var *longipinnatus*	Japanese or Chinese radish, Daikon	Brouk, 1975
var *radicula*	common red globe radish	Brouk, 1975
var *raphinistroides*	Chinese radish	Harlan, 1992
var *sativus*	" " " "	Crockett, 1972
R. caudatus	rat-tailed radish (pods eaten), India	Brouk, 1975
R. raphinistrum	wild radish	Henderson and Henderson, 1966

The genetic relationships between the various *Brassica* species has been established in terms of the chromosome pairs of each species, as shown in Fig. 1.2.2.A.

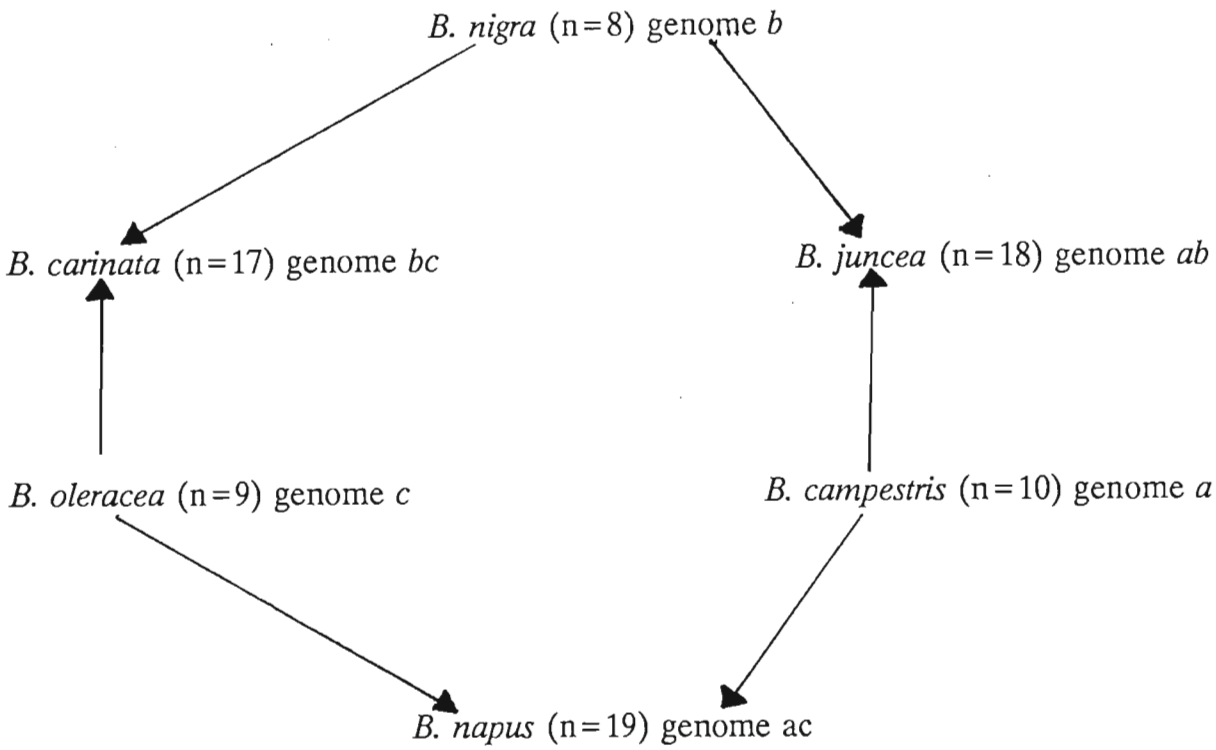

Fig. 1.2.2.A Relationship of *Brassica* Taxa Based on Chromosome Numbers

(after Dickson and Wallace, 1986)

Three major *Brassica* crops are grown in the developed world and have been studied extensively with regard to their susceptibility to crucifer blackleg: cabbage (*B. oleracea* var. *capitata*) and related vegetables such as cauliflower and broccoli, the Swede turnip (*B. campestris* var. *napobrassica*), and canola (*B. campestris* and *B. napus* var. *oleifera*). The fourth major *Brassica* crop, Chinese cabbage (*B. pekinensis)* is little mentioned in the Western literature in its relationship with blackleg, except that it is highly susceptible (Pound, 1946; Kenaga and Kiesling, 1957; Chupp and Sherf, 1960).

1.2.3 Crucifer Vegetables

1.2.3.1 International Perspective

Crucifer production is ubiquitous. Major production areas are Asia, Europe, the former USSR territories and North America (see Table 1.2.3.1.A for details).

Table 1.2.3.1.A: World Crucifer Vegetable Production in 1994

	Cabbage			Cauliflower		
Country	1000 Ha	YIELD kg/ha	1000 T	1000 Ha	YIELD kg/ha	1000 T
World	1713	23 496	40 250	606	17 956	10 888
Africa	33	25 373	841	9	17 645	160
N. & C. America	106	20 133	2 139	28	13 709	383
S. America	25	23 169	569	5	13 749	67
Asia	928	24 031	22 299	407	18 930	7 704
Europe	265	25 264	6 685	148	16 494	2 445
Oceania	4	32 640	127	5	20 980	107
China	419	23 520	9 850	88	25 708	2 265
India	200	16 500	3 300	270	17 778	4 800
Russian Fed.	180	26 000	4 680	3	5 000	15
USA	76	21 625	1 650	22	13 616	294
Ukraine	69	12 942	893	1	3 000	3
Japan	68	40 000	2 700	12	11 667	140
UK	20	31 331	633	26	15 674	414
Egypt	16	28 174	440	4	20 000	70
South Africa	6	33 788	216	1	25 017	30

Notes

1. Cabbage production as a percentage of world vegetable production ≈ 10%
2. Production values given reflect the quantities sold through formal marketing channels; they do not reflect the informal sector or home consumption. This results in underestimates of production in countries with a strong informal marketing sector.
3. The term "cabbage" is taken to include "red, white and savoy cabbages; Chinese cabbages, Brussels sprouts; green kale; and sprouting broccoli"
4. Cauliflower production as a percentage of world vegetable production ≈ 1.3%.
5. Cauliflower is taken to include heading broccoli.

(FAO, 1995).

The data presented in Table 1.2.3.1.A are abstracted from the FAO Production Yearbook 1995. The editors of the FAO Production Yearbooks comment in the NOTES ON THE TABLES, "In general, it appears that the estimates refer to crops grown in fields and market gardens for sale, thus excluding crops cultivated in kitchen gardens or small family gardens mainly for household consumption. In Austria, for example, reported data relate to field crops only and in Cuba they refer to procurement from state and private farms. Production from family and other small gardens not included in current statistical surveys and consequently not included in the tables of the Yearbook constitute quite an important part of the estimated total production in certain countries: for example in Austria, France and the Federal Republic of Germany, it constitutes about 40%, in Italy, almost 20% and in the United States, 10%".

For these reasons, regional, continental and world totals are far from representative of the total production of the various kinds of cruciferous vegetables cultivated.

1.2.3.2 Cabbage Intake in South Africa

Black people are the predominant community in South Africa (Fig. 1.2.3.2.A).

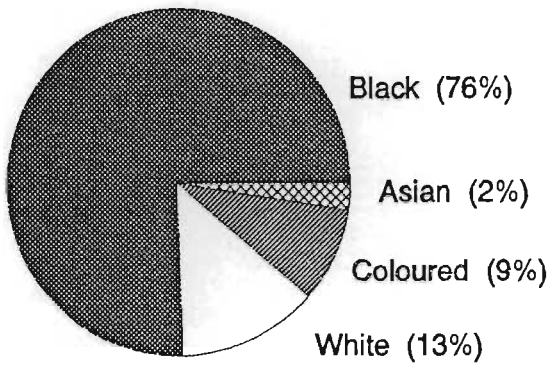

Fig. 1.2.3.2.A South Africa: Population Estimate, 1990
Central Statistical Services, Pretoria, 1992

Cabbage plays an extremely important role in their nutrition, being a staple food. As such, it is an important vegetable crop in South African agriculture, both in value and quantity produced annually (Fig. 1.2.3.2.B - 1.2.3.2.D).

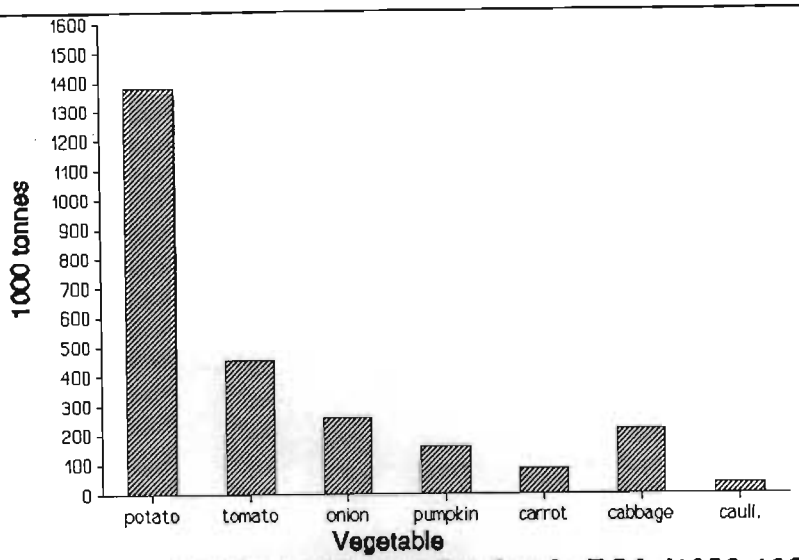

Fig. 1.2.3.2.B Vegetable Production in RSA (1989-1990) Central Statistical Services Estimates

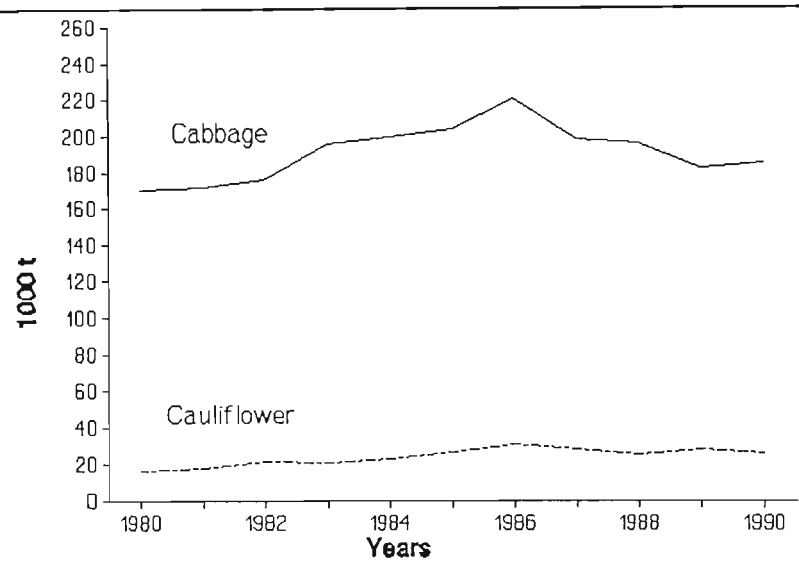

Fig. 1.2.3.2.D Quantities of Cruciferous Sold on National Municipal Markets

Cabbage is the staple vegetable in the diet of most black South Africans, in both the urban and rural populations, mainly because of its high nutritive value and because it keeps without refrigeration. Fernandes-Costa *et al.* (1984) commented on the diet of black residents of Cape Town, "the diet consists predominantly of maize (*Zea mays* L.), supplemented with small and irregular quantities of meat and vegetables, a diet which resembles that of the black peoples inhabiting the rural areas of Southern Africa".

supplemented with small and irregular quantities of meat and vegetables, a diet which resembles that of the black peoples inhabiting the rural areas of Southern Africa".

Bourne (pers. comm.) supplied unpublished data on the daily diet of urban blacks residing in Cape Town which is presented in Table 1.2.3.2.A. The figures arise from a survey conducted in 1990 on 1507 individuals.

Table 1.2.3.2.A: Daily Intake of Vegetables by 1507 Urban Blacks, Cape Town, 1990

Vegetables	Persons Eating Vegetables	Percentage
potato	580	38.5
cabbage	215	14.3
onion	118	7.8
tomato	117	7.8
carrot	113	7.5
pumpkin	86	5.7
beetroot	15	1.0
spinach	11	0.7
cauliflower	6	0.4
lettuce	6	0.4

Bourne (pers. comm.)

Bourne commented, "Certainly there is a general popularity amongst blacks of leafy vegetables or imfino, and cabbage is a favourite as it is comparatively inexpensive, stores well without refrigeration and goes further than other leafy vegetables when chopped. (This is because of its density and relatively little shrinkage during cooking.)" (Bourne, pers. comm.).

Data in earlier surveys suggest that in the past vegetables played a predominant role in the diet of rural blacks, and they remain a significant component in the diet of urban blacks today (Lubbe, 1971; Manning *et al.*, 1974; Vorster *et al.*, 1987).

Personal interviews with nine Zulu labourers suggested that three meals dominate black diets in KwaZulu-Natal:

1. a meat and cabbage stew eaten with "putu" (stiff boiled maize meal)
2. a meat and potato (*Solanum tuberosum* L.) stew also eaten with "putu"
3. "samp-and-bean" (coarsely crushed maize cooked together with dry beans (*Phaseolus vulgaris* L.).

Onions (*Allium cepa* L.) and tomatoes (*Lycopersicon esculentum* Mill.) may be added to the stews and chilies (*Capsicum annuum* L.) are sometimes used as a substitute for meat. When available, leaves of *Amaranthus* spp. (*mfino*) or pumpkin (*Cucurbita pepo* L.) shoots are collected to be used as substitutes for cabbage. Swiss chard (*Beta vulgaris* L.) is not well liked because of its high oxalic acid content and its poor storage life if stored without refrigeration. In contrast, half a cabbage would be used in a single meal for 2-3 people, and the other half used the next day. No other vegetable was viewed as a suitable substitute for cabbage, especially in winter.

The nutritive value of foodstuffs can be expressed using a Nutritional Index, calculated as follows:

$$\textbf{Nutritive Index} = \frac{\text{essential amino acid index} \times \text{\% protein}}{100}$$

The essential amino acid index is calculated as a ratio of essential amino acids in a foodstuff, relative to the requirements in an adult human diet (Royse and Schisler, 1980).

Table 1.2.3.2.B shows that cabbage has a nutritional index nearly as high as kidney beans and peanuts, and about double that of potatoes, tomatoes and carrots.

Table 1.2.3.2.B: Nutritive Value of a Variety of Foodstuffs, Including Cabbage

Foodstuff	Nutritional Index
Beef	45
Pork	35
Soybeans	31
Spinach	26
Milk	25
Mushroom (*Agaricus bisporus* (Lange) Singer)	22
Kidney beans	21
Groundnuts	20
Cabbage	**17**
Maize	11
Potato	9
Tomato	8
Carrot	8

After Eiker (1993)

1.2.3.3 The Economics of Cabbage Production in KwaZulu-Natal

After potatoes, cabbage is the second most important vegetable grown in KwaZulu-Natal, in terms of area farmed (Fotheringham, 1981). The volume and monetary value of the cabbage crop produced from this area (2696 ha) are moot points, and several yield figures have been calculated (Table 1.2.3.3.A).

Table 1.2.3.3.A: Yield Values of Cabbage for KwaZulu-Natal

Mean Yield (t ha^{-1})	**Rands** ha^{-1}	**Total Yield in KwaZulu-Natal** (on 2 696 ha)	**Total Value in KwaZulu-Natal** (on 2696 ha)	**Source**
34	8 334	91 664	R22 610 453	FAO, 1995
52.3	14 794	141 001	R33 884 624	Anon., 1995b
82.5	20 350	222 420	R54 863 600	Own calculation: Conservative
99.0	24 420	266 904	R65 836 320	Own calculation: High
130	32 064	350 480	R86 445 083	Smith, 1986a: maximum potential

Calculation Based on FAO Figure

Value of cabbage crop: 34 t ha^{-1} ÷ 30kg x R7.4 per bag x 2 696 ha = R22 610 453

Calculation Based on Anon 1995b Figure

Value of cabbage crop: 2 696 ha @ R14 794 ha^{-1} = R33 884 624

Own Calculation:

Conservative Estimate

at a spacing of 60 x 30 cm	= 55 000 cabbages ha^{-1}
75% harvest of cabbage heads	= 41 250 cabbages ha^{-1}
average weight of cabbage	= 2 kg (a minimum)
yield	= 82.5 t ha^{-1}
no. of 30 kg cabbage bags	= 2 750
@ R7.4 mean price per bag in 1995	= 20 350 rands ha^{-1}
total area under cabbage in KwaZulu-Natal	= 2 696 ha
total cabbage value	= 54 863 600

High Estimate

at a spacing of 60 x 30 cm	= 55 000 cabbages ha^{-1}
90% harvest of cabbage heads	= 49 500 cabbages ha^{-1}
average weight of cabbage	= 2 kg (a minimum)
yield	= 99 t ha^{-1}
no. of 30 kg cabbage bags	= 3 300
@ R7.40 mean price per bag in 1995	= 24 420 rands ha^{-1}
total area under cabbage in KwaZulu-Natal	= 2 696 ha
total cabbage value	= 65 836 320

Calculation of Maximum Potential Yield (Smith, 1986a):

130 t ha^{-1} ÷ 30kg = 4 333 bags @ R7.40 = R32 064 ha^{-1} x 2 696 ha = R86 440 000

The difficulty in establishing the exact value of the crop in KwaZulu-Natal is due to the predominant role informal marketing plays in the sale of the cabbage crop, and hence a lack of accurate statistics. 1995 municipal market statistics reflect that, at an average price of R220 t^{-1}, 15 000 t of cabbage were sold (Anon., 1995c). This constitutes only 11% of the total tonnage of the government estimate of 141 001 t, and 7% of a conservative personal estimate of 222 420 t.

The immediate incentives for a farmer to sell on the informal market are :

i) There are no transport or marketing costs involved;

ii) It is a cash transfer and therefore largely escapes taxation;

iii) There is no delay in cash flow, as opposed to an approximately 1 wk delay in payment coming from the municipal market;

iv) It is a direct sale, the price being set by agreement (not by a market agent).

v) Just under 50% of production costs are post-harvest marketing expenses (Anon. 1995b).

There has been dissatisfaction amongst vegetable farmers with the operation of local municipal markets for many years (Piccione, 1985; Anon., 1988; 1996b; Cordes, 1996). Many vegetable farmers believe that the marketing operation is run primarily to benefit the buyers, as the prices are set by market agents, with no genuine auction occurring. Farmers consider many market agents to be colluding with buyers to create artificially low prices, the market agent then receiving kick-backs from the buyers, an allegation which remains unproven. In contrast, on-farm sales are largely controlled by the cabbage farmer.

In the KwaZulu-Natal Midlands, the crop is grown throughout the year, with demand peaking in late winter and early spring when there are no alternative indigenous *mfino* (wild spinach) plants available to rural communities. Demand is extended during drought years.

1.2.4 Crucifer Vegetable Production Patterns in KwaZulu-Natal

The usual production pattern in KwaZulu-Natal in the 1970s and early 1980s was to develop carefully prepared and tended seedbeds in close proximity to the production lands (Fig. 1.2.4.A - B). Mature seedlings were then pulled from a heavily irrigated seedbed, packed into plastic or wooden boxes (Fig. 1.2.4.C) and carried to the production lands where they were hand-planted into prepared furrows. Some farmers used planters for direct seeding of crucifer crops. However, even when direct seeding was used, some transplanting was also done, using excess seedlings to fill gaps, or to plant alternate rows left unseeded.

Fig. 1.2.4.A
Production of cabbage seedlings in seedbeds, Tala Valley.

Fig. 1.2.4.B
Production of cabbage seedlings in seedbeds on the left. The empty field in centre is a harvested seedbed. Note proximity of the production land at the back.

Fig. 1.2.4.C Harvested cabbage seedbed transplants placed into a wooden crate.

In KwaZulu-Natal, Richards (1982) showed that the maximum tonnage is achieved with a planting rate of around 55 000 plants per ha, with a plant spacing of 600 x 300 mm. However, when cabbages are grown for the black community, which generally wants large cabbages of over 2.5 kg, a lower plant population is used, approximately 40 000 plants per ha, with a plant spacing of 500 x 500 mm.

The F1 hybrids used locally are harvested in 75-100 d in summer, and 100-140 d in winter. The heads are cut off the stalks with a machete and packed into plastic string bags for sale at a market, or tossed loose onto hawkers trucks, when sold on the farm. This harvesting method remains the standard process followed to this day. From the disease management point of view, it is important to note that this harvesting method leaves the stalk and roots in the soil, and that small plants are left unharvested. There are strong disincentives against pulling plants out of the ground before cutting their heads off:

a. it requires considerable physical effort to do so, especially if the ground is dry
b. snakes, including deadly puff adders, commonly curl around the base of cabbages (Fleming, pers, comm.).

These two factors result in considerable opposition by farm labourers to the task of clearing cabbage debris from cabbage lands.

Little or no sanitation, or rotation has ever been practised. Farm size militates against effective rotation:

90% of KwaZulu-Natal farms cover only 46.7% of the vegetable growing area;
10% of KwaZulu-Natal farms cover 53% of the vegetable growing area;
74% of KwaZulu-Natal's vegetable farms have less than 7 ha under cultivation (Fotheringham, 1981).

Furthermore, only 17% of agricultural land is arable (Anon., 1995a).

On smaller farms a decision to rotate demands a total cessation of cabbage production, a decision few farmers are prepared to take because there is no economic alternative to winter production of cabbages.

In 1975, the Speedling® system of seedling production was invented by Todd in the USA (Laffe and Koranski, 1985). In 1979, the system was introduced to South Africa and in the next decade developed exponentially, to become the dominant technology of seedling production (Smith, 1986b; Laing, 1988) (Fig. 1.2.4.D - E). The use of containerized seedlings, like the use of F1 hybrid seed, introduced a revolution in the field of vegetable production. The two seedling production systems, and their relative advantages and disadvantages are discussed in Section 1.2.4.

Fig. 1.2.4.D.
Open air production of containerized cabbage seedlings In South Africa, using the Speedling® system.

Fig. 1.2.4.E.
Production of containerized cabbage seedlings using the Speedling® system under 20% shadecloth.

Apart from the shift to containerized seedlings, no major changes have occurred in the systems used for cabbage production in KwaZulu-Natal in the last 15 yr. Even the cabbage cultivars have not significantly changed in the last decade. The two seed companies that dominate cabbage sales in South Africa, Mayfords Seeds and Starke-Ayres Seeds, are linked to the Japanese seed companies Taki and Sakata Seeds, respectively. In the last 15 yr, the two companies have introduced only about twelve new cultivars which have been successful. Some old cultivars, such as Gloria Osena, have now been used successfully for more than 20 yr in particular production niches.

Cabbage production in most of the USA is very different from that in South Africa in that the growing season is very short and therefore the seedlings are raised in the warm southern states such as Georgia (Jaworski *et al.*, 1982). Grown well before spring, the seedlings are trucked to the main production areas in the eastern and midwestern states to wait for the spring thaw after which they are promptly transplanted. The crop grows rapidly under the hot conditions (day temperatures reaching 40^0C in much of the Mid-West), and is harvested in time to plant a second crop before onset of winter (Williams, pers. comm.). In Europe, a similar pattern is followed, except that the seedlings are raised in commercial nurseries, heated seedbeds, or unheated coldframes (low brick seedbeds covered with glass to create a glasshouse effect) which allows seedlings to be produced in time for spring plantings. Crucifer vegetables may be produced in three ways: from direct-drilled plants (DDP), from seedbed transplants (SBT) or from container-grown seedlings (CGS). In South Africa, direct drilling of crucifers is relatively uncommon. The use of SBT was the norm in South Africa until the 1980s. However, in the 1980s the use of CGS became increasingly popular, and is the dominant technology today. Discussions with seed companies and seedling nurseries confirm that there are no large cabbage farmers still using seedbeds in KwaZulu-Natal today.

1.2.4.1 Direct-Drilled Plants (DDP)

Two farmers applying direct drilling of cabbage seeds in the 1980s in KwaZulu-Natal followed similar practices. The land was ploughed and fertilized and the seeds were drilled into the ground in rows using a tractor-drawn Stanhay® planter. After about 6-8 wk the land was heavily irrigated, excess seedlings were pulled out, and were transplanted into gaps in the rows of seedlings, a process called "blanking up" in KwaZulu-Natal. The process of blanking up with excess seedlings introduced elements of the process involved in seedbed transplants: wet seedlings are pulled out of the soil, stored in bulk and then transplanted, the same as seedbed transplants. The importance of these steps lies in the fact that these are believed to be the key steps in the transmission of *L. maculans* between seedlings, and severe blackleg epidemics have been recorded with this system of DDP followed by blanking up.

1.2.4.2 Seedbed Transplants (SBT)

The standard technique of crucifer planting in South Africa was based around the production of seedlings in seedbeds. When the seedlings were about 6 wk old, with an above-ground height of approximately 150 mm, the seedlings were pulled, transported to a production field, transplanted and grown to maturity. In KwaZulu-Natal, the process was as follows:

1. A land was chosen with established irrigation and good soils. In most cases, this meant that a production land was used for the seedbed and therefore, the chance for debris from prior crucifer crops being in the seedbed were high (Fig. 1.2.4.F). The result was frequent infection of seedbed plants by *L. maculans* Fig 1.2.4.G). In 15 yr observation of cabbage production in KwaZulu-Natal, I never saw or heard of a farmer fumigating his seedbed lands. The soil was ploughed and fertilized, and seed sown at the rate of approximately 600 kg ha^{-1}, in rows 150 mm apart. The seedbed was regularly irrigated, weeded by hand, insecticides applied for the control of aphids and caterpillars and in some cases, fungicides applied for the control of downy mildew (*Peronospora parasitica* Pers.

parasitica Pers. ex. Fr.). After 4-8 wk the seedlings grew to 100-300 mm in length, at which stage they were transplanted.

2. The seedbed was heavily irrigated before pulling of seedlings commenced, to ensure that some roots remained on the seedling after extraction from the seedbed. This was a key step in the epidemiology of blackleg and black rot pathosystems, as it left the pulled seedlings extremely wet. The pulled seedlings were then stored in a container. The containers used in KwaZulu-Natal ranged from plastic fertilizer bags, to wooden or polyethylene crates. Counts showed the numbers of seedlings in one farmer's wooden crates (dimensions: 400 x 275 x 200 mm) ranged from 380 to 450. Slightly more seedlings could be carried in the standard plastic crates used in KwaZulu-Natal (dimensions: 500 x 325 x 270 mm), and counts ranged from 550 to 660. The seedlings were then transported to the production field. This was another key step in the epidemiology of blackleg because a large number of wet seedlings remained packed tightly together for some time. Since the pycnidiospores of *L. maculans* are slime spores requiring wet conditions for release and infection this practice maximizes disease spread. Furthermore, the seedlings were highly stressed plants, which were fairly severely injured in the process of losing a substantial portion of their roots. Therefore, they would be particularly vulnerable to infection by blackleg (Alabouvette, 1970; Alabouvette *et al.*, 1974).

Fig. 1.2.4.F.
Cabbage debris in a cabbage seedbed.

Fig. 1.2.4.G.
Seedbed cabbage seedlings infected with *L. maculans*. Note the distinct purple lesion on the bottom left seedling's stem, from which the disease derives its common name.

3. The seedlings were then planted by hand into a ploughed, fertilized and herbicide-treated land. The planting process usually involved three people per row of seedlings. First, a tractor with chisels set on a toolbar drew furrows in the soil at the correct between-row spacings. Then a labourer with hand trowel or a hoe dug small pits in the row itself. Spacing within the row was usually based on a measuring stick carried by the digger. The second labourer carried a box or bag of seedlings, and dropped one into each planting hole. The third labourer followed behind and actually planted each seedling.
4. After transplanting, the seedlings were heavily irrigated for the first week and then usually given 25 mm wk^{-1} of rain or sprinkler irrigation.

The SBT process is described here in detail because the process is considered to be central to understanding the patterns of blackleg incidence in the field observed in many cases. Furthermore, it is a plant production system which has disappeared from commercial cabbage farming over the past decade, although it is still practised by many small-scale farmers.

1.2.4.3 Container-Grown Seedlings (CGS)

In 1979, a seed company with strong business links in the USA, Roode Lyon, first introduced the "Speedling" system of seedling production into South Africa. Invented and patented by Todd of Sun City, Florida, the Speedling® System uses polystyrene, "waffle" trays filled with artificial growing media for the production of containerized seedlings. It was soon realized that the system had advantages over seedbed production of seedlings. It is the seedling production system that now totally dominates all vegetable, ornamental and forestry seedling production in South Africa.

1.2.4.4 Relative Qualities of DDP, SBT AND CGS

The relative popularity of the three plant establishment technologies used today presents a different pattern for subsistence and commercial farmers.

Table 1.2.4.4: Use of Different Crucifer Plant Technologies in KwaZulu-Natal in 1995

Technology	Subsistence Farmer	Commercial Farmers
DDS	10%	none
SBT	60%	10%
CGS	30%	90%

(Askew, pers. comm.).

In the last 15 yr, most commercial farmers in KwaZulu-Natal have switched from SBT and DDP to using CGS because they have intuitively quantified the pros and cons of using CGS, a trend which has occurred worldwide. There are a number of reasons for the switch, several of which were identified by British researchers Hiron and Symonds (1985):

1. DDS and SBT technologies are both inefficient in their use of seed: about 1kg ha^{-1} of seed is used with DDP, 600 g ha^{-1} with SBT, and 200 g with CGS.
2. In the CGS system, seedling growth can be managed, using controlled fertilization and watering.
3. CGS are quicker and easier to transplant than SBT, particularly by mechanical planting because CGS are more even in size and shape.
4. Planting CGS results in more even crop establishment than either DDS or SBT, especially under adverse conditions, providing for greater crop uniformity and therefore shorter harvesting periods.
5. DDS require that the farmer irrigate large areas of land heavily during the initial growth stages; much of this water is wasted.
6. The use of CGS allows for the more efficient use of production lands for actual crop production.

Further issues are:

a. With DDS, the planting of seed directly into production lands exposes seedlings to stress at the initial, most vulnerable stage.

b. If pathogen-infected debris is left on the production land, then both the DDS and SBT system expose the plants to the pathogen for the maximum period of time. Final disease levels are a function of duration of pathogen exposure and inoculum levels. Use of CGS would reduce the period for disease development by about 6 wk. CGS are usually produced at sites isolated from production lands. Therefore, there is little risk of transmission of pathogens from production lands into CGS nurseries. This is a marked contrast to SBT, which tend to be produced in the middle of production lands.

c. Both DDS and SBT (as practised in KwaZulu-Natal) have a stage of pulling out wet seedlings and transplanting them. This is a critical step in the local blackleg pathosystem, avoided when CGS are used.

d. Damping off is a major problem with both DDS and SBT because damping-off organisms are commonly found in the soil. In contrast, CGS are usually produced in pathogen-free, composted bark and are therefore less likely to pick up soil-borne diseases such as *Pythium* spp. and *Rhizoctonia solani* Kühn via seed, irrigation water and dust (Stephens *et al.*, 1983; Laing, 1985).

In KwaZulu-Natal, CGS of a hybrid cabbage cultivar cost around R56.50 per thousand in 1996. In contrast, an estimate of the direct costs of SBT was R25.00 per thousand transplants, about half the price of plugs (Hillier, pers. comm.). The difference between R3 107 for CGS compared with R1 375 for SBT (55 000 plants ha^{-1}) is R1 732 ha^{-1}. The gross costs of producing and marketing a crop of cabbages is currently estimated at approximately R12 000 ha^{-1} when using CGS and R9 000 when using SBT (Stamp, pers. comm.). (An agricultural economist estimated cabbage production costs using CGS to be R13 209 (Anon. 1995b.)). Thus, with a planting rate of 55 000 ha^{-1}, the respective costs of CGS and SBT is estimated at 26% and 15% of overall production costs, a difference of 11%.

The uniform quality of CGS results in three important advantages over SBT and DDS operations:

1. The number of cutting operations required to remove all harvestable heads is lower: 1-3 with CGS, whereas with SBT, 5-9 cuts are common. With DDS, the number is even greater, 9-15 cutting operations being necessary (Smith, pers. comm.). Cutting operations also have management, transport and labour costs, and therefore, the fewer the cuts, the more profitable the crop. With fewer cuts, a CGS-planted field can be replanted sooner, and therefore there is a greater turnover per hectare per annum, and a lower opportunity cost to each crop.
2. Using CGS, the percentage of harvestable heads is usually in the region of 75-95%. In contrast, fields planted with SBT usually have counts of 50-75% harvestable heads.
3. When planted under hostile environmental conditions, percentage survival of seedlings is higher with CGS than SBT because CGS root plugs tend to have more roots at the time of transplanting, and these roots are undamaged by the transplanting process. In contrast, many roots are physically injured or lost when SBT are removed from seedbeds.

The different susceptibilities of SBT and CGS to crucifer blackleg were determined in a factorial trial, where the other factors examined were the form and quantity of inoculum of *L. maculans*. The trial and the results are discussed in Chapter 8.

A common problem with CGS is that farmers often take delivery of seedlings before their fields are fully prepared, or take delivery of more seedlings than they can transplant in 2-3 d. The CGS are then stored in boxes for days and even weeks. The result is that the CGS lose condition, becoming yellow and etiolated. Further, unusual seedling diseases such as *Botrytis* may develop under these conditions to cause severe losses. In contrast, plants in a seedbed can be left more or less until they are required.

severe losses. In contrast, plants in a seedbed can be left more or less until they are required.

The storage of CGS in cold rooms at low temperatures is an effective technique to solve this problem. The use of refrigerated trucks to move and store CGS is a development which will possibly become a common practice in the future (Hiron and Symonds, 1985; Smith, 1986b).

There are many hidden costs to SBT:

1. The yield from a field of cabbages is determined by the original quality of the seedlings, irrespective of subsequent management (Richards, 1982). A farmer should therefore spend considerable time and effort optimizing seedbed management. This management cost needs to be added to the overall cost of SBT.
2. The farmer should also use his best lands for the seedbeds, which renders these lands unavailable for crop production, an opportunity cost. Both these costs are absent when CGS are used: the farmer simply faxes or telephones his order to a specialist nursery and takes delivery on due date.
3. Another cost of SBT, which is difficult to put a value to, is risk. Seedbeds are regularly wiped out by hail or late frost. In contrast, most CGS nurseries have hailnetting, or are situated in non-hail and non-frost areas. Therefore, the risk of all CGS seedlings being destroyed by one event is unlikely. The major risk at a nursery is that the nurseryman will make a mistake with an input such as fertilizer levels or growing medium, and lose the crop that way. However, professional nurseries generally do not make catastrophic errors of these kinds. Further, losses to the farmer as a result a nurseryman's mishaps can be recovered from the nurseryman.

   In sum, the farmer's exposure to environmental catastrophes are shared with the seedling nursery.

4. Another identifiable risk is pathogen transfer from debris in seedbeds or from surrounding production lands.

However, a specialist CGS industry also carries risks:

1. Whilst blackleg appears to have almost disappeared in KwaZulu-Natal, other diseases such as downy mildew (*P. parasitica*) in crucifers (Brophy and Laing, 1992) and bacterial speck (*Pseudomonas syringae* pv. *tomato* (Okabe) Young *et al.*) and bacterial spot (*X. campestris* pv. *vesicatoria* (Doidge) Dye) in tomatoes (*Lycopersicon esculentum* Mill.) (Laing and Girdwood, 1991; Lowe and Laing, 1996) have become increasingly severe. This was predictable given that there is no break in time or space (Robinson, 1979) in the production of seedlings at commercial seedling nurseries.
2. The nurseries provide ideal environmental conditions for many pathogens, which readily spread from older seedlings to younger seedlings.
3. By concentrating the production of seedlings in the hands of a few, large commercial nurseries, extreme centralization of seedling supply has occurred. Thus, a single seedling supplier could supply many different farmers with infected seedlings and thereby initiate a widespread epidemic. In 1973, in the USA infected cabbage seedlots initiated seedbed infection of blackleg at low levels in southern seedling production areas. When the wet seedlings were uprooted and packed into crates for trucking to the production area an extensive, in transit spread of the fungus occurred, affecting most seedlings. The final result was a general epidemic, the worst yet recorded on cabbage in the USA (Williams, 1974; Williams pers. comm.; Gabrielson and Maguire, 1977). In 1984, bacterial canker (*Clavibacter michiganense* pv. *michiganense* (Smith) Davis *et. al.* caused severe epidemics in the midwestern USA and Canada in tomato crops (*L. esculentum*) as a direct result of contaminated seedlings (Chang *et al.*, 1991).

The epidemiologically significant point is that crop dispersion, and breaks in crop production in time and space within a pathosystem, produce an ecologically stable system and reduce the chances of widespread epidemics occurring. Centralization of any cropping procedure reduces crop dispersion and therefore increases the crop's vulnerability to disease. It is only justified if the potential gains outweigh the risks involved.

However, with pragmatic phytosanitary practices, it should be possible to reduce these risks substantially at the nursery level. Initially, the issues are to identify the important pathological problems, and to find effective solutions to them. Subsequently, it is a question of communication, of convincing the farmers, seedling producers and seed suppliers of the nature and magnitude of the pathological problems. The entire chain of supply has to be convinced to apply effective control measures at each stage. In particular, farmers need to demand clean seedlings, produced under phytosanitary conditions. However, this may be difficult to achieve with some diseases, such as tomato bacterial canker, and tomato speck and spot, which have latent phases which are extremely difficult to detect.

In just over a decade, there was an almost total change in the technology of commercial vegetable production, from the use of SBT to CGS, a minor agricultural revolution. From a plant pathological perspective, it resulted in a shift in the significance of various diseases, virtually eliminating crucifer blackleg in KwaZulu-Natal. However, there was a concurrent rise in the damage caused by other diseases with epidemiological competence in the environment of commercial CGS nurseries, such as downy mildew of crucifers.

1.2.5 Crucifer Oilseeds (Canola / Rapeseed / Oilseed Rape / Sarson)

Two canola species are widely grown, *Brassica napus* L. var. *oleifera* (Metzer) Sink and *B. campestris* L. *Brassica juncea* L. is also grown for its seed oil in India (sarson), but is considered a minor crop (Holmes, 1980). Both canola species are highly susceptible to blackleg and are usually considered together.

A confusing aspect of the literature is that many references are not clear as to the annual or biennial nature of the crop being studied. The crop grown in Europe is almost entirely of the biennial variety of either species. In the rest of the world, the quicker, annual varieties are used (Holmes, 1980). In Europe, the biennial varieties are direct-seeded in spring, grown to maturity in summer and vernalized over winter. In the subsequent spring, flowering occurs and the seed produced is harvested in the second summer. In Canada, the crop is grown as a short summer crop, often with irrigation, whereas the Australian crop is grown in winter to take advantage of mild, wet Mediterranean conditions that prevail in winter in the southern and western regions (Holmes, 1980). Since 1992 farmers in South Africa have begun to take advantage of a similar climate in the Cape, rotating annual canola crops with wheat and barley, as in Australia. An initial 300-500 ha were planted to canola in the 1992 winter season (Viljoen, 1992).

Cultivar trials have been conducted on canola varieties in South Africa, specifically looking for blackleg resistance (Laing and Aveling, 1995).

1.2.6 Crucifer Fodder Crops

B. napus var. *napobrassica*, the Swede turnip, is extensively grown in cold/temperate regions such as New Zealand as a winter fodder, the crop being grown over summer to reach maturity by autumn. The mature roots are either left in the field, or stacked into clamps or pits for winter storage.

Another crucifer, Japanese Radish (or daikon) (*R. sativus* var. *longipinnatus*), fulfils much the same role in the KwaZulu-Natal Midlands and is a vital winter fodder for a large population of beef and sheep. Other fodder crops such as turnips, swedes and kale are of minor significance in KwaZulu-Natal.

The potential role of cruciferous fodder crops as alternate hosts of *L. maculans* in the epidemiology of cabbage blackleg closely parallels that of weed hosts, as discussed in Section 1.2.7.3.

Table 1.2.6.A: Crucifer Fodder Crops Production in KwaZulu-Natal 1981

Crop	Area (ha)	No. of Farms
Japanese radish	14 312	1 374
Fodder roots	4 326	173
Kale	249	46
TOTAL	18 887	1 593

(Fotheringham, 1981)

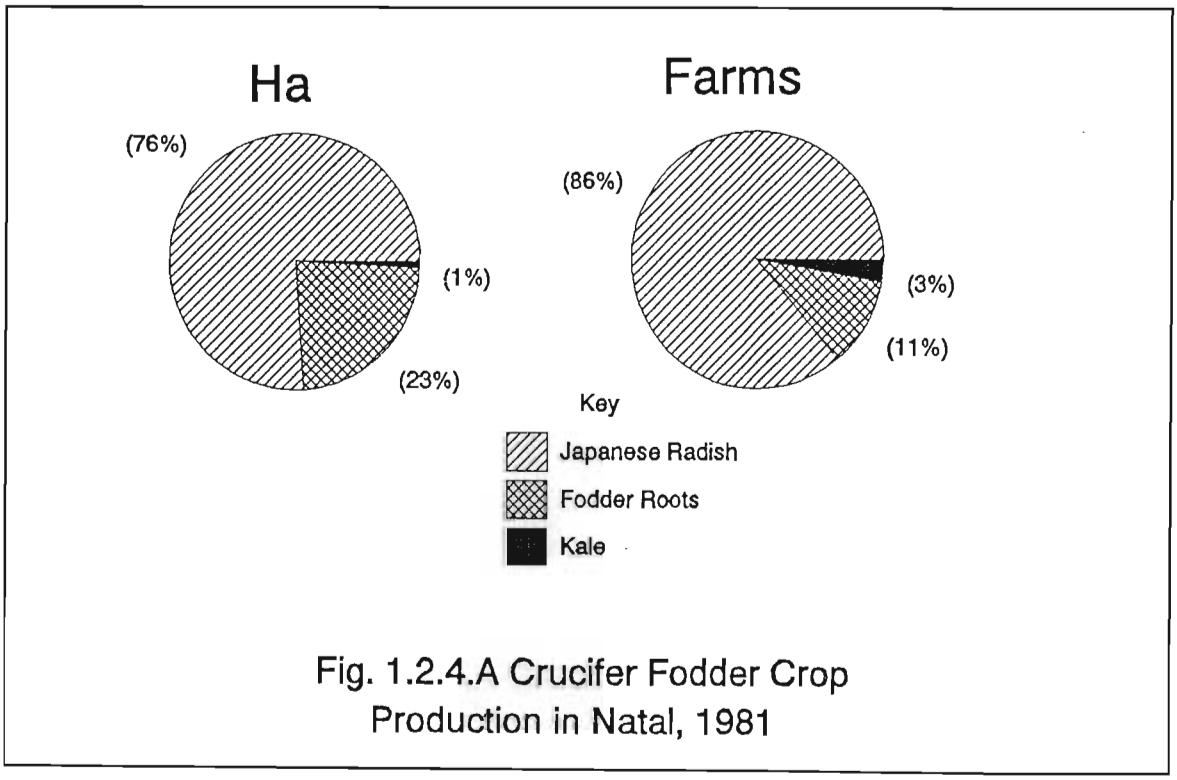

Fig. 1.2.4.A Crucifer Fodder Crop Production in Natal, 1981

1.2.7 Crucifer Weeds

1.2.7.1 Reported Weed Hosts of *Leptosphaeria maculans*

Numerous weed hosts of *L. maculans* are reported in the literature, as shown in Table 1.2.7.A.

Table 1.2.7.A: Reported Weed Hosts of *L. maculans*

Host	Common Name	Author
Allaria officinalis		Holm, 1957
Arabis albida		Gibbs and Brien, 1935
Brassica alba	white mustard	Henderson, 1918; Buddin, 1934; MacKay, 1956
B. arvensis	charlock	Henderson, 1918; Buddin, 1934; Gibbs and Brien, 1935; MacKay, 1956
B. campestris	wild turnip	Gibbs and Brien, 1935
B. hirta	mustard	Petrie and Vanterpool, 1965
B. juncea	Chinese mustard	Henderson, 1918
B. carinata	mustard	Sjodin and Glimelius, 1988
B. nigra	black mustard	Sjodin and Glimelius, 1988
B. napus	rape fodder	De Wolf *et al.*, 1987
B. kaber	wild mustard	Petrie, 1979
B. nigra	black mustard	Henderson, 1918
B. tournefortii	wild turnip	Barbetti, 1978
Camelina sativa	false flax	Henderson, 1918; Petrie and Vanterpool, 1965
Capsella bursa-pastoralis	shepherds' purse	Henderson, 1918; Gibbs and Brien, 1935; Dennis, 1939
Cheiranthus cheiri	wallflower	Gibbs and Brien, 1935; MacKay, 1956; Petrie and Vanterpool, 1965
Descurainia sophia		Petrie and Vanterpool, 1965
Diplotaxis muralis		Petrie and Vanterpool, 1965
Isatis tinctoris		Muller, 1953
Iberis umbellata		Snyder and Baker, 1950
Lepidium sativum	garden cress	Henderson, 1918; Petrie, 1975
Lobularia maritima	sweet alyssum	Henderson, 1918; Snyder and Baker, 1950
Mathiola incana	stock	Henderson, 1918; Gibbs and Brien, 1935
Neslia paniculata	ball mustard	Henderson, 1918
Raphanus raphinistrum	wild radish	Henderson, 1918; Snyder and Baker, 1950; Chupp and Sherf 1960; Barbetti, 1978
Thlaspi arvense	stinkweed	Petrie and Vanterpool, 1965; Petrie, 1975; McGee and Petrie, 1978; Gugel *et al.*, 1990

The largest genus affected is *Brassica*, and it is also the genus with the greatest susceptibility to *L. maculans*. Of the rest, it is important to realize that many of the earlier researchers used extremely artificial inoculation techniques, such as the injection of inoculum into the potential host with a hypodermic syringe (Henderson, 1918), which may have generated unrealistic data. Table 1.2.7.B lists the weed hosts observed to have been naturally infected by *L. maculans*.

Table 1.2.7.B: Weed Hosts Infected Naturally by *L. maculans*

Host	Common Name	Author
Brassica campestris	wild turnip	Gibbs and Brien, 1935
Camelina sativus	false flax	Petrie and Vanterpool, 1965
Capsella bursa-pastoralis	shepherd's purse	Petrie, 1995
Cheirathus cheiri	wallflower	MacKay, 1956; Petrie and Vanterpool, 1965
Descurainia sophia	flixweed	Petrie and Vanterpool, 1965; Petrie, 1995
Diplotaxis muralis	stinking diplotaxis	Petrie and Vanterpool, 1965
Draba nemorosa	yellow whitlow-grass	Petrie, 1995
Isatoris tinctoris		Muller, 1953
Iberis umbellata		Snyder and Baker, 1950
Lepidium sativus	garden cress	Petrie, 1975
Lobularia maritima	sweet alyssum	Snyder and Baker, 1950
Sinapsis arvensis	wild mustard	Petrie, 1995
Sisymbrium altissimum	tumbleweed	Muller, 1953; Petrie and Vanterpool, 1965; Petrie, 1995
S. loeslii	penny cress	Petrie and Vanterpool, 1965
S. officinale	hedge mustard	Muller, 1953; Petrie and Vanterpool, 1965
Raphanus raphanistrum	wild radish	Snyder and Baker, 1950; Barbetti, 1978
Thlaspi arvense	stinkweed	Petrie and Vanterpool, 1965; McGee and Petrie, 1978; Petrie, 1995

Wild turnip (*B. campestris*) and wild radish (*R. raphanistrum*) both appear to be relatively susceptible to *L. maculans*. In Australia, Barbetti (1978) considered *R. raphanistrum* to be a significant alternative host of *L. maculans*. In contrast, in New Zealand, Gibbs and Brien (1935) expressed the view that the wild turnip (*B. campestris*) played no role in the swede and turnip stem canker epidemics there. However, the same authors also considered the highly susceptible crucifers cabbage, cauliflower, chou moullier and foliar kale to be insignificant in the frequent canker outbreaks. Their contribution on this matter is therefore dubious. In contradiction of their opinions, another New Zealand pathologist, Smith (1956) established the conspecificity of *L. maculans* and *Phoma lingam* (Tode: Fr.) Desmaz. using infected chou moullier debris, one of the crops Gibbs and Brien (1935) said was not susceptible.

Of particular note are the findings of Petrie (1969), that five major biotypes of *L. maculans* existed in Saskatchewan, Canada:

i) "Brassica"
ii) "Sisymbrium"
iii) "Thlaspi"
iv) "Lepidium"
v) "Richardsonii".

Petrie (1975) differentiated the strains on the basis of their morphology and pathogenicity, with only the "Brassica" and "Sisymbrium" strains being highly pathogenic on canola. It is unclear whether mating occurs between these different biotypes.

Table 1.2.7.C: Cruciferous Weeds in South Africa

Host	Common Name	Author
Capsella bursa-pastoralis	shepherd's purse	Henderson and Henderson, 1966
Cardaria draba	hoary cardaria	Henderson and Henderson, 1966
Coronopsis didymus	carrot weed, wild carrot	Bromilow, 1995
Diplotaxis muralis	stinking diplotaxis	Henderson and Henderson, 1966
Diplotaxis virgata	mustard diplotaxis	Henderson and Henderson, 1966
Lepidium africanum	bird-seed, pepperweed	Henderson and Henderson, 1966
Lepidium bonariense	bird-seed, pepperweed	Henderson and Henderson, 1966
Raphanus raphanistrum	wild radish	Henderson and Henderson, 1966
Rapistrum rugosum	wild mustard	Bromilow, 1995
Sisymbrium capense	Cape wild mustard	Bromilow, 1995
Sisymbrium orientale	Indian hedge mustard	Bromilow, 1995
Sisymbrium thellungii syn *Brassica pachypodia*	wild mustard	Henderson and Henderson, 1966

Of the above cruciferous weeds, two species, *Diplotaxis muralis* and *Raphanus raphanistrum*, are recorded hosts of *L. maculans*. *Lepidium africanum*, *L. bonariense* and *Sisymbrium thellungii* may also be natural hosts to *L. maculans*. In KwaZulu-Natal, the *Lepidium* spp. *R. raphanistrum* and *Sisymbrium* spp. are widespread and common weeds, particularly in the cabbage-growing areas. *R. raphanistrum* is a particularly common weed in the Cape and is perhaps the most serious weed in winter wheat production areas of the Cape, exactly the area now being used for the production of canola.

1.2.7.2 The Effect of Herbicides on Selective Weed Survival

Herbicides are commonly used in cabbage production. At present local farmers have a choice of seven registered herbicides to use against annual grasses and broad-leafed weeds (Vermeulen *et al.*, 1991). However, Bromilow (1995) commented that cruciferous weeds are not susceptible to the acetanilide herbicides, "which are often used for broad-leafed weed control in vegetable crops". One of these, alachlor (Lasso 483 EC), has been observed to selectively kill non-cruciferous broad-leafed weeds. This creates a vacuum in the weed niche which only the cruciferous weeds are able to exploit, which they do, *R. raphanistrum* and *S. thellungii*, in particular. This removal

of weed competition may be epidemiologically significant; if the cruciferous weeds are alternate hosts of biotypes of *L. maculans* virulent on cabbage, then the role of cruciferous weeds in the disease cycle of *L. maculans* would be expanded. Incidence of blackleg would increase:

1. Weed hosts would become reservoirs of *L. maculans* during crop rotations with non-crucifers, either by providing a continuous population of living hosts, or in the provision of a seed population, which once infected, would provide a long term survival mechanism.
2. Alternative biotypes living in weed hosts may cross with *Brassica* biotypes to produce strains of *L. maculans* more virulent than the predominant *L. maculans* strains which are mildly virulent (Pound, 1946; 1947; Bonman *et al.*, 1981).
3. Weed sources can provide a large primary inoculum for either ascosporial or pycnidiosporial infection of a crucifer crop. In such a case, the epidemic would 'start' in the second cycle of a compound interest disease (*sensu* Vanderplank, 1963), giving a higher disease incidence at harvest.

1.2.7.3 The Epidemiological Role of Weed Hosts

The concept of ecological continuity is paramount to understanding the role of alternate hosts in pathosystems. The ecological status of a pathogen is dependent upon several factors, including:

1. Its various hosts' individual status in the particular pathosystem
2. The percentage of the total plant population made up by host plants
3. Its own pathogenic characteristics
4. Abiotic environmental factors, such as weather conditions.

Consider Case 1, a system with a single pathogen parasitic on a single annual crop. The pathogen's ecological status will oscillate in a series of "S" curves parallel to, but behind that of its host, in a characteristic predator / prey relationship (as in the standard Volterra-Lotka relationship (Lotka, 1925; Volterra, 1926; Cole, 1951; Charnov and Schaffer, 1973).

Consider Case 2, a system with a single pathogen and two hosts, an annual crop and a perennial weed, on which it is equally pathogenic and on which there are no strain effects. Again the pathogen's population growth curve will parallel that of the annual crop, in a series of "S" curves. However, the minimum status of the pathogen will be an asymptote, the level that is maintained on the weed host in the absence of the crop host. This minimum will be higher than that found in Case 1.

Epidemiologically, Case 1 provides a simple compound interest disease equation,

$$X_i = X_0 \cdot e^{r.t} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 1$$

where
X_i = final level of disease
X_0 = initial level of disease; i.e., initial inoculum
r = apparent infection rate
t = time between initial infection and final evaluation.

In this case, X_{0i} is effectively the minimum level of status oscillation of the pathogen, and is generated from survival mechanisms of the pathogen, such as mycelium in debris or seed-borne inoculum. During this survival period the pathogen is immobilized, its population status drops continually and it has little chance of adapting to changes in its environment; i.e., it is "out-of-touch". This explains why many fungi emerge from the survival state as the sexually reproductive form. This way, a maximum variability of propagules is immediately achieved. Therefore, if a change has occurred in the local environment, some of these variant propagules will probably be able to colonize fresh host material, which may or may not have changed from the previous cycle. The significant point is that there is a break in the activity level of the pathogen, a lack of ecological continuity and therefore a period of vulnerability.

Case 2 is a more stable pathosystem: the pathogen is always present in a live host, reproducing asexually, producing large quantities of infectious structures. It is, therefore, more adaptable, and if changes do occur in the cropping system, the pathogen will be maintained on the alternate host. Some of the progeny produced on the alternate host will be genetic variants, able to colonize new crop cultivars; i.e., the pathogen is constantly able to "test-the-water". Epidemiologically Case 2 may be represented by the equation:

$$X_{ii} = (X_0 + X_w).e^{r.t} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad 2$$

where X_w is the inoculum level maintained on the alternate host, the other factors being the same as in Equation 1.

Case 3 caters for "n" alternate hosts, either annual weeds or other annual crops, which provide a continuous source of host material for the pathogen to live on. This translates into the epidemiological equation:

$$X_{iii} = (X_0 + X_a + X_b - - - + X_n).e^{r.t} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad 3$$

where X_a, X_b, X_n are the inoculum levels maintained on the alternate hosts **a, b and n**, all other factors being the same as in Equation 1.

In the situation described by Case 1; initial inoculum will always be less than or equal to that found in the situation presented in Case 2, and this will be less than or equal to that occurring in the situation presented in Case 3; i.e.,

$$X_{oi} < X_{oii} << X_{oiii}$$

And hence, *ceteris paribus*,

$$X_i < X_{ii} << X_{iii}$$

However, the overall impact that alternate hosts, whether weed hosts or other crucifers, and the compounding effect which the use of selective herbicides will have on the blackleg pathosystem, may be masked by the other factors affecting the pathosystem (weather, farming patterns, etc.).

Another confounding factor is that there may be strain differentiation, and movement of strains from one plant onto another may be hindered by differential pathogenicity. Petrie (1969) and McGee and Petrie (1979) have shown this to be the case with some weed strains of *L. maculans*, which do not retain their pathogenicity for canola.

1.2.8 The Effect of Insects on Crucifers

Insects may play a role in transmission of *L. maculans* (Neill, 1929). Cottier (1930; 1932) found occasional transmission of *L. maculans* between infected and uninfected swedes by a staphylinid beetle (*Atheta pseudocoraria*) and a drosophilid fly (*Drosophila rubostriata*), both very common insects in New Zealand. However, the low rates of transmission of *L. maculans* reported suggested that this means of dissemination is relatively insignificant. Buddin (1934), working in England, found no evidence of *L. maculans* being transmitted by insects. This may reflect the different insect populations of the two countries, or simply that transmission levels are so low that Buddin was unable to detect their occurrence.

A number of insects are important parasites of cruciferous crops. One or more insecticides are registered for the control of American bollworm (*Heliothus armigera*), various aphid species, Bagrada bug (*Bagrada hilaris*), diamond-back moth (*Plutella xylostella*), greater cabbage moth (*Crocidolomia binotalis*) and twin spotted red spider mite (*Tetranychus cinnabarinus*) in South Africa (Nel *et al.* 1993). All of these insects are important pests of cabbage in KwaZulu-Natal (Bell, 1985). However, in this study the interactions of insects and *L. maculans* were not studied because most farmers apply insecticides to their crops routinely, and insect damage is therefore rare.

1.3 The Economic Impact of *Leptosphaeria maculans* in the World

Dry-rot disease is still a major field crop disease of swedes after 55 years of investigation.

Smith (1960)

1.3.1 Introduction

Crucifers are intensively cultivated all over the world. The major crucifer diseases such as blackleg, black rot and clubroot (the latter caused by *Plasmodiophora brassicae* Woronin) are ubiquitous pathogens and affect most of the members of the Cruciferae. Disease is therefore a perennial and widespread problem in crucifer production, occasionally characterized by disastrous epidemics. Most major crucifer production areas of the world have experienced severe blackleg and black rot epidemics and endure clubroot as an endemic problem.

In New Zealand at the turn of the century, the swede turnip used to be the major winter fodder. However successive blackleg epidemics caused regular losses of 50-100% and it ceased to be a profitable crop in many areas (Levy, 1919; 1920; 1922). The quote above from Smith (1960) suggests that blackleg continued to plague swede production in New Zealand, despite intensive research.

Canola has proved to be particularly vulnerable to *L. maculans*, and in Australia and France the disease has been the limiting factor to its production. In 1969, western Australia's canola production area was only 120 ha. This jumped to 49 200 ha in 1972, but crashed to 2 000 ha by 1974: successive epidemics in 1972 and 1973 had made it an unprofitable crop, with yields in 1972 reduced from an expected 1 000 kg ha^{-1} to 159 kg ha^{-1} (Barbetti, 1975; Bokor *et al.*, 1975). Resistant cultivars have since returned this crop to profitability (Roy, 1978). In France also, the popularity of canola as a rotation crop with wheat increased dramatically in the 1960s (94 000 ha in 1965 to

240 000 ha in 1969). However, it was also matched by widespread epidemics of blackleg, resulting in an overall drop in yield, from 2 190 kg ha^{-1} in 1964 to 1 300 kg ha^{-1} in 1966 (Alabouvette and Brunin, 1970). As in Australia, the planting of resistant cultivars has subsequently returned the crop to large areas of France (Anon., 1982).

Trials in South Africa show that several of the currently available canola cultivars are highly susceptible to *L. maculans*. However, totally resistant cultivars are available (Laing and Aveling, 1995).

In 1973, the USA lost more than 10% of its total cabbage production to blackleg (Gabrielson and Maguire, 1977). The value of cabbage produced in 1973 in the USA totalled US $124.1 million (Ware and McCollum, 1975), and the monetary loss to blackleg was in excess of US $13.8 million [1].

In KwaZulu-Natal, losses in cabbage production directly due to blackleg in the early 1980s have been estimated as follows:

> Cabbages were produced on some 2 696 ha p.a. (Fotheringham, 1981) and grossed an estimated R9-15 million p.a. (see details in Section 1.2.3.3). In the periods April 1980-81, 1981-82 and 1982-83, areas of cabbage production lost to blackleg in KwaZulu-Natal were estimated at 25 ha, 25 ha and 250 ha, respectively. These amounted to annual losses of R140 000, R140 000 and R1.5 million respectively; i.e., losses of 1%, 1% and 10%, based on 1981 market prices. The estimates were based on personal visits to affected farms. There may have been other cabbage farms in KwaZulu-Natal which were affected by blackleg but which were not visited, especially at isolated locations. The above is therefore a conservative estimate.

The simple monetary value of the losses, however, does not communicate the true significance of the crop losses to blackleg. Consider an overall crop loss in KwaZulu-

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[1] $124.1 mill. x (100 ÷ 90) x 0.10 = $13.8 mill.

The simple monetary value of the losses, however, does not communicate the true significance of the crop losses to blackleg. Consider an overall crop loss in KwaZulu-Natal of 10%, typically caused by an endemic disease such as *Rhizoctonia solani* Kühn. If this were spread over all farms, then these would operate at 90% efficiency; the difference in productivity would be compensated for by market pressures pushing the commodity price up. The net result would be that the farmers would earn the same returns for the crop. The situation is different with an epidemic disease, such as cabbage blackleg or black rot. If 10% of the cabbage farms, by area, were to lose their crop to blackleg, the cabbage price would rise, and by the same amount as in the first scenario, but the affected farmers would not benefit as they would have no crop to sell at the higher price.

Cabbage is a popular cash crop because it can be grown all year, and because the financial returns are relatively rapid. It is therefore used widely to maintain healthy cash flows, particularly for the payment of staff salaries. The loss of an entire cabbage crop can have serious financial implications for farmers. Indeed, in 1983 two KwaZulu-Natal farmers faced bankruptcy as a result of the loss of their cabbage crop to blackleg. In 1986, a cabbage farmer at Currie's Post, KwaZulu Natal went bankrupt as a result of total crop losses caused by hail, black rot and blackleg.

A further effect of the disease is to disrupt normal farming patterns. Cabbage fills a unique economic and agronomic niche in the KwaZulu-Natal Midlands farming environment; there is no economically viable alternative winter cash crop.

In the early 1980s, low incidences of blackleg were observed within many of the cabbage crops examined. Localized epidemic outbreaks were experienced in all production areas, and virulent biotypes were found to be present. Given that,

1. the years 1980-81, '81-82 and '82-83 were drought years in KwaZulu-Natal;
2. blackleg epidemics are most prevalent in warm, wet years (Walker, 1922);
3. inoculum was present in the production areas;

4. large areas were continuously under cabbage cultivation;
5. sanitation, rotation, seed treatment and any other disease control measures were not being applied;

then it was entirely feasible that in a subsequent season, with suitable climatic conditions, a *widespread* blackleg epidemic could have developed. This did not occur and a major part of this thesis is devoted to explaining why.

1.3.2 The Fungus

1.3.2.1 Taxonomy

Leptosphaeria maculans is distributed throughout the world, wherever cruciferous crops are grown, as can be expected with a fungus which is very successfully seed-borne. For details of its distribution and taxonomy, see the C.M.I. Descriptions of Pathogenic Fungi and Bacteria No. 331 (Punithalingam and Holliday, 1972), reproduced below with permission from CAB, Fig. 1.3.2.1.A-C.

Table 1.3.2.1.A: Taxonomy of *Leptosphaeria maculans*
(after Alexopoulos and Mims, 1979)

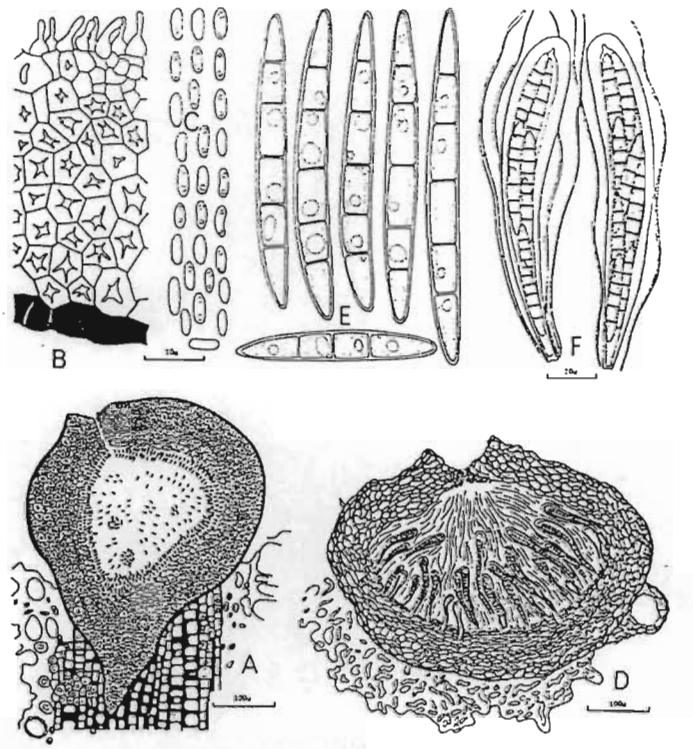
	PERFECT STAGE		IMPERFECT STAGE
kingdom :	Myceteae		Myceteae
division :	Amastigomycota		Amastigomycota
subdivision :	Ascomycotina		Deuteromycotina
class :	Ascomycetes	form class	Deuteromycetes
subclass :	Loculoascomycetidae	form subclass	Coelomycetidae
order :	Pleosporales	form order	Sphaeropsidales
family :	Pleosporaceae	form family	Sphaeropsidaceae
genus :	*Leptosphaeria*		*Phoma*
species :	*maculans*		*lingam*

Nomenclature of the Causal Organism of Crucifer Blackleg

Perfect Stage:	*Leptosphaeria maculans* (Desm.) Ces. et De Not. (1863)
Basinomen:	*Sphaeria maculans* Desm. (1846)
Synonyms:	five obsolete synonyms were listed by Boerema and Van Kesteren (1964).
Imperfect Stage:	*Phoma lingam* (Tode: Fr.) Desmaz. (1849)
Basinomen:	*Sphaeria lingam* Tode (1791)

CMI Descriptions of
Pathogenic Fungi and Bacteria
No. 331

LEPTOSPHAERIA MACULANS



A, Vertical section of pycnidium; B, part of pycnidial wall; C, conidia; D, v.s. of ascocarp; E, ascospores; F, asci and pseudoparaphyses.

Leptosphaeria maculans (Desm.) Ces. & de Not., *Comment. Soc. Crittogam. Ital.* 1 (4): 235, 1863
≡ *Sphaeria maculans* (Desm.) *Ann. Sci. nat.*, ser. 3, 6: 77, 1846
≡ *Pleospora maculans* (Desm.) Tul., 1863.

Conidial state: *Phoma lingam* (Tode ex Fr.) Desm., 1849
≡ *Sphaeria lingam* Tode, 1791
≡ *Sphaeria lingam* Tode ex Fr., 1823
= *Phyllosticta brassicae* Westend., 1851
= *Phoma brassicae* (Thüm.) Sacc., 1884
= *Phoma lingam* var. *napobrassicae* (Rostrup) Grove, 1935.

For full nomenclatural synonyms see Boerema & van Kesteren, 1964.

Ascocorps on stems and leaves, immersed, becoming erumpent, globose, black, with protruding ostioles, 300–500µ diam. *Asci* cylindrical to clavate, sessile or short stipitate, 8 spored, 80–125 × 15–22µ; ascus wall bitunicate. *Ascospores* biseriate, cylindrical to ellipsoidal, ends mostly rounded,

Fig. 1.3.2.1.A CMI Description of *Leptosphaeria maculans*

(Punithalingam and Holliday, 1972)

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yellow brown, slightly or not constricted at the central septum, guttulate, 35–70 × 5–8μ. Pseudoparaphyses filiform, hyaline and septate.

Pycnidia on stems and leaves, of two types. Type I (sclerotioid form) immersed, becoming erumpent, gregarious, variable in shape, convex, soon becoming depressed and concave sometimes without any definite shape, with narrow ostioles (or pores), 200–500μ across; wall composed of several layers of thick-walled cells (sclerenchymatous). Type II globose, black, 200–600μ diam., wall composed of several layers of cells, thick walled on the outermost layer. *Conidia* hyaline, shortly cylindrical, mostly straight, some curved, guttulate, with one guttule at each end of the conidium, unicellular, 3–5 × 1·5–2μ.

On *Brassica* spp.

DISEASE: Variously known as canker, dry rot and black leg, mainly of *Brassica oleracea*, *B. rapa* and *B. napobrassica*. Several Cruciferous genera are attacked. The first symptoms are seen on seedlings as pale lesions on the stem, cotyledons and first true leaves. These become greyish with the pycnidia developing in their centres. On older plants in the field lesions on the leaves and other above-ground parts often have purplish margins. The stem, root and bulb are attacked causing necrotic, girdling cankers and transverse splits; severe infection of stem or root leads to wilt or the plant toppling over. Pycnidia develop abundantly on all infected areas. The perfect state has been reported from Australia, Canada, England and the Republic of Ireland (RAM 44, 65; 45, 1568; 48, 51).

GEOGRAPHICAL DISTRIBUTION: Widespread but mostly in temperate regions (CMI Map 73, ed. 3, 1969). Records not yet mapped are: Australia (NT), Brazil, Costa Rica, Salvador and USSR (Ukraine).

PHYSIOLOGIC SPECIALIZATION: Variation has been reported, both in the symptoms caused and in growth characteristics *in vitro* but it is not clear of what practical importance this is (12: 481; 27: 305; 28: 609; 29: 282). The existence of strains may in part account for variations in the importance of the disease in vars. and spp. of *Brassica* in different geographical areas.

TRANSMISSION: The seed is invaded, dormant mycelium forming beneath the seed coat (11: 489; 19: 58). A recent survey in Denmark (48, 2641) showed seed of *B. oleracea* var. *capitata* to be most frequently infected and a longevity of 3 yr 8 months reported. From New Zealand a longevity of 14 months was found (39: 200). Seed treatment for control of conidial infection has led to the recognition that the disease may also be introduced by air-borne ascospores from host debris (42: 62; 49, 1822; 50, 2027). A persistence of 3 yr in soil organic matter can occur (9: 218; 29: 448).

NOTES: The pathogen is serious in cool climates and in the tropics only at high altitudes (31: 414). It can cause a rot in storage (39: 358). General accounts of the disease have been given for Australia (42: 712), England (13: 487; 24: 259), Netherlands (47, 1996), New Zealand (7: 70), Republic of Ireland (12: 481) and USA (7: 610). The host range, including wild hosts, has been studied (14: 546; 36: 225; 48, 51). With the production of disease-free seed, careful handling in the seedbed and rotation (3–4 yr) *L. maculans* is much less severe than formerly. The standard hot water treatment for seed (25–30 min at 50°C), although sometimes unreliable, has been frequently recommended (3: 74; 7: 758; 13: 488; 20: 239; 30: 133; 37: 428). A thiram soak has been found effective and complete control in seed was given by germisan (0·2%) for 5 min at 50°C (48, 2641). Treatment for 24 hr in 0·2% suspensions of benomyl and thiabendazole is also effective (51, 811). Little work on host resistance has been done except on *B. napobrassica* in New Zealand (34: 502).

LITERATURE: Boerema & van Kesteren, *Persoonia* 3: 20, 1964; Butler & Jones, *Plant Pathology*, 1949; Walker, *Diseases of vegetable crops*, 1952; Chupp & Sherf, *Vegetable diseases and their control*, 1960; Western (editor), *Diseases of crop plants*, 1971.

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Fig. 1.3.2.1.B CMI Description of *Leptosphaeria maculans (continued)* (Punithalingam and Holliday, 1972). Reproduced with permission.

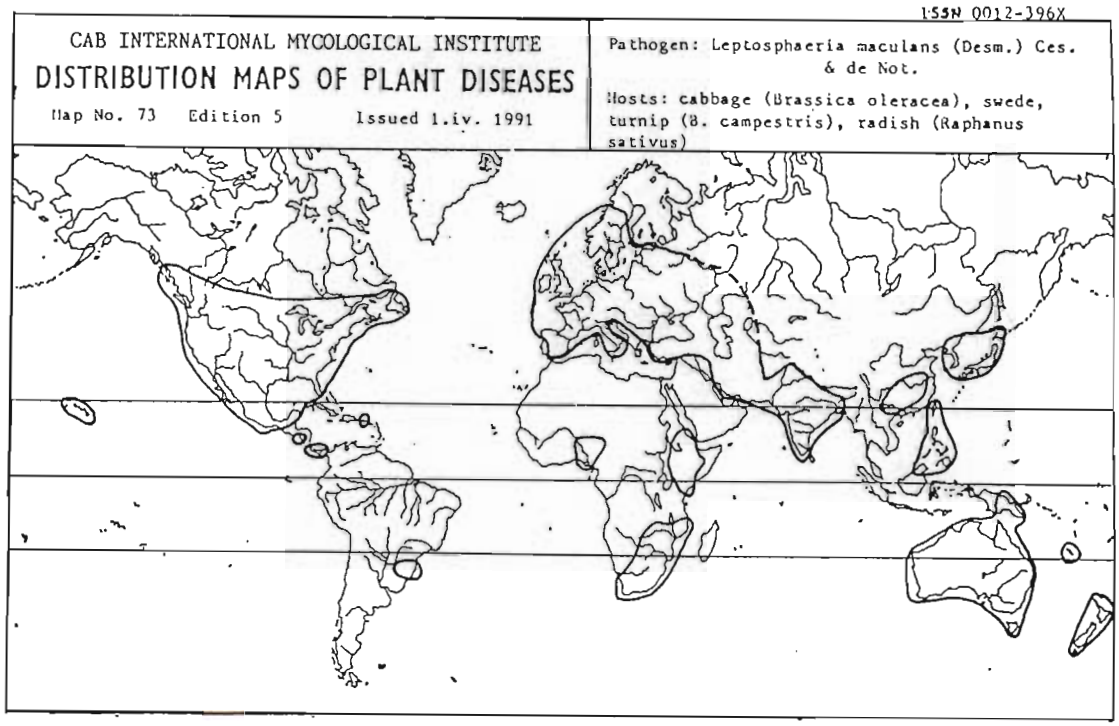

Fig. 1.3.2.1.C CMI Distribution Map for *Leptosphaeria maculans* (Punithalingam and Holliday, 1972). Reproduced with permission.

Synonyms: fourteen obsolete synonyms were listed by Boerema and Van Kesteren (1964).

Conspecificity was established by Smith (1956), and confirmed by Muller and Thomasevic (1957) and Smith and Sutton (1964). Interestingly, Tulasne and Tulasne (1863) first suggested the association of *L. maculans* and *P. lingam*, and Cunningham (1927) even drew the pseudothecia of *L. maculans*, but described them as resistant pycnidia of *P. lingam*.

Whilst the name of the perfect stage is the primary name of an Ascomycetous fungus, the name of the imperfect stage is also valid if used in reference to the conidial stage of its life cycle.

For a detailed discussion of the taxonomy and mycology of *L. maculans* see Boerema (1976); Boerema and Bollen (1975); Boerema and Van Kesteren (1964); Dennis (1946); Diedicke (1911); Grimes *et al.* (1932); Luttrell (1973); Muller (1950); Smith and Sutton (1964) and Ndimande (1976).

Table 1.3.2.1.B lists the three common names for the disease caused by *L. maculans*, namely, blackleg, canker and dry rot.

Table 1.3.2.1.B: Common Names of the Disease

Name	Crop
blackleg	*B. oleracea* vegetable crops canola (recent literature)
canker	*Brassica* crops with a swollen root canola (older literature)
dry rot	*Brassica* crops with a swollen root (archaic)

1.3.2.2 Environmental Requirements

Ascospore release is relatively amenable to study, using spore traps, and has been recorded in relation to rainfall, temperature and humidity. Lacoste *et al.* (1969) established that in France the optimum temperature for ascospore release was 15^0C, and that ascospore release occurred within an hour of rainfall and continued for 4-6 hr after the rain ceased. Their humidity parameters are of little value as they used unconverted % rH values (not adjusted for temperature and barometric pressures, parameters which affect rH); consequently, no conclusions can be drawn from their rH data.

They found that even at 100% rH, no ascospore release occurred unless free water was present. However, high humidities extended the release period following rainfall. Conversely, high humidity has been reported to be essential to successful germination of ascospores (Gladders and Musa, 1980). Other workers in France (Alabouvette and Brunin, 1970) and England (Maude and Humpherson-Jones, 1978) found similar environmental constraints affecting ascospore release. Alabouvette and Brunin (1970) also established that ascospore release only occurs in a limited temperature range of 10-20^0C, above or below which very few ascospores are released. In Australia, Bokor (1972) found ascospore discharge to be at a maximum between June and August, which coincided with the main Australian canola crop, which is sown in May. The mild, wet Mediterranean winters there are ideal for both the crop and the pathogen. Also in Australia, McGee (1974) found that ascospore discharges started in April and continued through till August when they tailed off. Thus the early April crop received the maximum dose of ascospores. He also established that:

1. Ascospore discharges occurred after precipitation, large discharges occurring after >1 mm of rain, and minor discharges occurring in the presence of dew;
2. Spore discharge occurred day and night;
3. Optimum temperature for spore discharge was 8-12^0C, which was significantly lower than that reported for the French isolates.

The canola debris he studied decreased in mass by 90% p.a., a finding similar to that made by made by MacNish (1979). However, discharges of ascospores continued throughout the 3 yr period of study.

In Canada, McGee and Petrie (1979) found a summer-to-autumn discharge pattern of ascospores from canola debris which was totally different to the winter-to-summer discharge pattern of the *Thlaspi arvense* strain of *L. maculans*. The weed strain was fortunately not pathogenic on canola or it would have provided a continuous source of *L. maculans* inoculum (as discussed in Section 1.2.7.3, Case 2). The ascospore discharge temperature for both strains occurred in the range 12-18^{0}C.

Petrie (1995) found ascospore discharges from infected canola debris to be positively correlated with the number of days with measurable rainfall in April-June. They were also negatively correlated with the number of days in April-July with a temperature of 30^{0}C or more.

The distance travelled by *L. maculans* ascospores has not been established definitively, but Bokor *et al.* (1975) found that a 5-8 km separation between fields eliminated detectable interplot interference. A single source of ascospores could therefore infect 7 854 - 20 106 ha. The effective dispersal of ascospores, combined with their high infectivity (Brunin and Lacoste, 1970), result in the high epidemiological competence of the perfect stage of this pathogen.

The subsequent infectious process, the latent period in particular, is very variable, depending primarily on the temperature. Brunin and Lacoste (1970) found the minimum incubation period to be 12 d on leaves at a 12^{0}C-18^{0}C regimen (rain on 9 of the 12 d), and 16 d on stems at an 11.5^{0}C - 17.3^{0}C regimen (rain on 11 of the 16 d). Their longest observed incubation period was 34 d, ascribed to low temperatures (4.7^{0}C - 11^{0}C) and few rainy days (9 of 34 d). Gladders and Musa (1980) found the incubation period of *L. maculans* ascospores to be 4 d at 20^{0}C under continuous light, but 42 d under winter conditions of 3^{0}C and 7 hr light per day. Nathaniels and Taylor (1983) discovered a symptomless stage in the growth of *L. maculans* on canola when

plants were kept at low temperatures (<5.3^{0}C). Such plants rapidly developed typical cankers when moved into a 18-20^{0}C environment. This latent phase of the fungus is of major epidemiological significance in Europe, where heavy autumn infection of canola will only be detected in the following spring and summer.

McGee (1977) found a positive correlation between stem infection and temperature (8^{0}C, 12^{0}C and 15^{0}C), but a negative correlation (r = -0.90) between cotyledonary infection and temperature. Analysis of his data also shows a negative correlation coefficient between cotyledonary and stem infections by *L. maculans*.

Following initial infection, *L. maculans* ramifies deeply into host tissue (Nathaniels and Taylor, 1983) and starts to produce pycnidia from which pycnidiospores are extruded in a gelatinous, pink/mauve filament. *L. maculans* produces pycnidia readily in culture, the fungal mycelium growing over a wide range of temperatures (4^{0}C-30^{0}C) with an optimum of 25^{0}C (Limasset, 1955).

The epidemiological significance of pycnidiospores in the development of an epidemic is a moot point. Firstly, all pre-1956 work on the significance of pycnidiospores is suspect because the researchers were unaware of the perfect stage of the fungus. Secondly, it is very difficult to study the role of pycnidiospores without the presence of ascospores (Alabouvette, 1970). However, existing research literature does give some information on the issue. Bokor *et al.* (1975), working on canola in Australia, considered pycnidiospores to be the main cause of secondary disease spread and the infection of canola stems. They suggested that pycnidiospores were washed down onto the stems from foliar lesions initiated by ascospores.

Alabouvette *et al.* (1974) also studied the role of *L. maculans* pycnidiospores in blackleg epidemics of canola, but in France. Working in the laboratory and the field, they used various inoculation techniques including aerosols of pycnidiospores. They were able to initiate blackleg epidemics at various temperature regimes (18^{0}C-26^{0}C, 10^{0}C and 4^{0}C-9^{0}C). Symptoms appeared sooner at higher temperatures and more

frequently on wounded than unwounded plants, although more than 50% successful inoculation of unwounded plants occurred with a pycnidiospore aerosol.

In contrast, McGee (1977) trapped no pycnidiospores of *L. maculans* with a Burkhard spore trap, despite the presence of many pycnidia in infected canola debris in the field being spore-trapped. However, this may only be a reflection on the efficiency of the Burkhard spore trap in catching waterborne pycnidiospores, as opposed to airborne ascospores which it catches well.

Barbetti (1976) found the maximum distance of pycnidiosporial disease spread by splash in canola to be 1.5 m, which is very close to the spread distance of *Phoma. exigua* Desm. var. *foveata* (Foister) Boerema of 1.4 m (Logan, 1976). The distance of 1.5 m is greater than between-plant and between-row distances commonly used in crucifer planting, and therefore would give rise to limited secondary disease spread by splash. McGee (1977), measuring disease spread in canola, found *L. maculans* to spread similar distances, even though both ascospores and pycnidiospores were present. An interpretation of this result is that the primary epidemiological role of ascospores is disease dissemination between crops, the exodemic, whereas the primary epidemiological role of pycnidiospores is disease spread within crops, the esodemic.

Barbetti (1975) found the incubation period of pycnidiospores to be around 14 d. He also found that disease spread due to pycnidiospore infection is a function of both rainfall volume and number of wet days/week. Another factor affecting secondary disease spread of *L. maculans* is the seedling maturity effect observed by Brunin and Lacoste (1970) and Barbetti (1976). Barbetti (1976) showed that 3 wk old seedlings were significantly more susceptible to pycnidiospore infection than 5 and 8 wk old seedlings.

For much of the year the ambient temperature in the KwaZulu-Natal Midlands is higher than the 8-12^{0}C maximum (for spore discharge) determined by McGee (1977) for Australian biotypes. However, the temperature range for much of autumn, winter and spring is similar to the range of temperature (10-20^{0}C) in which *L. maculans* will sporulate in France (Alabouvette and Brunin, 1970). Further, the above-mentioned differences in optimal and cardinal temperatures for the fungus in different parts of the world indicate that there are various biotypes of *L. maculans*, including sub-tropical biotypes, adapted to exploit their local environment. The seaboard of the Western Cape has a temperate Mediterranean climate very similar to that of western Australia where blackleg has devastated canola crops. A blackleg epidemic is reported to have occurred in canola crops there in the 1995/96 summer crop (Aveling, pers. comm.), some 3 yr after the crop started to be grown on a commercial scale.

1.3.2.3 Polymorphism of *L. maculans*

As early as 1849 Desmazière noted that *L. maculans* was highly polymorphic. Cunningham (1927) and Pound (1946; 1947) subsequently established cultural and pathogenic criteria for the separation of isolates. Workers in the USA (Bonman *et al.*, 1981; Koch *et al.*, 1991; Mengistu *et al.*, 1991), Australia (Thurling and Venn, 1977; Cargeeg and Thurling, 1980a; 1980b; Ballinger *et al.*, 1991), Germany (Koch *et al.*, 1989, 1991), France (Somda *et al.*, 1995) and the U.K. (Newman, 1981; 1984a; 1984b; Hammond and Lewis, 1987) have shown that there are numerous pathotypes of *L. maculans*. With the advent of molecular techniques such as RFLP and PCR, many papers have been published on this subject (Rouxel *et al.*, 1994; 1995a; 1995b; Sippell and Hall, 1995). Rouxel *et al.* (1994) reviewed the issue of how many different pathogens actually cause blackleg in crucifers. Using morphological, cultural and pathogenic characteristics, early blackleg researchers (Cunningham, 1927; Petrie, 1969; Pound, 1946; 1947) separated blackleg isolates into three primary groups: (a) a wea kly pathogenic group; (b) a highly pathogenic group; and (c) a heterogenous group pathogenic on cruciferous weeds in Canada. Recent isozymic, molecular and genetic analyses have shown that the separation of the strongly pathogenic group and the weakly pathogenic group is correct, and that they can be considered two distinct "species". The variation within these two "species" has been shown to be substantial.

Further, that isolates from different geographic sources may differ considerably in morphological, pathogenic and genetic make-up (Rouxel *et al.*, 1994). More work will need to be done in this research sphere to provide clarity to the genetic relationships between isolates of *L. maculans* pathogenic on *Brassica* crops, and *Brassica* weeds. To achieve these goals, Séguin-Swartz (Agriculture Canada, Saskatoon, Canada) established the International Blackleg Network (IBN) in the 1990's to facilitate international communication between blackleg researchers, and to facilitate movement of isolates of *L. maculans* internationally. Using this network, South African isolates of *L. maculans* are being analysed using a full range of molecular techniques, to establish their relatedness to isolates from other countries.

1.3.3 The Disease

Crucifers have three morphologically distinct parts that are regularly infected by *L. maculans*, the leaves, the stem, and the siliques. Foliar lesions are usually circular, approx. 2 cm in diameter, with distinct, non-chlorotic margins. The centre of the lesion is usually a pale fawn colour, spotted with relatively few, distinct, black pycnidia (Fig. 1.3.3.A). Ringspot foliar lesions (caused by *Mycosphaerella brassicicola* (Duby) Lindau) are similar but have many, small pycnidia and usually have chlorotic halos. Alternaria lesions (caused by either *Alternaria brassicicola* (Schw.) Wiltshire or *A. brassicae* (Berk.) Sacc.) are also similar in size and shape, but a layer of conidia and conidiophores creates a smoothly dark centre. When wet, a clear, ruby red cirrhus is extruded from mature pycnidia of *L. maculans* (Fig. 1.3.3.B). An unusual foliar symptom can develop when rapid progress of the fungus down a vein or petiole results in a large lesion affecting most of the leaf (Fig. 1.3.3.C).

Crucifer siliques are directly infested by ascospores or pycnidiospores, resulting in localized lesions usually confined to the stigmatic end. Infected siliques show internal blackening of the locular walls and/or the suture (Bonman and Gabrielson, 1981). The external symptoms are usually small, black lesions, 1-2 mm in diameter. Pycnidia of *L. maculans* are commonly present on infected siliques but are not readily observed (Bonman and Gabrielson, 1981).

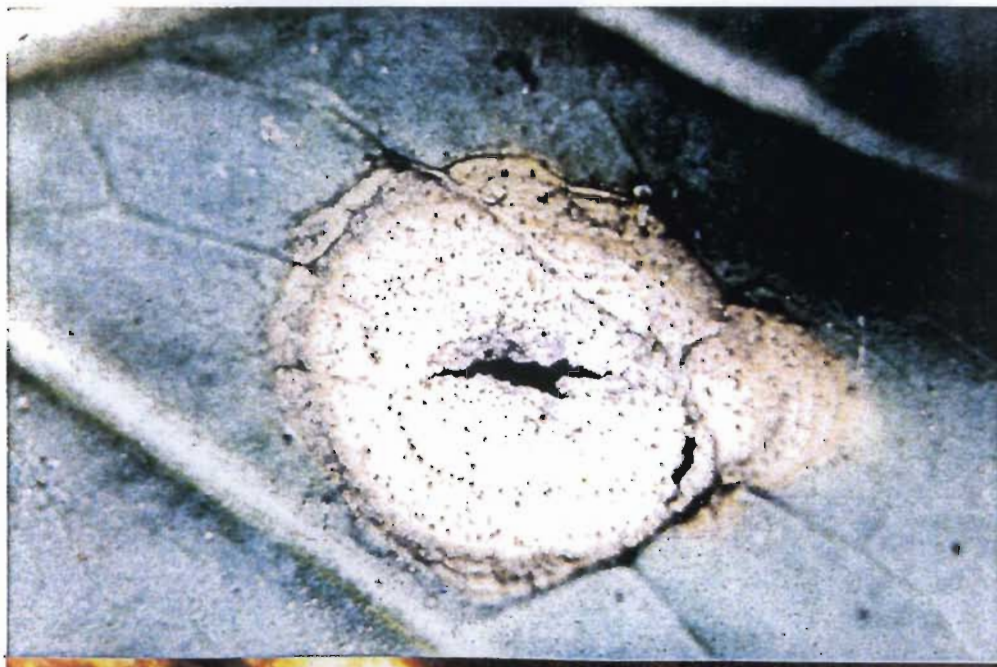

Fig. 1.3.3.A
Foliar lesion of cabbage caused by *L. maculans*. Note the relatively few, distinct, pycnidia.

x 20

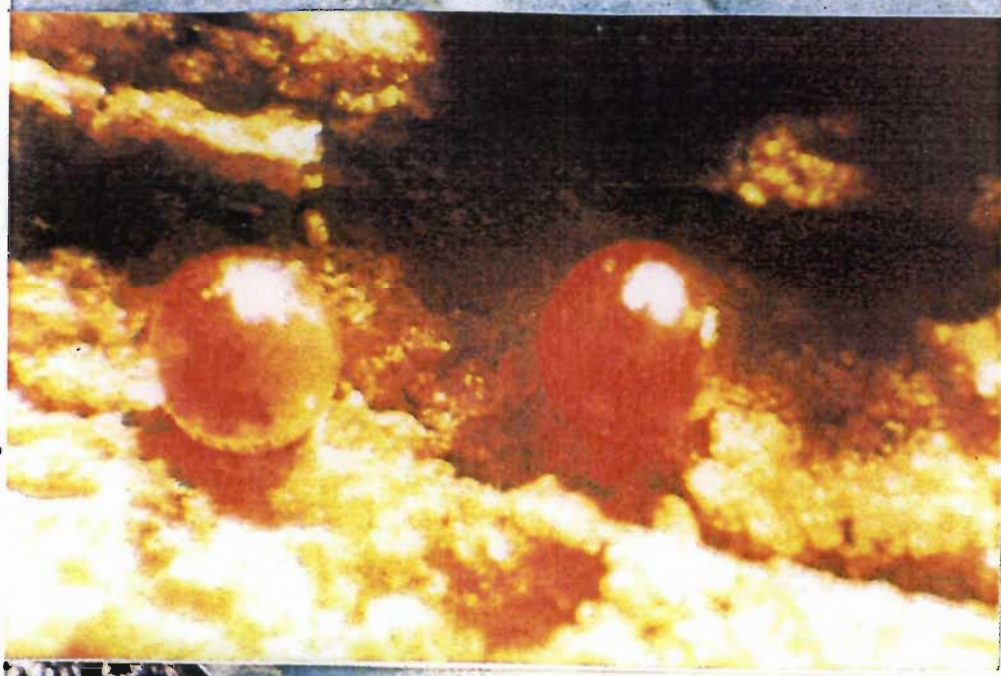

Fig. 1.3.3.B
A clear, ruby red cirrhus is extruded from mature pycnidia when they absorb water.

x 80

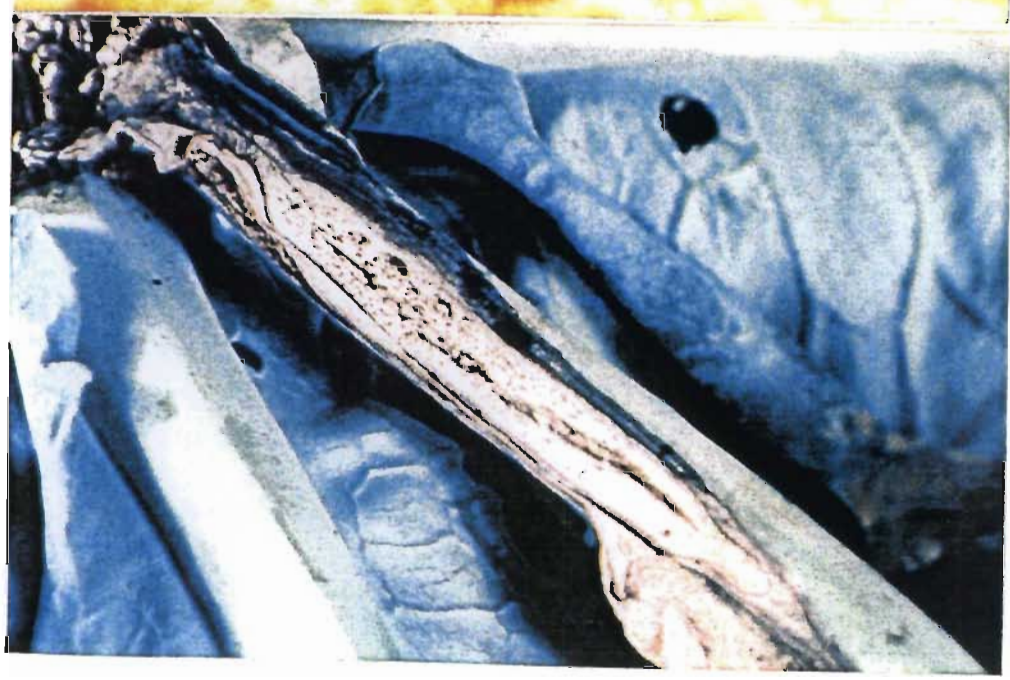

Fig. 1.3.3.C
A severe foliar lesion of cabbage caused by *L. maculans*. Note the pycnidia.

Symptoms of stem lesions on mature, live plants are initially a blackening of the affected spot, followed by a woodiness developing in this region, and a dark purple/black collar delineating the perimeter of lignification (Fig. 1.3.3.D). The lesion usually develops in a circular or elliptical manner, 20-30 mm diameter, the center region having a khaki colour with a superficial sheen (Fig. 1.3.3.E). The infected area may then crack open as further lignification occurs (Fig. 1.3.3.F) and black and brown pycnidia liberally pepper the area, between the cellulosic fibres. The lesion may engulf the entire stem, in which case plant death occurs rapidly, usually preceded by wilting (Fig. 1.3.3.G - H) and occasionally by the collection of red and purple anthocyanins in the leaves. In many cases, the presence of a blackleg lesion is not immediately fatal, and the lesion reduces the vascular transport system of the affected plant (Fig. 1.3.3.I). Such a plant may survive to head, often flowering prematurely, but not developing a saleable head. Characteristically, a plant killed by blackleg stem infection has a woody, shortened tap root with no lateral roots. The lignification of the stem must result in a reduced flow of photosynthates reaching the roots through the constricted vascular bundles, causing lateral roots die. These are removed by saprophytes, leaving only the tap root.

It is only on dead stem tissues that pseudothecia of *L. maculans* develop, along with pycnidia, the fungus mixing its survival options by producing both pycnidiospores and ascospores on weathered stem debris (Chapter 2).

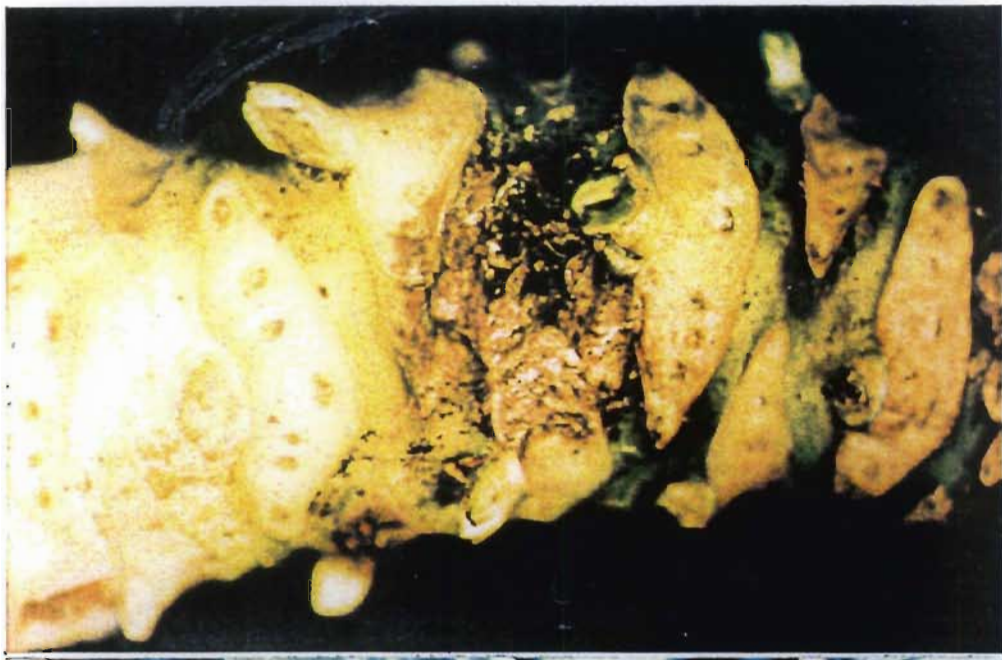

Fig. 1.3.3.D
A stem lesion of cabbage caused by *L. maculans*. Note the purple/black colour of the lesion.

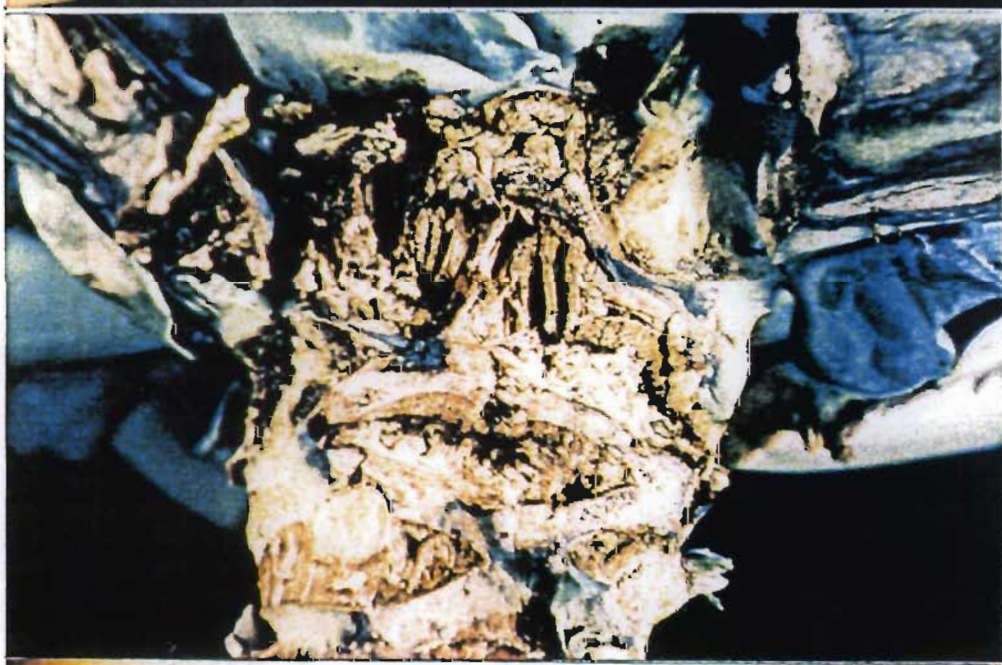

Fig. 1.3.3.E
A stem lesion of cabbage caused by *L. maculans*. Cracking of the lesion follows lignification.

Fig. 1.3.3.F
Pycnidia on a cabbage stem lesion caused by *L. maculans*.

Fig. 1.3.3.G
Wilting and death of cabbage caused by *L. maculans*.

Fig. 1.3.3.H
Wilting and death of cabbage caused by *L. maculans*.

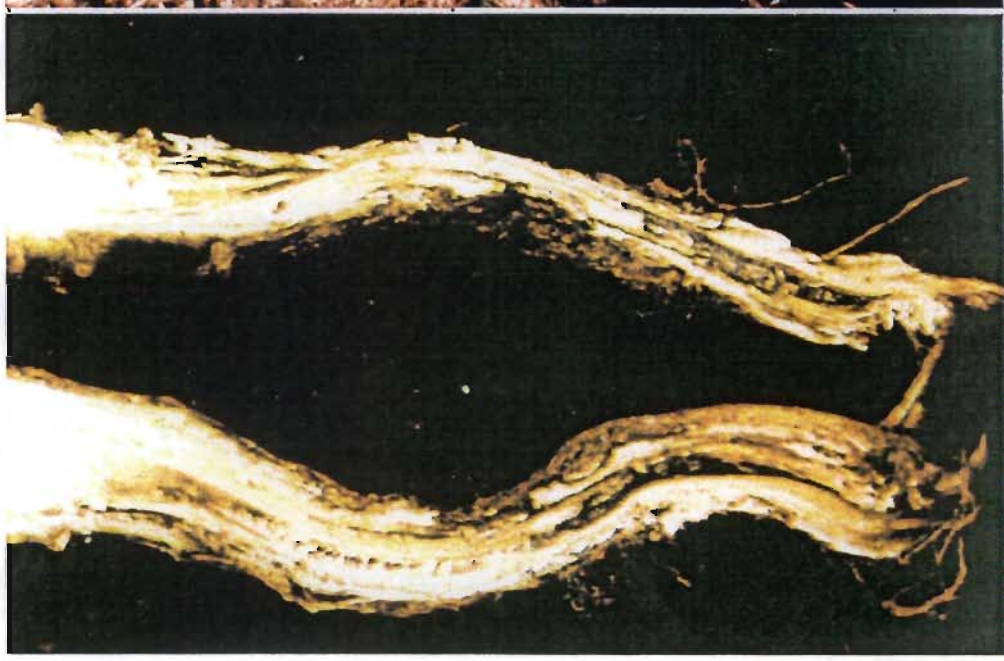

Fig. 1.3.3.I
Cross-section of a cabbage stem and tap root infected by *L. maculans*. Note the extensive lignification and lack of lateral roots.

Fig 4

1.4 References

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CHAPTER 2. MYCOLOGICAL STUDIES ON *LEPTOSPHAERIA MACULANS* IN KWAZULU-NATAL

The Ascomycetes in general have two distinct reproductive phases: the ascus or sexual stage, often called the ***ascigenous*** *or* ***perfect stage****, and the conidial or asexual stage, often designated as the* ***imperfect stage****.*

Alexopoulos and Mims, 1979

Abstract

The perfect stage is reported for the first time in South Africa. Koch's postulates were performed using local isolates of *L. maculans*. Pycnidia, but no pseudothecia, were commonly found on live tissue. Viable pseudothecia and pycnidia were found on dead, weathered tissue, sometimes physically attached to each other. A theory is proposed to explain the dual ***r*** and ***K*** strategies adopted by this fungus in its reproductive cycle.

2.1 Introduction

The imperfect stage of *L. maculans* is readily observed on live material in the middle of blackleg lesions. Examination of live tissue lesions of local material under the microscope usually reveals pycnidia. However, pseudothecia of the perfect stage develop naturally only on weathered debris (Smith, 1956; Smith and Sutton, 1964), and the perfect stage had never been reported in South Africa, prior to the work reported here.

2.2 Materials and Methods

2.2.1 Discovery of the Perfect Stage

Infected cabbage stems were collected from Howick, KwaZulu-Natal. These stems were left in a field, exposed to the weather, for 3 mo during winter. In spring, they were examined with dissecting and transmission microscopes for the presence of pseudothecia, asci and ascospores. A series of photographs, and video material were taken of both the sexual and asexual reproductive structures found on the debris.

Pseudothecia, asci and ascospores were readily observed. Figs 2.2.1.A - I are photographs of *L. maculans* reproductive structures. In particular, they captures the existence of a pycnidium attached to a pseudothecium (Fig. 2.2.1.E - F). This provides an integrated form of mixed reproductive strategies.

It is notable that pycnidia containing viable pycnidiospores were found on all debris on which pseudothecia were also observed.

It was relatively easy to produce pure cultures of *L. maculans* from pseudothecia using the technique originally described by Mengistu *et al.* (1991), and after 7 d the cultures produced pycnidia prolifically, containing typical pycnidiospores of *P. lingam*.

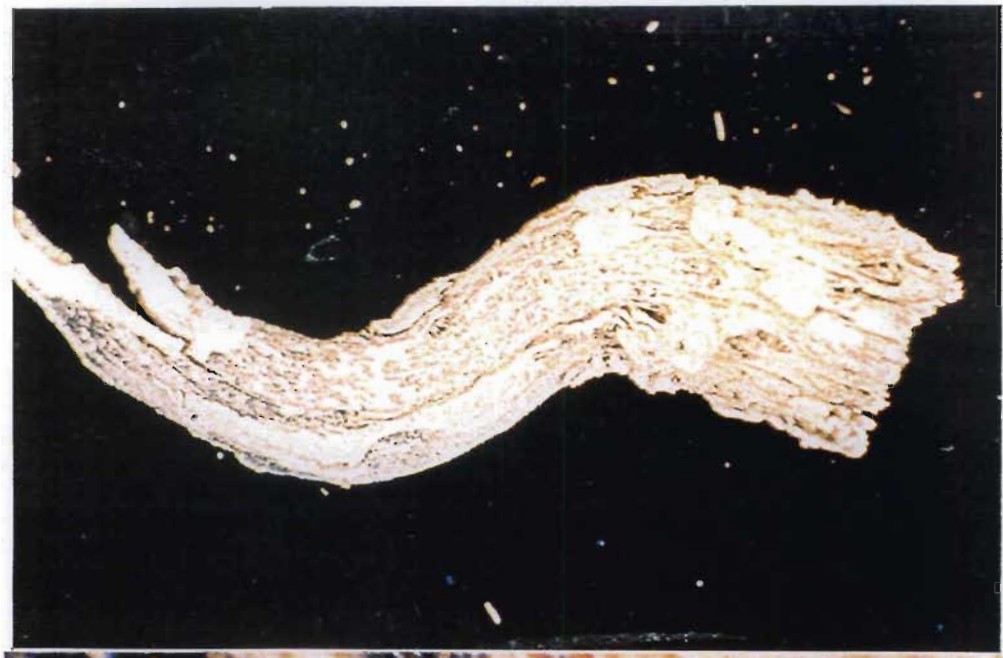

Fig. 2.2.1.A
A weathered cabbage stem, peppered with pynidia and pseudothecia.

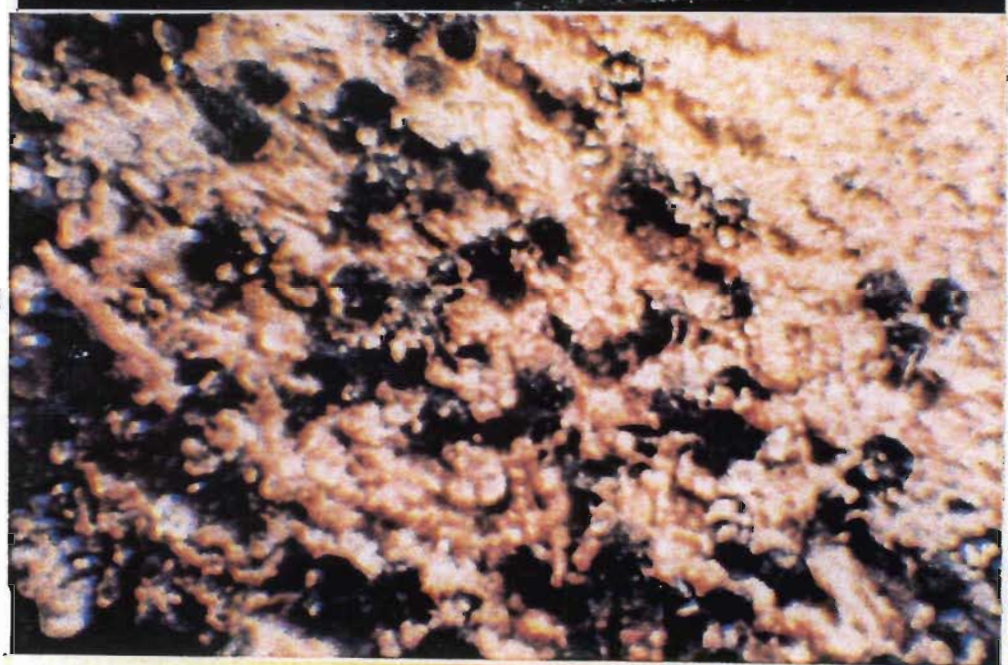

Fig. 2.2.1.B
A close-up of a weathered cabbage stem, peppered with pynidia and pseudothecia.

x 40

Fig. 2.2.1.C
Pseudothecia of *L. maculans* on a weathered cabbage stem.

x 80

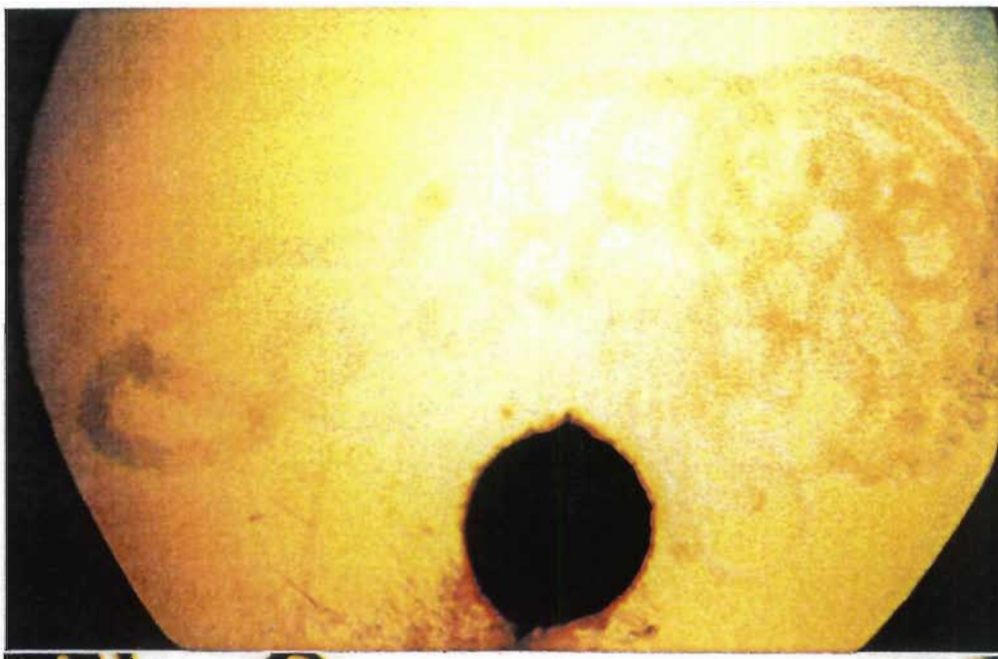

Fig. 2.2.1.D
Conidia released in a ribbon-shaped cirrhus from a pycnidium.

x 125

Fig. 2.2.1.E
Conidia released in a ribbon-shaped cirrhus from a pycnidium joined to a pseudothecium.

x 400

Fig. 2.2.1.F
Asci released from a pseudothecium. Note the pycnidium attached to the bottom right side of the pseudothecium.

x 400

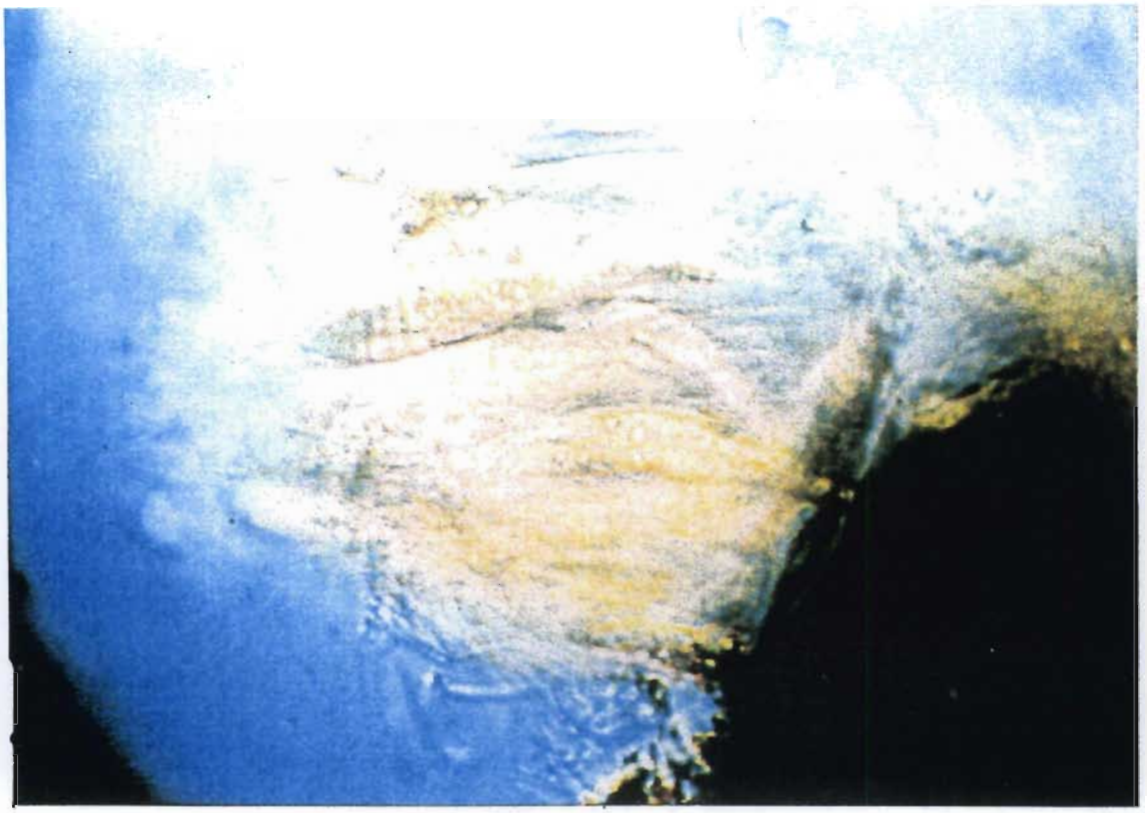

Fig. 2.2.1.G Asci containing ascospores of *L. maculans*.

x 1250

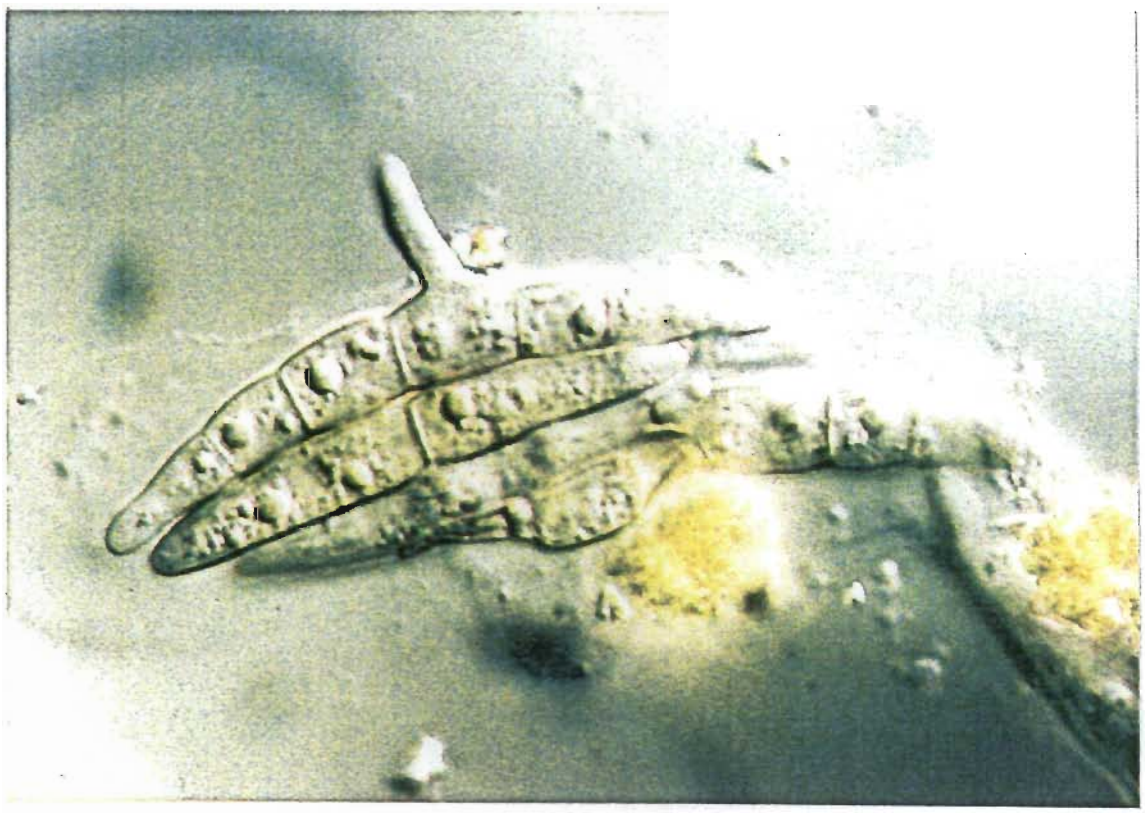

Fig. 2.2.1.H Ascospores of *L. maculans*. A germ tube develops from the top ascospore.

x 1250

2.2.2 Confirmation of Koch's Postulates

To test Koch's postulates, 2 ml of sterile distilled water was added to a petri dish with a mature culture of *L. maculans* on oatmeal agar (OMA). The plate was then hockey-sticked vigorously and the conidial suspension sucked into a hypodermic syringe. This was injected into the leaves and stems of 4 wk old cabbage seedlings (cultivar Gloria Osena, McDonald's Seeds), which were kept in a greenhouse at approximately 25^{0}C. These seedlings were observed until 28 d after inoculation for disease symptoms, after which the pathogen was reisolated from lesions onto V8 agar from surface sterilized, infected leaves.

2.3 Results

Typical blackleg symptoms were observed on the leaves after 12 d. After 15 d, the stems typically developed blackening, and after 20 d lignified lesions developed. At 28 d pycnidia were observed in the centres of some stem lesions. The fungus was readily isolated from infected leaves, and it produced prolific pycnidia when it was transferred to OMA.

2.4 Discussion

The discovery of the sexual stage of *L. maculans* in KwaZulu-Natal is significant for several reasons. Firstly, whilst blackleg of crucifers had been reported in South Africa as early as 1931, only the asexual stage of the fungus, *P. lingam*, was discovered at that stage (Doidge and Bottomly, 1931). Given that *L. maculans* is a heterothallic fungus, it was feasible that the sexual stage might not occur in South Africa. However, this point is now clarified and makes the record complete for this region for the CMI maps and other plant pathological data.

Secondly, when canola was introduced into Australia, Germany and France, blackleg had a devastating effect on the crop before resistant cultivars were developed. Canola was introduced into South Africa in 1992, an initial 500 ha being cultivated in the winter rainfall region of the Cape (Viljoen, 1992). It is therefore important for the agronomists and plant pathologists in southern Africa to be aware that the sexual stage of the fungus is present in the region, given *L. maculans*' great epidemiological competence on canola in cool, wet environments. For example, both French and Australian researchers have shown that the ascospores of *L. maculans* are much more infective than its pycnidiospores (see Section 1.3.2). Furthermore, they have also shown that ascospores can travel at least 8 km from their inoculum source, whereas other researchers have shown pycnidiospores to travel only 1.5 - 2.0 m (see Sections 1.3.2 and 7.3).

This prediction of canola's susceptibility to *L. maculans* in the Cape appears to have been well directed as Aveling (pers. comm.) reported the first blackleg epidemic in canola in the Cape in the 1995/96 season.

Thirdly, the fungus has been found to generate a wide range of pathotypes, active at the species (Petrie, 1975) and cultivar level (see Section 1.3.2.3). The presence of the sexual stage will ensure that sexual reproduction generates a genetic diversity within the species in this country, and within each pathotope (Putter, 1980). The potential of the sexual stage, *L. maculans*, to create blackleg epidemics in cruciferous crops should be substantially greater therefore, than that of the asexual stage alone.

An interesting feature of this fungus, and indeed many Ascomycetes and Basidiomycetes, is that the perfect and imperfect stage may appear concurrently (Gabrielson *et al.*, 1978) and may even emerge from the same mycelial base, with pseudothecia and pycnidia attached to each other (Fig. 2.2.1.E - F). Given the larger size and higher infective capacity of the ascospores but their relatively low numbers, and the small size but large numbers of pycnidiospores (Alabouvette, 1970), it is possible to classify the ascospores and pycnidiospores as **K** and **r** strategies adopted

by an organism at the same time (Zadoks and Schein, 1979). The role adopted by each stage is clear: the ascospores are larger, tougher, more infective and airborne. Their role is to be produced from debris after winter, and to initiate infection in a new field of crucifers, near or far from the original infected field. However, because they are resource-expensive, the fungus is forced to produce less of this spore type. In contrast, pycnidiospores are small, less infectious and are splash-dispersed only short distances. Their role is to spread the disease rapidly, after long or medium distance dissemination has occurred by infected seed or ascospores, respectively. Because they are small and therefore relatively resource-cheap, they can be produced in large numbers by the fungus. It is notable that on live plants *L. maculans* only produces pycnidiospores, not ascospores. This is understandable from an energetic perspective: it would be less efficient to produce ascospores once the fungus was already established in a crop, than to produce pycnidiospores which would be splash dispersed efficiently onto neighbouring plants within a crop.

The fitness value of producing pycnidiospores together with ascospores on the same overwintered crucifer material was initially considered an anomaly. It was later realized that it would be a fitness advantage to the fungus if it had splash-spores if a new crop arose from seeds of the previous generation (wild pathosystem) or if a farmer replanted the same lands with a fresh crucifer crop (crop pathosystem). The advantage would be that rapid and efficient inoculation of the crop would occur using resource-efficient pycnidiospores. If the nearest crop was planted some distance away, then the production of airborne ascospore would provide some chance of the fungus infecting that crop also.

Whilst many plants also mix *r* and *K* reproductive strategies (in the form of sexual and asexual reproductive structures), most higher organisms are forced to adopt primarily one strategy or the other, and are classified as either **r** or **K** strategists (Zadoks and

Schein, 1979; Smith, 1990). Fungi with two reproductive structures, such as *L. maculans*, may adopt a dual strategy to cover a range of survival challenges.

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CHAPTER 3. AN OVERVIEW OF THE EPIDEMIOLOGY OF *LEPTOSPHAERIA MACULANS*

Epidemiological analysis has come to stay.

Vanderplank (1963)

3.1 An Ethograph of *Leptosphaeria maculans*

The aim of plant pathology is to arrive at a clear understanding of each disease triangle or quadrangle studied (Robinson, 1976). Each contributing component studied is functionally irrelevant until it is integrated into the overall picture. This may become difficult when much is known of the disease. Putter (1980) developed the concept of the disease ethograph. An ethograph is a graphic integration of epidemiological information available on the disease quadrangle concerned. The ethograph starts from a central core of information and is built up as a series of concentric spheres of information covering each systems level, from the molecular in the centre to the population level on the outside. This approach has been taken with the crucifer blackleg pathosystem in KwaZulu-Natal and a basic ethograph is included here (Fig. 3.1.A).

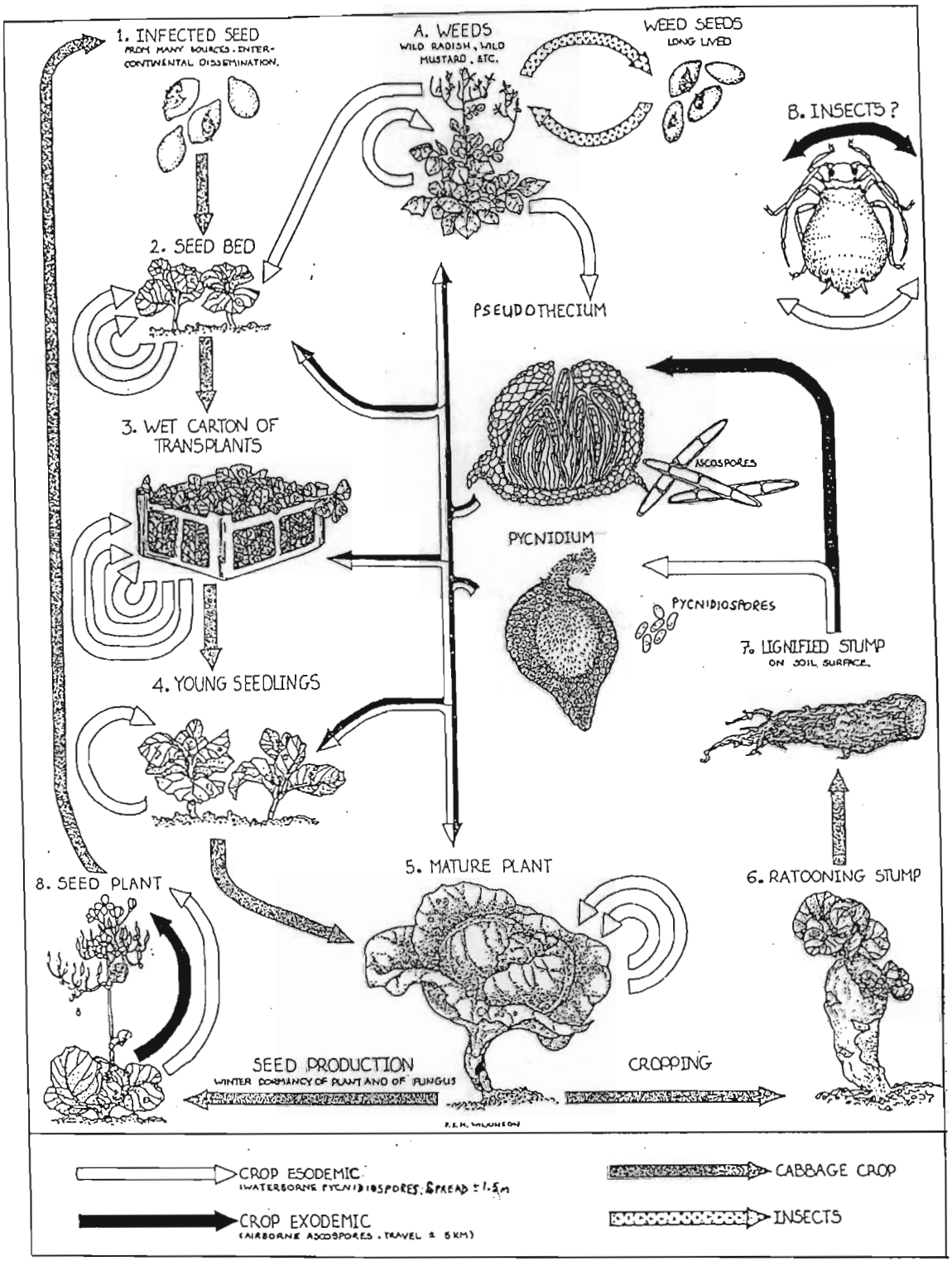
Convergent evolution of pathogens and their hosts tends to clump disease ethographs into only two broad types (foliar and soilborne pathogens), within which are shared a common evolutionary stable strategy (ESS) (Maynard-Smith, 1974). For example, cabbage blackleg and black rot are two crucifer diseases caused by dissimilar organisms, a fungus and a bacterium. However, comparison of their ethographs shows that they share many common feature (Figs 3.1.A and 3.1.B).

The net value of an ethograph is that one is able to examine and integrate all the contributing facets of a disease in one figure. Furthermore, it is a dynamic system, integrating new information as it becomes available.

Based on an understanding of the key components of an ethograph, a series of intervention points can be identified at which disease control measures could be applied (Laing, 1987). The significance of each of these intervention points is related to the quantitative contribution of each step to the epidemic process. The efficacy of a given practical control measure will therefore be determined by factors such as the reproductive rate of the pathogen and the influence of the environment.

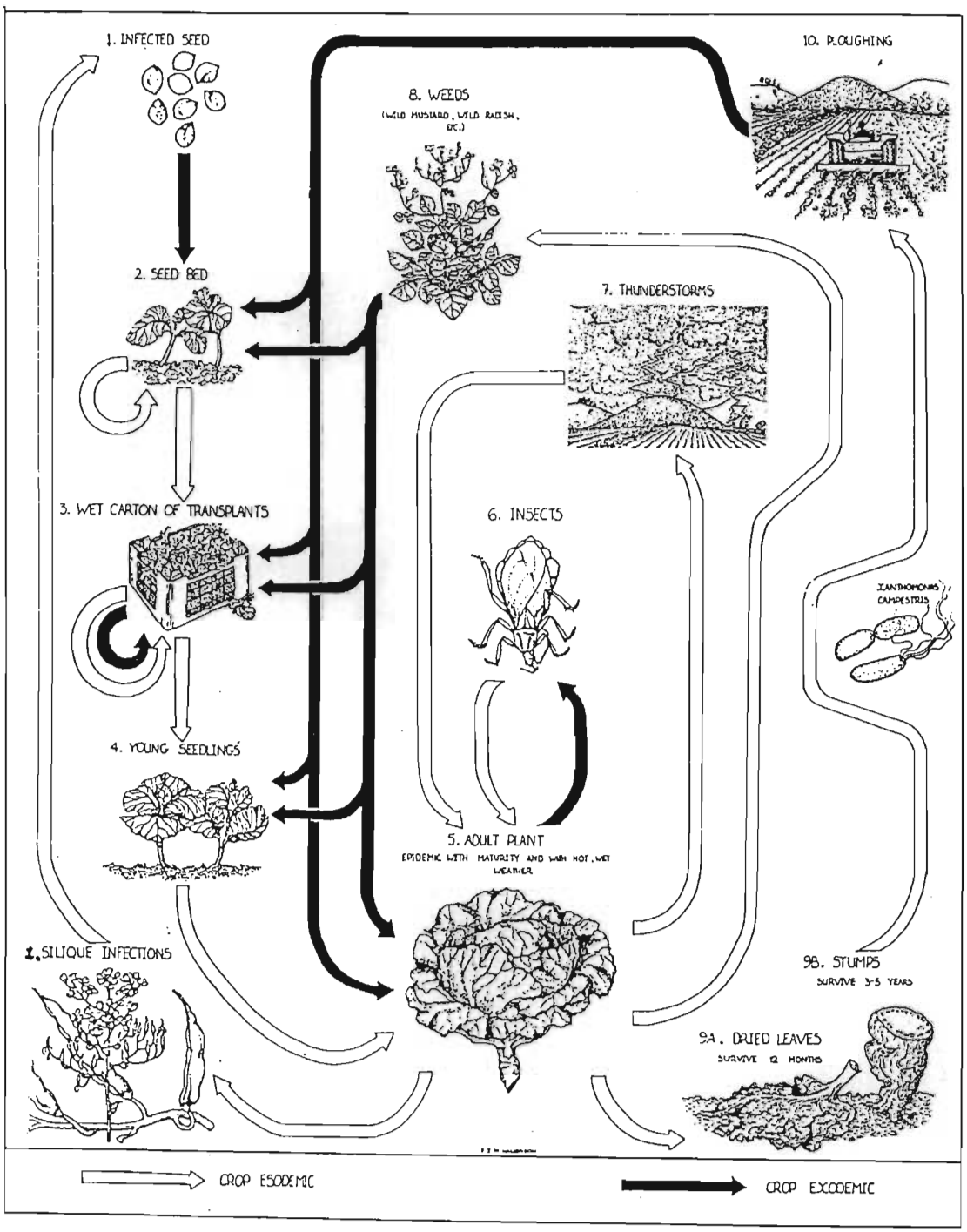
The disease ethograph in Fig 3.1.A presents a concise overview of the interaction of *L. maculans* with a transplanted crucifer crop, focusing on the crop systems level. The ethograph of a direct-seeded crop is identical if any transplanting occurs to fill gaps in the rows of plants. Otherwise, Steps 3 and 4 are left out. With containerized seedlings, the ethograph remains largely the same. However, the very significant transfer of inoculum in Steps 2 and 3 which occurs with transplanted seedlings was discovered later to be substantially reduced.

The ethograph (Fig. 3.1.A) provided the basis for the investigations undertaken to unravel the crucifer blackleg pathosystem in the KwaZulu-Natal Midlands, and to identify control options.



ETHOGRAPH OF CABBAGE BLACKLEG

Fig. 3.1A An Ethograph of the Crucifer Blackleg Pathosystem in KwaZulu-Natal



AN ETHOGRAPH OF CABBAGE BLACK ROT

Fig. 3.1.B An Ethograph of the Crucifer Black Rot Pathosystem in KwaZulu-Natal

3.2 References

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CHAPTER 4. STUDIES ON THE PRESENCE AND CONTROL OF *LEPTOSPHAERIA MACULANS* IN CRUCIFER SEED

Seed, cuttings, transplants and even produce contaminated or infected by pathogens are major sources of inoculum and a serious impediment to local, national and international trade. Voluntary or official regulation of commerce, coupled with therapeutic measures, is an essential part of disease-free production.

Jarvis (1992)

Abstract

Importation of crucifer seed infected by *L. maculans* is significant in South Africa because:

1. It has the potential to introduce the disease to new areas of crucifer production.
2. When suitable environmental conditions prevail, it has the potential to initiate localized epidemics in crucifer production areas when either seedbeds or direct-drilling are used to produce seedlings.
3. Infected seedlots of cabbage and Japanese radish seed have been imported and sold in South Africa, despite phytosanitary regulations requiring crucifer seed to be effectively free of *L. maculans*.

However, control of the disease as a result of seed-borne inoculum is now possible because systemic fungicides can be applied to crucifer seed to ensure the total elimination of seed-borne *L. maculans*. In particular, dicarboximide fungicides (e.g., iprodione) and sterol-biosynthesis inhibitor fungicides (e.g., triforine, propiconazole) were shown to be effective in this role.

4.1 Introduction

4.1.1 Pathological Significance of Seed Infection

Seed infected and infested with *L. maculans* has long been recognized as an important means of dissemination of blackleg around the world (Henderson, 1918; Allen and Smith, 1961). The fundamental significance of seed-borne *L. maculans* is that crucifer crops are grown entirely from seed. Therefore, wherever infected seed is sown, the disease will appear; it creates an umbilical relationship between the cultivation of crucifers and the incidence of blackleg from season to season. It also gives the pathogen a powerful means of universal dissemination, one by which *L. maculans* has been introduced into all regions of crucifer production throughout the world.

Initial examinations of crucifer seed for the presence of *L. maculans* located it in the testa only (Cunningham, 1927; Hughes, 1933; Van Bakel, 1968). However, Jacobsen and Williams (1971) found the fungus to be carried both superficially on the seed coat and internally. Internally, it was most commonly located in the outer epidermis and the subepidermal parenchyma. Occasionally the mycelium was found in the outer integument, the inner integument, the aleurone layer and the endosperm. Profuse mycelial growth was common at the funicular attachment. In 23% of infected seeds studied, hyphae penetrated the embryo, usually only into the cotyledon but occasionally into the radicle.

The significance of seed-borne infection is much disputed, the basic question posed by early workers being, "is seed-borne infection more or less important than soil-borne debris in providing initial inoculum for an epidemic ?". Whitehead and Jones (1929), working on blackleg of swedes in Ireland, initially considered soil infection to be the primary inoculum source. However, Whitehead (1930) changed his mind the next year as he was unable to explain a widespread blackleg epidemic in Ireland except on the basis of infected seed. One wonders what his explanation would have been if he had known of the ascosporial stage of the fungus, discovered in Ireland in 1965 (Comerford and Mangan, 1965). Was the epidemic Whitehead observed the result of high levels of seed-borne infection coupled with favourable weather conditions ? Or was it the

result of a large and well-dispersed cloud of ascospores covering the affected area, as occurred in New Zealand blackleg epidemics on swedes (Smith, 1956) and on Australian canola (Bokor *et al.*, 1975) ? In retrospect, the latter scenario appears more likely.

Another widespread epidemic, in 1973 in the USA, arose from infected seed lots planted out without eradicant treatment and caused massive losses in the midwestern and eastern states of the USA (Williams and Wade, 1973; Williams, 1974; Gabrielson, 1974; Gabrielson and Maguire, 1977).

4.1.2 Epidemiological Significance of Seed-borne Inoculum

Vanderplank's (1963) logarithmic equation allows for the calculation of the exponential disease progress in the initial stages of an epidemic, when disease levels are below 5%. It is a useful context within which to discuss the factors that affect disease progress resulting from seed-borne inoculum. Mentioned previously in Section 1.2.7.3, Case 1, it is repeated here for clarity:

$$X_1 = X_0 \cdot e^{r.t} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 1$$

Any factor reducing X_0, r, or t will reduce the X_h, the level of disease at harvest. Four primary factors govern the development of disease from seed-borne inoculum:

1. The initial inoculum level required to initiate infection, X_0: i.e., the threshold inoculum level (TIL) (as discussed in Vanderplank, 1975). This, in turn, is a function of variations in the individual biotype's epidemiological competence. This will be a function of the virulence and aggressiveness of the biotypes involved (*sensu* Vanderplank, 1978), interacting with the host species and cultivar resistance levels (for example, in the case discussed by Mengistu *et al.*,

1991). In terms of Equation 1, these factors affect the *r* of the disease progress curve.

2. The influence of the environment on disease progress (Vanderplank, 1968; 1975; Zadoks and Schein, 1979): Windspeed, temperature, rainfall and duration of leaf wetness are environmental factors which profoundly affect the rate of disease progress in blackleg (McGee, 1977; Kruger and Wittern, 1985; Vanniasingham and Gilligan, 1989). The environment impacts directly on the *r* of Equation 1.
3. The effect of farming practices: For example, the use of direct seeding or transplanting seedbed seedlings affects blackleg severity (Gabrielson, 1988). Kruger and Wittern (1985) noted the reduction of *L. maculans* leaf infections in canola in Germany was partly as a result of the practice of deep ploughing in autumn for wheat sowing. Farming practices such as seed treatment and field sanitation impact on the $\mathbf{X_0}$ of Equation 1. Other practices, such as the application of overhead irrigation, may impact on the *r* value. In contrast, the use of early-maturing cultivars or the use of externally grown seedlings may reduce the time, *t*, for disease to develop.
4. The actual level of seed-borne inoculum present: This clearly impacts on the $\mathbf{X_0}$ value.

The paper of Taylor *et al.* (1979), entitled "Epidemiology and strategy for the control of halo-blight of beans", discussed these relationships in detail, and in the epidemiological context of the logistic equation (the logistic equation is similar to the logarithmic equation but includes a correction factor (1-X) for X > 0.5). The pathosystem discussed concerns a polycyclic disease, where disease progress is indeed a function of $\mathbf{X^0}$, *r* and *t* within a single season.

The key epidemiological issues that their study raises, with respect to *L. maculans*, are that:

1. Seed treatment reduces X_0, and is therefore a sanitation practice. Its value as a disease control measure is inversely related to the level of other sources of inoculum:

If $$X_0 = X_{0s} + X_{0d} + X_{0w} + X_{0a},$$

where X_0 = total initial inoculum
X_{0s} = seed-borne inoculum
X_{0d} = inoculum arising from debris within the field
X_{0w} = inoculum from weeds and alternate hosts
X_{0a} = airborne ascospores arriving from external sources,

and if $$(X_{0d} + X_{0w} + X_{0a}) \rightarrow 0,$$

then $$X_{0s} \rightarrow X_0,$$

and the significance of seed-borne inoculum increases.

Conversely,

if $$(X_{0d} + X_{0w} + X_{0a}) \rightarrow X_0,$$

then $$X_{0s} \rightarrow 0,$$

and the significance of seed-borne inoculum decreases.

McGee (1977) showed the latter situation to be true with blackleg on canola in Australia. Seidel *et al.* (1984) observed that chemical and heat treatment of seed had no effect on final levels of blackleg incidence, in the presence of high levels of field inoculum.

In the situation of a land planted for the first time to a crucifer crop and isolated from other crucifer plantings, then

$$X_0 = X_{0s}$$

because $$X_{0d} + X_{0w} = 0,$$

and assuming that the isolation of the crop is complete and therefore that

$$X_{0a} = 0.$$

Therefore, the significance of seed-borne inoculum would be at a maximum. As subsequent susceptible crops are planted, the two other factors, $\mathbf{X_{0d}}$ and $\mathbf{X_{0w}}$ would start to play an increasingly significant role (unless excellent sanitation practices were followed), and $\mathbf{X_{0a}}$ would start to play a role in the pathotope (Putter, 1980).

2. The value of seed treatment is inversely related to the pathogenicity of the pathogen. As pathogenicity increases, so does the ***r*** value, and hence the value of seed treatment is reduced. Vanderplank (1963) put it this way: "a fast infection rate and a long period over which infection can mount are the primary factors that make sanitation inadequate in some seasons and perhaps unnecessary in others".

3. The value of seed treatment is inversely related to the susceptibility of the host species and cultivar. As susceptibility increases, so does the ***r*** value, and hence the value of seed treatment is reduced.

The International Seed Testing Association (ISTA) set the maximum level of seed-borne *L. maculans* in crucifer seed at 0 in 20 000 (0.005%) (Anon., 1966). A comparison with two bacterial diseases reveals that TIL's may be as small as this with some seed-borne diseases. Firstly, Trigalet and Bidaud (1978) worked on the epidemiology of halo blight of beans, caused by *Pseudomonas phaseolicola* (Burkh.) Dowson. They established that seed infection of 1 in 20 000 did not initiate disease epidemics, even under optimum climatological conditions. Secondly, Guthrie *et al.* (1965), working on the same disease, found that 1 seed in 16 000 could initiate an epidemic, which indicates that *ceteris paribus*, the inoculum threshold lies somewhere between 1 in 16 000 and 1 in 20 000. Wharton (1967), also working with *P. phaseolicola*, found the TIL to be as low as 1 in 10 000. Williams and his associates at the University of Madison, Wisconsin, have found that under suitable conditions, and with a virulent biotype of *X. campestris.* pv. *campestris*, a black rot epidemic in cabbages could be initiated with 1 infected seed in 100 000 (Williams, pers. comm.).

However, the TIL for most fungal diseases is lower than that for most bacterial diseases, owing to their lower *r* values under optimum conditions. In New Zealand, Allen and Smith (1961) found the relatively high TIL of 1 in 200 was needed to initiate blackleg outbreaks in swedes. Subsequently, Gabrielson (1983) determined a lower TIL for *L. maculans* in cabbage, of 1 in 10 000. Higher TIL values have been determined for other seed-borne fungi: the TIL of *Phoma betae* (Oudem.) Frank on beets is considered to be 0 in 200 (Anon., 1985). *Phoma medicaginensis* Malbr. on lucerne (*Medicago sativa* L.), and *Phoma medicaginensis* var. *pinodella* (L.K. Jones) Boerema on peas (*Pisum sativum* L.) have similar TIL values (Neergaard, 1979). Gabrielson (1988) has reviewed the issue of TIL values for several fungal diseases on various crops, and *L. maculans* in crucifers in particular.

The environment has a profound influence on the TIL of crucifer blackleg. For example, Allen and Smith (1961) studied canker of swedes in New Zealand over a 3 yr period, comparing results for two wet seasons and one dry season. They found the TIL to have increased from less than 0.5% in the wet seasons to more than 3% in the dry season.

The level of *L. maculans* infection in crucifer seed varies widely. Allen and Smith (1961) found the infection level in a batch of New Zealand swede seed to be less than 0.1%, which was less than the TIL they observed. Hence, they concluded that seed-borne infection was secondary to ascospores as the primary inoculum initiating annual blackleg epidemics. Williams (1967), however, has reported a cabbage seed lot with 18% *L. maculans* contamination, which is certainly well above any reported TIL. At higher levels, seed infection may initiate a local epidemic. Bennett (1939) observed one seed lot (infection level unknown) to produce 60% blackleg incidence at harvest. In a neighbouring plot, using a different seedlot, only 1% infection resulted. Wood and Barbetti (1977) reported that in two separate trials using seed with infection levels of 0.08% and 0.5%, only 0.08 and 0.061% field infection was measured at harvest, respectively; i.e., no epidemic developed. In another trial utilizing a seed lot with an infection level of 5.9%, they recorded a 19% field infection. The authors chose their experimental site very carefully to exclude airborne ascospores, using an isolated

Indian Ocean island off Australia. They also tested for the arrival of air-borne ascospores, using plots of susceptible plants as spore traps. Their primary conclusion was significant: that seed infection introduced the disease into an area and in this sense, constituted the "original" initial inoculum source. In subsequent seasons, the initial inoculum would be a combination of pycnidiospores and ascospores from debris sources, and pycnidiospores from contaminated seed sources. The role of seed-borne inoculum would probably decrease in significance.

In sum, seed-borne infection is the first step of a serial succession of the disease cycle, as graphically shown in the ethograph (Fig. 3.1.A). Low level seed-borne inoculum alone is not enough to initiate a full epidemic. There has to be a multiplication step in-between. This may be the spread of pycnidiospores from a few infected seedlings to many others during a transplanting operation involving seedbed seedlings, or the slow generation of widespread inoculum in the form of infected stem and leaf debris in a field.

One of the difficulties in evaluating research on the TIL of *L. maculans* and other seed-borne pathogens is that the results may not be directly comparable. It is not always clear from the publications that environmental conditions during the trials were similar, or that the pathogenicities of the involved biotypes of *L. maculans* were equivalent, or that host susceptibilities were similar. For example, in the publication by Gabrielson *et al.* (1977), a single, naturally-infected seedlot of Brussels sprout seed was used. It is not possible to judge whether the trial is representative, and hence whether the data is representative and can be used to generate a generally applicable TIL, because there is no measure of the crop's relative susceptibility, nor of the pathogenicity of the strain of *L. maculans* in the seed. For example, if the strain present in the seed was only mildly pathogenic and the Brussels sprout cultivar was highly resistant, then the results would have provided an underestimate of a worst-case TIL. Alternatively, if the Brussels sprout cultivar was highly susceptible and the strain of *L.maculans* present in the seed was highly virulent, then a worst-case TIL would have developed. The trial of Heald (1921) on *Tilletia caries* (DC.) Tul. resulting from infection of wheat seed provided a clear example of this. The TIL of a resistant

cultivar, Marquis, was found to be between 542 and 5043 spores/seed, compared to <104 spores/seed with the susceptible cultivar, Jenkins Club.

A spectrum of *L. maculans* biotypes has been found, varying both in virulence (Helms and Cruikshank, 1979) and aggressiveness (Pound, 1946; 1947; Bonman *et al.*, 1981; Mengistu *et al.*, 1991; Rouxel *et al.*, 1994; 1995a; 1995b; Pang and Halloran, 1995). As discussed in Section 1.3.2.3, considerable effort has been expended by pathologists worldwide in clarifying the issue of different pathogenicity groupings. Variable pathogenicity will obviously have a profound effect on the TIL, interacting with host resistance and the environment to give a specific TIL. When determining effective TIL values from field trials, it is therefore essential that they are conducted with a number of cultivars displaying a spectrum of resistance, with pathogenic strains with defined pathogenicities, and with careful monitoring of environmental parameters for the duration of the trial. Without these precautions, it is difficult to extrapolate from the results with any confidence, or to compare the results of different trials.

4.1.3 Seed Treatments

Shuring (1971) briefly reviewed the history of seed treatments, going back to Remnant in 1635 who recommended "brining" or "steeping" of wheat grains in sea water to reduce infection by wheat bunt (*Tilletia caries*). Subsequently, hot water treatments were discovered as an effective seed treatment: of oats by Jensen in 1888, of celery by Krout in 1921 and of peas by Ogilvie in 1932. Ever since the importance of the seed-borne phase of *L. maculans*' disease cycle was established (Henderson, 1918), control of this source of inoculum has been sought on crucifer seed. Furthermore, investigations on the control of seed-borne fungi are amenable to laboratory studies, which always gives impetus to research. The net result is a profusion of papers on this subject. Table 4.1.3.A summarizes the research conducted on the control of seedborne *L. maculans*.

Table 4.1.3.A: Treatments for the Control of Seedborne *L. maculans* [2]

Chemical	Group	Efficacy	Author
hot water	physical	excellent excellent partial partial partial excellent partial poor excellent	Norton, 1919 Walker, 1923 Clayton 1928 Williams 1967 van Bakel 1968 Pauvert 1971 Klinkovskaya, 1976 Gabrielson *et al.*, 1977 Holtzhausen, 1978
mercuric chloride	mercuric	partial partial partial ineffective	Walker, 1923 Clayton, 1928 van Bakel and De Kraker, 1963 Gabrielson *et al.*, 1977
mercuric chloride, hot soak	mercuric/physical	excellent	Giessmann and Daebeler, 1973
phenyl mercury acetate	mercuric	excellent	Holtzhausen, 1978
hot cupric acetate soak	copper/physical	partial	Schaad *et al.*, 1980
quintozene	chlorinated hydrocarbon	excellent	Holtzhausen, 1978
captan soak	carboximide	excellent	Holtzhausen, 1978
maneb, hot soak	dithiocarbamate	excellent	Giessmann and Daebeler, 1973
mancozeb	dithiocarbamate	excellent	Holtzhausen, 1978
thiram soak	dithiocarbamate	excellent excellent partial excellent excellent but phytotoxic partial excellent partial excellent	Maude and Keyworth, 1967 Maude *et al.*, 1969 Jacobsen and Williams, 1971 Maude *et al.*, 1973 Maude 1977 Gabrielson *et al.*, 1977 Holtzhausen, 1978 Harman and Nash, 1978 Humaydan *et al.*, 1980
thiram soak with vacuum	dithiocarbamate	excellent excellent	Harman and Nash, 1978 Humaydan *et al.*, 1980
zineb	dithiocarbamate	partial	Klinkovskaya, 1976
benomyl wp or slurry	benzimidazole	excellent ineffective excellent excellent	Pauvert, P.A. 1971 Shuring, 1972 Gabrielson *et al.*, 1977 Harman and Nash, 1978
benomyl in acetone	benzimidazole	excellent excellent	Harman and Nash, 1978 Humaydan *et al.*, 1980
benomyl soak	benzimidazole	excellent excellent	Jacobsen and Williams, 1971 Winter and Huber, 1978

[2] *Continued on next page*

Chemical	Group	Efficacy	Author
thiabendazole	benzimidazole	excellent but phytotoxic excellent excellent partial excellent excellent	Jacobsen and Williams, 1971 Shuring, 1972 Maude *et al.*, 1973 Gabrielson *et al.*, 1977 Humpherson-Jones *et al.*, 1983 Maude *et al.*, 1984
iprodione	dicarboximide	excellent	Humpherson-Jones *et al.*, 1980 Humpherson-Jones *et al.*, 1983 Maude *at al.*, 1984
tridemorph	morpholine	excellent but phytotoxic	Maude *et al.*, 1984
fenpropimorph	morpholine	excellent	Humpherson-Jones *et al.*, 1983 Humpherson-Jones *et al.*, 1984Maude *et al.*, 1984
imazalil	imidazole	excellent but phytotoxic	Maude *et al.*, 1984
triarimol	pyrimidine	excellent but phytotoxic	Maude *et al.*, 1984
nuarimol	pyrimidine	excellent but phytotoxic	Maude *et al.*, 1984
fenarimol	pyrimidine	excellent but phytotoxic	Maude *et al.*, 1984
Benomyl/ thiram	mixed	partial	Rawlinson and Muthyalu, 1979
fenpropimorph/ thiram/ hexachloro-cyclohexane	mixed	excellent	Humpherson-Jones *et al.*, 1984

In general, early workers concentrated on the use of hot-water treatments (Norton, 1919; Walker, 1923) and mercuric fungicides (Walker, 1923; Clayton, 1928; Van Bakel and De Kraker, 1963). Mercuric fungicides were not particularly effective (Walker, 1923; Clayton, 1928) and are now banned in most countries of the world (Saha, 1972).

The 'standard' hot-water treatment (30 min at 50^0C) (Walker, 1922;, 1923) has been fairly effective at eliminating both seed-borne fungi and bacteria. To this day it is a widely recommended treatment of crucifer seed against *X. campestris* pv. *campestris*, the seed-borne causal organism of crucifer black rot. However, it does not entirely eliminate seed-borne blackleg in crucifer seeds (Clayton, 1928; Van Bakel, 1968; Robocker, 1976). Williams (1967) used a hot-water treatment (25 or 30 min at 50^0C)

on a batch of infected Australian cabbage seed. Starting with 18% infection, the treatment reduced the infection level to 2%. The ISTA standard for *L. maculans* in cabbage seed was zero in 20 000 (Anon., 1966), or <0.005%, so a 2% infection was still 400 times the accepted level for seed-borne infection.

The other disadvantage of the hot-water treatment is reduced seed viability. It can reduce germination of seed by 18%, particularly with older seed (Walker, 1923). This makes it unattractive to seedsmen, especially on new hybrids which often show greatly reduced germination after heat treatment (Williams, 1974; Robocker, 1976; Cox, 1980).

The treatment is also technically difficult to perform as exact times and temperatures are critical to its success. A large-volume, thermostatically controlled waterbath is essential. The large volume is necessary so that the addition of a quantity of seeds does not drop the water temperature significantly. The thermostat is essential to keep the water temperature within a narrow range lethal to the seed-borne pathogens, but not the seed. Williams (pers. comm.) considered the treatment to be technically too difficult for the layman to perform without either "boiling" the seed or failing to kill the pathogens.

Research in the last two decades has concentrated on the use of organic chemicals. In particular, the benzimidazoles, thiabendazole and benomyl, have proved effective in the control of seed-borne *L. maculans*. Jacobsen and Williams (1971) first tested these fungicides, together with thiram (0.2% aqueous suspension for 24 hr at 30^{0}C). Whilst both thiabendazole and benomyl eliminated the seed-borne infection (control 21.5% infection), the thiabendazole also reduced germination by 8%. Conversely, Shuring (1971) found thiabendazole at 2.5 g a.i. kg^{-1} seed to be an effective seed slurry treatment, whereas benomyl was ineffective under these conditions. Maude *et al* (1973) also found thiabendazole effective when applied as a seed slurry at 1.0, 2.5 and 5.0 g a.i. kg^{-1} seed. In contrast, in personal experience with seedling nurseries, the locally formulated thiabendazole has proved to be fairly phytotoxic. The company supplying the product locally considered the phytotoxicity to be the result of

compounds such as xylene, which are added in the formulation process, and not to be the result of the active ingredient.

Gabrielson *et al.* (1977) found a benomyl treatment (125-250 g per 100 kg seed) entirely eliminated seed-borne *L. maculans*. Thiabendazole was also effective, but less so than benomyl. Their final recommended seed treatment was the slurry application of 125 or 250 g benomyl plus 250 g thiram per 100 kg seed. The thiram was added as a preventative fungicide against damping-off.

Slurry treatments have a number of inherent advantages over seed soaks:

1. Seeds are not wet for long and, therefore, do not require drying, nor is the germination process initiated, both problems occurring with seed soaks.
2. The technology for rapid high-volume slurry treatment is available, whereas seed soaks require large, expensive tanks and the through-volume is small.
3. Seed-borne pathogens of crucifers are carried both superficially and internally. In the process of a seed soak, it is possible that non-target pathogens such as *X. campestris* pv. *campestris* could be transmitted to the seed coats of clean seed which would be counter-productive. This problem could not arise with slurry treatments (Gabrielson *et al.*, 1977).

Harman and Nash (1978) attempted to overcome the limitations of aqueous seed soaks by using acetone as the fungicide solvent (5 g ℓ^{-1} solution of benomyl in acetone), and successfully eliminated *L. maculans* after a 5 hr seed soak, with no reduction in germination. Notably, they reported the absence of any control of *Alternaria* spp. They also reported the control of 2.4% seed-borne *L. maculans* with the use of a benomyl/thiram dust (Benomyl T; 30% a.i. each of benomyl and thiram) at 2.5 g a.i. kg^{-1}. Holtzhausen (1978) also reported eliminating seed-borne infection of *L. maculans* at 1.2% using a benomyl dust treatment. He also found mancozeb, quintozene and phenyl mercury acetate to be effective. These results are difficult to evaluate because of the low infection levels in the seed being tested. The results are also in question because Gabrielson *et al.* (1977) reported the ineffectiveness of

mercuric chloride, and both mancozeb and quintozene were found to be ineffective in research for this thesis (Section 4.3.3).

The use of benzimidazoles was preceded by the use of thiram. It was first applied as a long seed soak (0.2% at 30^0C for 24 hr) (Maude and Keyworth, 1967; Maude *et al.*, 1969), and later as a rapid seed soak (30 min), with a partial vacuum applied (400 mm mercury) to improve seed coat penetration (Harman and Nash, 1978). The long seed soak with thiram may not be totally effective (Jacobsen and Williams, 1971; Gabrielson *et al.*, 1977; Harman and Nash, 1978) and may reduce seed germination (Maude, 1977). Similarly, the vacuum seed soak technique may be only partially effective (Section 4.3.3).

In a search for control of seed-borne *Alternaria* spp. in crucifer seed, the fungicide iprodione (Rovral, using the EC formulation), applied as a seed slurry (2.5 a.i. kg^{-1} seed), was found to be effective against both *Alternaria* spp. and *L. maculans* (Humpherson-Jones and Ainsworth, 1981). This dual control achieved by iprodione is important as *Alternaria* spp. have been recognized as a serious problem in crucifer production (Babadoost-Kondri, 1979; Cox, 1980; Maude and Humpherson-Jones, 1977; 1980).

More recently, the sterol biosynthesis inhibitor fungicide, fenpropimorph, was successfully tested for control of seed-borne *L. maculans* and *Alternaria* in crucifer seed (Humpherson-Jones *et al.* 1984; Maude *et al.*, 1984).

Alternative treatments reported include 100 ppm aureofungin with 20 ppm $CuSO_4$ (Rahalker and Neergaard, 1969) and aerated steam (Baker, 1969; Navaratnam *et al.*, 1980), both of which kill a broad spectrum of seed-borne pathogens. The latter treatment, however, failed to eliminate *Ascochyta pisi* and *Mycosphaerella pinodes* from infected pea seed, reduced germination, and only allowed for a small throughput of seed (Maude, 1966). Schaad *et al.* (1980) developed a treatment against *X. campestris.* pv *campestris* which was also fairly effective against *L. maculans* and *Alternaria* contamination (95, 92 and 81% efficiency, respectively). The treatment comprised a

seed soak of 20 min at 40^{0}C in acidified cupric acetate (0.5% cupric acetate dissolved in 0.005N acetic acid). However, germination was negatively affected.

In sum, there appear to be several effective biocidal seed treatments which reduce, but do not eliminate seed-borne *L. maculans*, such as the hot-water treatment. Benomyl and thiabendazole are both systemic, benzimidazole fungicides which eliminate *L. maculans* in infected seed when applied as seed soaks or slurry treatments. Thiabendazole appears to reduce germination and to be slightly less effective than benomyl. Iprodione and fenpropimorph slurry treatments appear to eliminate both seed-borne *L. maculans* and *Alternaria* spp. From the literature, the current treatments of choice against *L. maculans* and *Alternaria* spp. are iprodione (Rovral EC) or fenpropimorph (Corbel) (2.5 g a.i. kg^{-1} seed), applied as a slurry.

4.2 Local Incidence of Seed-borne *L. maculans*

4.2.1 Introduction

Seed infection probably pays a key role in the disease cycle of *L. maculans* in KwaZulu-Natal in some cases by initiating the first infections. In that blackleg has occurred throughout KwaZulu-Natal, including some exceedingly isolated locations, it is probable that *L. maculans* was initially introduced in and on commercial seed. It is also quite feasible that seed-borne infection has initiated localized blackleg epidemics under suitable climatic conditions, since the levels of seed-borne infection in local seed exceeded the reported TILs in some cases, as discussed below. Such an epidemic would probably be localized because, firstly, KwaZulu-Natal cabbage farms are mostly small and relatively isolated, and secondly, many different cabbage cultivars are grown, the seed of which comes from many diverse sources. There is a further dilution factor, in that different seed lots of the same cultivar may be grown in different seed producing areas. For example, seed of the popular cultivar Gloria Osena was produced in both the USA and Italy (Larsen, pers. comm., 1984). Thus, one highly infected seed lot might only be distributed to a small proportion of the cabbage farming community. For the individual farmers affected, however, the result may be a destructive epidemic.

In South Africa, *L. maculans* has been detected on both imported and locally produced crucifer seed (Holtzhausen, 1978; Holtzhausen and Knox-Davies, 1974). Various *Alternaria* spp are also commonly found on crucifer seed (Babadoost-Kondri, 1979), the important pathogenic species being *A. brassicicola* (Schw.) Wilts. and *A. brassicae* (Berk.) Sacc. Whilst these two species are generally considered minor pathogens, they are considered important in inducing damping-off and for reducing germination of infected seed (Shuring, 1971; Babadoost-Kondri, 1979; Cox, 1980). They are also a major pathogen in canola (Petrie *et al.*, 1985).

The purpose of this trial was twofold:

1. To test local commercial crucifer seed for the presence of seed-borne *L. maculans* and *Alternaria* spp., to establish what level of seed-borne inoculum typically occurred;

2. To discover at least one infected seedlot on which to test fungicide treatments.

4.2.2 Materials and Methods

Green Coronet is a popular commercial cabbage hybrid from a Japanese company, Taki Seeds. Two seedlots (McDonald's Seeds Lot No 88233; Starke-Ayres Lot No. 2019/A/DJC) were tested for the presence of seed-borne fungi. Seed was surfaced sterilized in a 1:9 sodium hypochlorite solution (approx. 3% free chlorine) for 3 min, rinsed in sterile distilled water, individually transferred onto 1.5% water agar and incubated at 20^{0}C in the dark. Twenty seeds per plate and 60 plates per seedlot were tested. Observations were made every 3 d for 21 d. *L. maculans* was identified from brown cotyledonary lesions which contained typical pycnidia, isolations from which produced typical *P. lingam* colonies. *A. brassicicola* was easy to identify as infected seedling were soon covered with typical, dark *Alternaria* spores.

4.2.3 Results

Both seedlots were found to be infected with *L. maculans* and *A. brassicicola*, as presented in Table 4.2.A.

Table 4.2.A: Incidence of *Leptosphaeria maculans* and *Alternaria brassicicola* in Cabbage Seed

Seedlot	*L. maculans*	*A. brassicicola*
McDonald's 88233	1.5%	15.0%
Starke-Ayres 2019/A/DJC	5.3%	8.5%

4.2.4 Discussion

It was clear from this very limited survey that seed-borne inoculum of *L. maculans* was entering South Africa in at least one Japanese cabbage cultivar. The levels detected greatly exceeded the international phytosanitary limits, and exceeded the theoretical TIL, as determined by Gabrielson (1983).

A. brassicicola is a very rapidly growing fungus, highly aggressive towards cabbage seedlings. Examination of germinating seed therefore needed to be conducted several times, to ensure that *L. maculans* incidence was noted before *A. brassicicola* colonies on neighbouring seeds overwhelmed it.

The Starke-Ayres seedlot had higher levels of *L. maculans* and *A. brassicicola* (5.34% and 8.53%). Unfortunately, only limited quantities of this seed were available, and therefore the McDonald's seedlot was used to test fungicides for the control of seed-borne *L. maculans* and *A. brassicicola*.

4.3 Seed Treatment Trial 1

4.3.1 Introduction

Given that infected seed was entering South Africa and possibly contributing to the spread and incidence of blackleg, we considered it important to develop an effective treatment of crucifer seed to eliminate seed infection by *L. maculans*. Local efficacy trials have to be performed if a fungicidal treatment is to be registered, even if it has been shown to be effective elsewhere (Act 36 / 1947, Union of South Africa).

The objective of this trial was to identify effective, non-phytotoxic fungicides for the control of seed-borne *L. maculans* and *A. brassicicola*. Two criteria were used to select fungicides for testing:

1. Activity against Ascomycetes and *Fungi Imperfecti*
2. Local availability.

4.3.2 Materials and Methods

Thirty two fungicides (Table 4.5.1.A) were tested. A control treatment followed the same treatment as the fungicidal treatments, applying tap water only. Seed was surface sterilized by immersing into a 1:3 solution of commercial bleach (NaOCl, with approx. 3% free chlorine) for 3 min, followed by a wash in sterile distilled water. It was then treated with a fungicide slurry for 1 hr.

For each fungicide, a slurry was made up by suspending the equivalent of 5 g or 5 ml a.i. kg^{-1} seed in 2 ml water, for application to approximately 400 seeds. After this treatment, the seeds were air dried overnight. They were then washed for 30 sec under a tap and redried. This washing step was introduced to simulate the frequent irrigation cycles experienced by container-grown seedlings, the assumption being that water falling on the seed would wash off some of the applied fungicides. Finally the treated seeds were placed by hand, 20 per dish, onto 10 water agar plates; i.e., a total of 200 seeds per treatment were tested. The plates were incubated in a Conviron, with $10/20^{0}C$ night and day temperatures and 12 hr light / 12 hr dark. After 6 and 12 d

12 days the seeds were examined with a dissecting microscope. Counts were made of seed infected by the two target fungi. The fungi were identified as discussed Section 4.2.2 above.

The counts were analysed in terms of the χ^2 (Chi Squared) distribution of the control counts. A problem arises when scores are less than 5 infected seeds in 200. In such cases, one is forced to pool the counts for the presence of the two fungi and then test the totals (Rayner, 1967). This is not entirely satisfactory as the activity of some fungicides may be high against one fungus but not another; a sum of the two is then misleading. For example, benomyl is effective against *L. maculans* but ineffective against *Alternaria* species. However, in this trial there was no alternative but to adopt this approach. Using the χ^2 tables with P = 0.05, treatments were considered to be significantly different from the control if falling outside the range 4.5-9% infected seed.

4.3.3 Results

Results are tabulated in Table 4.3.3.A. Fig. 4.3.3.A graphically displays the results for the effective fungicides.

Table 4.3.3.A: Seed Treatment Trial 1: The Activity of 34 Fungicides Against Seed-borne *Leptosphaeria maculans* and *Alternaria brassicicola* [3]

Fungicide CATEGORY [1]	Fungicide Chemical Name	Fungicide Trade Name	% *L. maculans*	% *A. brassicicola*	Both Fungi	Rank
	Control		2.5	11.0	13.5	-
1	anilazine 750wp	Dyrene	1.5	6.0	7.5	10
1	dichlofluanid 500wp	Euparen	3.0	3.5	6.5	6
1	fermicyclox wp		3.0	2.0	4.0	5
1	fermicyclox +	captan 750wp	0.5	5.0	5.5	6
1	guazatine 400soln	Panoctine	3.5	4.5	8.0	11
1	mancozeb 800wp	Ifax	3.5	8.5	12.0	25
1	propineb 700wp	Antracol	1.0	14.5	15.5	29
1	quintozene 750wp	PCNB	4.5	13.5	18.0	32
1	TCMTD 330ec	Busan 30A	3.0	1.5	4.5	3

[3] *Continued on next page*

Fungicide CATEGORY [1]	Fungicide Chemical Name	Fungicide Trade Name	% *L. maculans*	% *A. brassicicola*	Both Fungi	Rank
1	thiram 750wp	Thiulin	1.5	11.0	12.5	27
2	BAS 3308 500wp	experimental	3.5	8.5	12.0	25
2	Bay SLJ wp	experimental	1.5	6.5	8.0	11
2	pyrocarbolid wp	experimental	3.0	9.0	12.0	24
3	iprodione 250sc	Rovral	2.0	2.5	4.5	3
3	procymidone 500wp	Sumisclex	2.5	10.0	12.5	27
4	carboxin 750wp	Vitavax	3.5	8.0	11.5	22
4	oxycarboxin 750wp	Plantvax	0.5	9.0	9.5	16
5	benomyl 500wp	Benlate	0.5	14.5	15.0	29
5	carbendazim 500wp	Bavistin	1.5	10.0	11.5	22
5	thiabendazole 450sc	Tecto	2.5	7.5	10.0	17
5	thiophanate 650wp	Topsin	2.5	14.0	16.5	31
6	benodanil 500wp	Calirus	3.0	7.5	10.5	18
6	bitertanol 250wp	Baycor	1.5	7.0	8.5	13
6	dodine 650wp	Venturol	0.5	10.0	10.5#	18
6	etaconazole 100wp	Sonax	1	7.5	8.5 *	13
6	fenarimol 120ec	Rubigan	3.0	2.5	5.5	6
6	nuarimol 90ec	Trimidal	1.5	9.5	11.0	21
6	imazalil 500ec	Fungazil	1.5	2.5	4.0 *	2
6	propiconazole 250ec	Tilt	0.5	2.0	2.5 *	1
6	triadimenol 150wp	Baytan	3.5	7.0	10.5	18
6	triadimefon 50wp	Bayleton	2.5	6.5	9.0	15
6	tridemorph 750ec	Calixin	3.0	3.5	6.5	9

Key:

# = phytotoxicity; yellowing of leaves * = stunting of germinated seedling
wp = wettable powder formulation sc = suspendable concentrate formulation
ec = emulsifiable concentrate formulation

[1] Fungicide Categories:
1 = contact fungicide
2 = experimental products of unknown action
3 = dicarboximide, translaminar
4 = carboxin, systemic
5 = benzimidazole, systemic
6 = sterol biosynthesis inhibitor, systemic

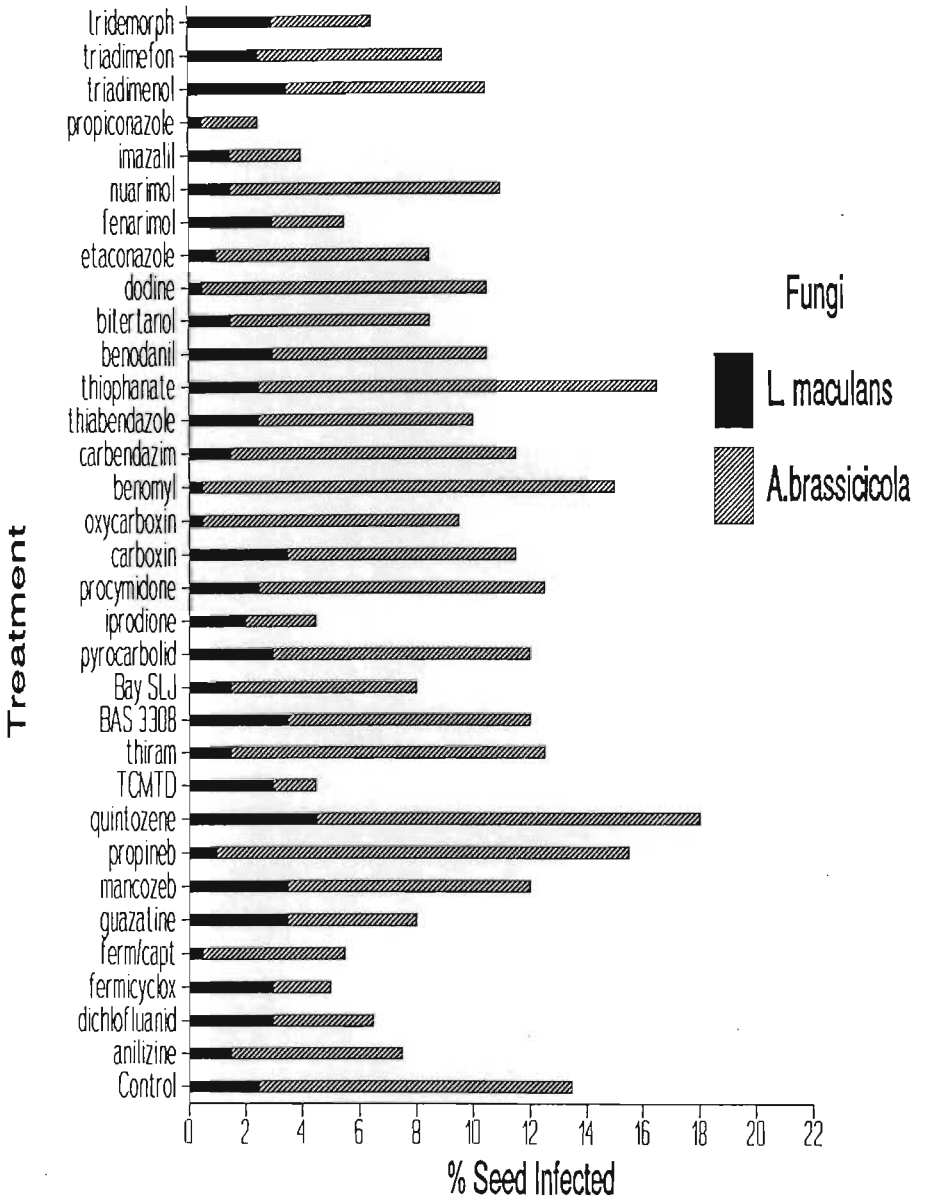

Fig 4.3.3.A Seed Fungicide Trial 1
Fungicidal Control of L.maculans and A.brassicicola in Cabbage Seed

4.3.4 Discussion

Fourteen fungicides showed significant activity against the two fungi. However, it was hard to evaluate activity against *L. maculans* because the level found in the Control treatment was low. A consistent result was the poor performance of the benzimidazoles (benomyl, thiabendazole, thiophanate-methyl and carbendazim) for both *L. maculans* and *Alternaria* control. The latter was not surprising because these fungicides do not control *Alternaria* spp. and even increase the competitive ability of Deuteromycetes. However, of the benzimidazoles, only the benomyl treatment gave a positive test for control of *L. maculans*. This contrasts with the findings of previous researchers who have found thiabendazole, in particular, to be effective against *L. maculans* (Jacobsen and Williams, 1971; Shuring, 1971; Maude *et al*, 1973).

Contrary to the findings of Holtzhausen (1978), neither mancozeb nor quintozene were effective against *L. maculans*. That mancozeb was ineffective may be ascribed to its strictly contact mode of action, and therefore an inability to kill internal fungi. Quintozene is also strictly a contact fungicide, and furthermore, it has a very restricted range of activity, being used almost exclusively as a soil fungicide against *Rhizoctonia solani*, a basidiomycete.

Among the dicarboximides, the excellent performance of iprodione relative to that of a very similar chemical, procymidone, was initially surprising. However, it was probably due to their different formulation, the iprodione tested being in a suspendable concentrate (sc) formulation, whereas the procymidone was in a wettable powder (wp) formulation. Humpherson-Jones and Ainsworth (1981) have shown that the addition of salad oil to the wp formulation of iprodione is necessary to achieve seed coat penetration, while the sc formulation can penetrate the seed coat on its own. It is therefore far more effective than the wp formulation in controlling *L. maculans*.

As a group, the sterol biosynthesis inhibitor (SBI) fungicides gave inconsistent results; some showed high levels of activity (propiconazole, imazalil, fenarimol, tridemorph), whereas others were ineffective (triadimenol, nuarimol). The plant growth regulatory activity of some SBI fungicides (propiconazole and imazalil) was not surprising as this group is closely related to a group of triazole plant growth regulators (PGR), which includes the chemicals uniconazole (Sumagic, Sumitomo Chemicals) and paclobutrazol (Bonzi, Zeneca Chemicals). Their mode of action is to block gibberellin synthesis, and hence cell division is reduced and growth retardation occurs without cytotoxicity. The general effect is to reduce vegetative growth and stimulate flower production (Carlile, 1988).

The activity of TCMTD (Busan 30A, Buckman Laboratories) was interesting because this chemical is a biocide which also kills bacteria and might therefore provide a single treatment to kill both seed-borne *L. maculans* and *X. campestris.* pv. *campestris*. It is registered by Buckman Laboratories to control *X. campestris.* pv. *malvacearum* (Smith) Dye in cotton seed.

4.4 Seed Treatment Trial 2

4.4.1 Introduction

Many of the fungicides tested in Seed Treatment Trial 1 showed sufficient activity to warrant further investigation. The second trial had several objectives:

1. To verify some results from the first trial;
2. To use higher dose rates than used in the first trial;
3. To test some other fungicidal treatments.

4.4.2 Materials and Methods

The Control treatment was the same as for Trial 1.

The doses of fungicides used were double those used in the first because some potentially effective fungicides may have been ineffective in the previous trial because their dose levels were too low. In practice, this meant that each fungicide was made up into a slurry with the equivalent of 10 g or 10 ml a.i. kg^{-1} seed. Enough was made to treat 400 seeds.

An additional fungicide was introduced, triforine, as it is systemic and has activity against the Ascomycetes and *Fungi Imperfecti*. An alternative application of thiram was also introduced, the thiram/vacuum treatment advocated by Maude *et al.* (1969). Mixtures of fungicides were also tested, with the objective of utilizing the reported high activity of the benzimidazoles against *L. maculans* and to use another fungicide to control *A. brassicicola*.

The trial protocol followed was similar to that used in the first trial. One change was that the number of seeds tested was increased by 50%, from 200 to 300 seeds per treatment.

The scores were again analysed in terms of the χ^2 distribution of the pooled scores for *L. maculans* and *Alternaria* spp. Treatments were considered to be significantly different to the control if falling outside the range >7.0 (at P = 0.05).

4.4.3. Results

Table 4.4.3.A contains the details of the results, represented graphically in Fig. 4.4.3.A.

Table 4.4.3.A: Seed Treatment Trial 2: Activity of Selected Fungicides Against *L. maculans* and *A. brassicicola* in Cabbage Seed

Fungicide Category	Treatment	Trade Name	% *L. maculans*	% *A. brassicicola*	Both Fungi	Rank	Comments
-	CONTROL	-	3.7	6.7	10.7	N/A	
1	TCMTD 330ec	Busan 30A	3.5	0	3.5	3	
1	Dichlofluanid 500wp	Euparen	1.0	2.5	3.5	3	
1	fermicyclox wp		1.0	4.5	5.5	11	
1	thiram 750wp + 133 Pa vacuum	Thiulin	4.5	2.0	6.5	13	good growth
2	iprodione 250sc	Rovral	1.5	1.5	3	2	
2	procymidone 500wp	Sumisclex	1.5	3.5	5	10	
3	bupirimate 233ec	Nimrod	6.0	2.5	8.5	16	
3	fenarimol 120ec	Rubigan	2.0	4.5	6.5	13	
3	imazalil 500ec	Fungazil	3.5	1.0	4.5	4	stunting
3	propiconazole 250ec	Tilt	2.0	0.5	2.5	1	stunting
3	triadimefon 50wp	Bayleton	1.0	4.5	5.5	11	
3	triforine 190ec	Saprol	0.5	3.0	3.5	3	
4	benomyl + imazalil		2.5	2.0	4.5	4	stunting
4	benomyl + iprodione		4.0	3.5	7.5	15	
4	benomyl + propiconazole		3.5	0.5	4.0	7	stunting
4	thiabendazole + iprodione		1.5	2.0	3.5	3	

[1] **Fungicide Categories:**

1 = contact fungicide
2 = dicarboximide, translaminar
3 = sterol biosynthesis inhibitor, systemic
4 = mixed systemic fungicides

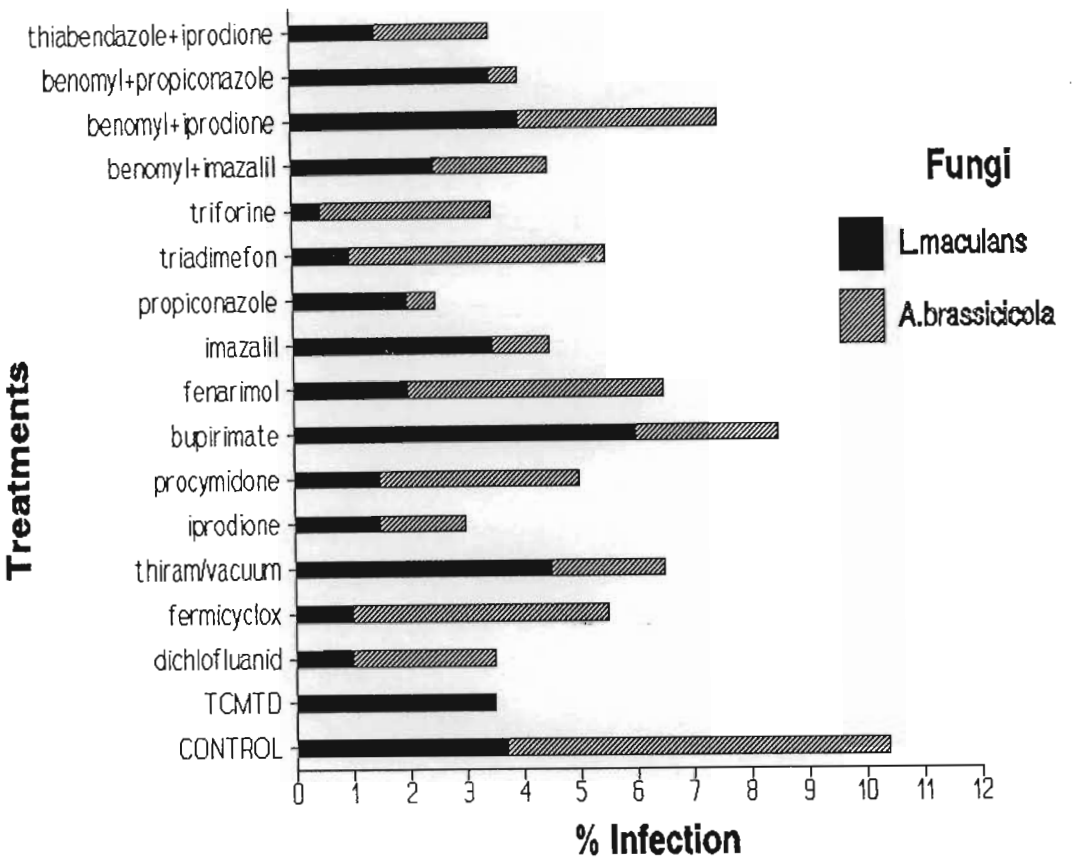

Fig. 4.4.3.A Seed Fungicide Trial 2

Fungicidal Control of L.maculans and A.brassicicola in Cabbage Seed

4.4.4 Discussion

All the fungicides tested, except the benomyl + iprodione mixture, were significantly active against the two test fungi. Given the proven activity of both the constituents against *L. maculans*, it is probable that the lack of activity was due to an incompatibility between the two very different formulations: a wettable powder and a suspendable concentrate.

Both triadimefon and procymidone were effective at the increased rate tested here, in contrast to their inactivity at half this rate tested in the previous trial. Propiconazole was again the most active chemical, but at the rate tested, it also caused stunted growth, as did imazalil. In contrast, the Maude treatment of thiram applied with a vacuum (Maude, 1966), stimulated germination and growth of the cabbage seedlings. The vacuum infiltration resulted in pre-germination priming of the seed and hence its excellent germination and early growth. However, it did not appear to improve the mediocre control of *L. maculans* and *A. brassicicola* by thiram.

4.5 Seed Treatment Trial 3

4.5.1 Introduction

Despite the careful treatment of the infected cabbage seed with a range of the most powerful fungicides available, complete control of neither of the test fungi was achieved in either Seed Treatment Trial 1 or 2. Further research was therefore needed to evaluate fully the most successful test fungicides at different concentrations, and with modifications to the application techniques used.

4.5.2 Materials and Methods

The Control treatment used was the same as for Trial 1 and 2.

An aspect of Trials 1 and 2 which may have reduced the efficacy of the fungicides was the wash applied to the seeds before their testing, a simulation of fungicide leaching considered to occur in container-grown seedling nurseries. This washing treatment was particularly detrimental for superficial, protectant fungicides; it would wash away the protective coat these fungicides had formed. It would have also reduced the amount of systemic fungicide left on seed coats, to be absorbed into the seeds when they started imbibing water during the germination process. However, a change in container-grown seedling technology occurred at this time in South Africa, whereby planted seed trays were no longer put onto production tables immediately; instead, seedling trays are planted with seed, given a single watering, and then placed in a

germination chamber kept at a constant, ideal temperature. Only after full germination are the trays moved out into the nursery where they are subjected to several irrigation cycles daily. The washing step of Seed Treatment Trials 1 and 2 was therefore omitted from Seed Treatment Trial 3.

A second change was to double the amount of water used in the fungicide slurry, from 2 to 4 ml per 400 seeds, and to soak the seed in the slurry for twice as long, for 2 hr.

A third change was to test more seeds, increasing the number from 200 to 1000 seeds per treatment. The increased number allowed for the separation of treatment effects against *L. maculans* and *A. brassicicola*, making it unnecessary to pool fungal counts. To cope with the larger numbers of seeds being tested, the trial was conducted using glass trays, with 200 seeds per tray. Rather than use water agar, seed was germinated on moist paper towels.

The concentrations of the fungicides used were adjusted according to the results obtained in Seed Treatment Trials 1 and 2, and according to manufacturers' recommendations. In particular, the advice of Ciba-Geigy (McKenzie, pers. comm.) was sought with respect to appropriate dose rates of propiconazole, to reduce its PGR effects.

4.5.3 Results

Table 4.5.3.A contains the details of the results, presented graphically in Fig. 4.5.3.A.

Table 4.5.3.A: Seed Treatment Trial 3: Activity of Four Fungicides Against *L. maculans* and *A. brassicicola* in Cabbage Seed

Treatment	Trade Name	a.i./kg seed	% *L. maculans*	% *A. brassicicola*	Comments
CONTROL			2.8	9.7	
TCMTD 330ec	Busan 30A	5 ml	3.1 NS	1.2 *	
"	"	10 ml	3.4 NS	0.2 **	
"	"	20 ml	2.8 NS	0 **	
iprodione 250sc	Rovral	2.5 ml	0.8 **	1.5 *	
"	"	5 ml	0 **	0 **	
"	"	10 ml	0 **	0 **	
propiconazole 250ec	Tilt	0.25 ml	0 **	0.2 **	
"	"	0.5 ml	0 **	0 **	slightly stunted
"	"	5.0 ml	0 **	0 **	stunted
triforine 190ec	Saprol	5 ml	0.5 **	0.3 **	
"	"	10 ml	0 **	0 **	
"	"	20 ml	0 **	0 **	yellowed

NS = not significant

* = significant at P = 0.05, Chi Squared Test

** = significant at P = 0.01, Chi Squared Test

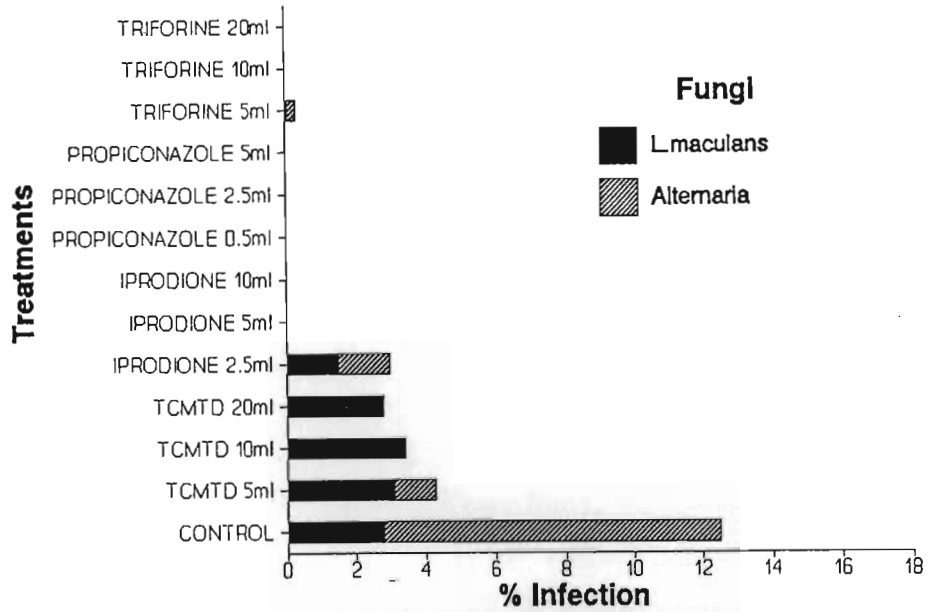

Fig. 4.5.3.A Seed Fungicide Trial 3
Control of L.maculans and Alternaria spp.

4.5.4. Discussion

The experimental protocol followed in Seed Treatment Trial 3 was an improvement on those followed in Trials 1 and 2, providing a greater degree of precision.

TCMTD was highly effective against the superficially-borne *A. brassicicola* but it had no effect on the internally-borne *L. maculans*. It is therefore unlikely to control seed-borne *X. campestris.* pv. *campestris*, a surprising finding, given that TCMTD is registered in the USA to control *X. campestris.* pv. *malvacearum* (Smith) Dye in cotton. One can surmise that *X. campestris.* pv. *malvacearum* is carried superficially on cotton seed, unlike *X. campestris.* pv. *campestris*.

Iprodione was highly effective against both fungi. However, the rate of 2.5 ml a.i. kg^{-1} seed recommended by Humpherson-Jones and Ainsworth (1981) appeared to be too low, and double this rate was more effective under the conditions of this trial.

Propiconazole was extremely effective against both fungi. However, at fully-effective rates there was a distinct dwarfing effect, which the seedlings eventually outgrew. At the lowest rate, which caused no PGR effect, *A. brassicicola* was not eliminated entirely. However, many SBI fungicides of the triazole class have subsequently been synthesized. Many of them have as great a fungicidal activity but much lower PGR activity than propiconazole (Thompson, 1991). It is therefore suggested that this group of fungicides be investigated extensively for the control of *L. maculans* and *Alternaria* spp., both in seed and in the field.

Triforine is a piperazine fungicide discovered in 1967. It is also an SBI fungicide, but without PGR effects, and acts against a wide spectrum of Basidiomycetes, Deuteromycetes and Ascomycetes (Thompson, 1991). The results obtained suggest that it has a wider potential as a seed treatment fungicide than its current registrations in this country would suggest. However, it is a fungicide long out of its patent period, and is, therefore, a commodity product produced and sold by a number of companies. As such, it is probably not being developed in new directions by the various manufacturers, an unfortunate commercial reality.

The most significant aspect of this trial was that treatments were found which eliminated all seed-borne inoculum of *L. maculans* and *Alternaria* from contaminated seedlots. This reflects similar results of Humpherson-Jones *et al.* (1984) and Maude *et al.* (1984). The epidemiological implication is that seedborne inoculum could be eliminated universally if there is the political and economic will and resources to do so.

A potential problem lies in the danger of fungal resistance to systemic fungicides. If the effective fungicides identified above were to be used universally on all crucifer seeds then there would be strong selection pressure for resistance to the dicarboximides or the triazoles (Cremlyn, 1991). Strains of *L. maculans* resistant to benzimidazoles have already appeared (Shuring, 1971). Practical solutions would be to alternate or to mix effective fungicides with different modes of action (Kable and Jeffrey, 1980; Skylakakis, 1981; 1982a; 1982b; 1984; Wade, 1988).

An aspect to consider is the meaninglessness of statistics in the above trial: a significant reduction in fungal contamination is irrelevant if the level of *L. maculans*' contamination stays above the TIL, determined by Gabrielson (1983) to be 0 in 10 000. For practical and epidemiological purposes then, there is a zero tolerance; any fungicidal treatment unable to produce this result would not be able to stop an epidemic developing from seed-borne inoculum.

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CHAPTER 5. CULTIVAR RESISTANCE STUDIES

Infectious disease is an unfortunate side effect of the struggle for survival. Ever since organisms climbed out of the primeval soup, they have been bumping into one another during their search for a place to stand and a bite to eat. When one has bumped into another that it can live upon, it has stayed to dinner. The entrance of the diner is infection, and disease is the cost of the dinner, paid by the host.

Waggoner, 1977

Abstract

In a field trial of eight cabbage and two cauliflower cultivars, incidence of stem infection by *L. maculans* ranged from 16-80%, cauliflower cultivars being far more susceptible than any of the cabbage cultivars. A horizontal resistance breeding programme is proposed, based on the broad range of quantitative resistance found. Two seedlots of the cabbage cultivar Gloria Osena differed in susceptibility to *L. maculans*. Correlations between either stem lesion incidence and foliar infection counts, or between stem lesion incidence and average days-to-harvest within each cultivar were tested but found non-significant.

In a second trial 15 cabbage, four cauliflower and one broccoli, kohl rabi and Brussels sprout cultivars were tested for blackleg resistance. Trials were also conducted on multiple seedlots of both cabbage and cauliflower cultivars. Incidence of stem infection ranged from 50% (Rotan) to 95% (Dynasty) in cabbage, and 64.2 to 96.6 in cauliflower. Brussels sprouts and broccoli were highly susceptible. No significant correlation was found between stem length and disease incidence. Significant variation in blackleg incidence was again found between seedlots of the same cultivar, in several cultivars of cabbage and cauliflower.

An observational trial on single cultivars of turnip, tyfon, swedes, Japanese radish, chou moulier and red cabbage showed the turnip and tyfon cultivars tested to be immune to blackleg but the other plants to be highly susceptible.
A third trial testing 10 replicates of four seedlots of the cabbage cultivar Hercules confirmed that different seedlots of a single cabbage cultivar may vary in susceptibility to blackleg.

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5.1 Introduction

The entire genus *Brassica* is recognized as being susceptible to the fungus *L. maculans*, as discussed in Section 1.2. Effective levels of genetic resistance have been developed in cultivars of canola (*B. campestris* and *B. napus*) (Alabouvette *et al.*, 1974; Thurling and Venn, 1977; Roy, 1978; Helms and Cruikshank, 1979; Cargeeg and Thurling, 1980a; 1980b). However, useful levels of resistance have not yet been bred into the *B. oleracea* group of crucifers. Van Marrewijk (1974), in a review of the literature on breeding for blackleg resistance, considered the chances for finding resistance in the *B. oleracea* group to be very slim. However, he was probably looking for complete Vertical Resistance (VR) (*sensu* Vanderplank, 1968; 1984), which does not appear to be present. However, in view of *L. maculans*'s known variability and the existence of pathogenic races (Thurling and Venn, 1977; Venn, 1979; Cargeeg and Thurling, 1980a; Koch *et al.*, 1989), and the development of a differential series in canola (Koch *et al.*, 1991), VR is clearly present in canola. However, the observations of Chupp and Sherf (1960), where the emphasis was on variations in susceptibility, indicated that potentially exploitable levels of Horizontal Resistance (HR) (*sensu* Vanderplank, 1984) exist in the *B. oleracea* group. Furthermore, crucifers are strongly outbreeding, which allows for recurrent selection methods and the selection for HR. A series of trials were therefore conducted to establish whether useful levels of blackleg resistance could be found in locally available commercial cabbage cultivars.

5.2 Cultivar Trial 1

5.2.1 Introduction

The first cultivar trial was conducted on a farm in the mistbelt region of the Karkloof Valley, 20 km north-east of Howick, a town which lies some 30 km north of Pietermaritzburg. The farmer had experienced an outbreak of blackleg in the preceding cabbage crop and was, therefore, prepared to assist in the trial on his lands.

5.2.2 Materials and Methods

The soils were of the Hutton Series with >35% clay content, and were fertilized with calcitic lime and 2.3.2 (28) fertilizer (Triomph) at the rate of 600 kg ha^{-1} each, preplant, rotovated into the soil.

A randomised complete blocks design was used, with 10 treatments allocated to eight cabbage and two cauliflower cultivars in four replicates, with 36 plants per plot.

Cabbage cultivars tested were Sunlander and Green Coronet (Starke-Ayres Seed (Pty) Ltd, Pietermaritzburg), Gloria F1 and Glory of Enkhuisen (McDonald's Seeds (Pty) Ltd, Pietermaritzburg), Rotan (Hygrotech Seeds (Pty) Ltd, Pretoria), Gloria Osena, Grand Slam and Hercules (Mayford Seeds (Pty) Ltd, Johannesburg). The cauliflower cultivars tested were Igea 65 and Snowcap (Starke-Ayres). Cultivars grown commercially were used, with the exception of Glory of Enkhuisen, which was chosen because it is an old, open-pollinated cabbage variety. Due to different local marketing names, two different seed lots of one cultivar, Gloria Osena, were tested; hereafter termed Gloria F1 and Gloria Osena. All samples were treated with a benlate/thiram slurry as reported by Gabrielson *et al.* (1977), to eliminate any endogenous seed-borne *L. maculans* inoculum.

The seedlings were grown in a commercial containerized seedling nursery. When 6 wk old they were hand transplanted at a standard field spacing of 0.5 m x 0.5 m into square plots of 3.5 m x 3.5 m, with a 1 m border between plots.

A topdressing of L.A.N. (28% N) was applied at transplanting and again at 4 wk post-planting, on both occasions at the rate of 150 kg N ha^{-1}. Boron, zinc and molybdenum were applied at rates of 1000 g Solubor®, 500 g zinc oxide and 125 g sodium molybdate in 500 ℓ water ha^{-1}, at 2, 4 and 6 wk after transplanting, in order to overcome micronutrient deficiencies common in KwaZulu-Natal soils. The rates used were as determined in trials by Richards (1982).

Weed control was provided by a preplant application of Lasso® (alachlor, Monsanto) and a single handweeding after 70 d. Insect control was provided with backpack spraying of Metacystox® (demeton-s-methyl, Bayer (SA)) and Ambush® (permethrin, Zeneca) applied every 14 d to control aphids and caterpillars, respectively.

In order to provide a uniform source of inoculum, the experimental area was artificially inoculated with blackleg-infected stems collected from other cabbage crops. This material was stored for 4 mo at 4^0C, then put through a commercial silage cutter to produce chunks of cabbage stem approximately 30 mm in length. The chopped debris was then distributed evenly over the experimental area, at a rate of approximately 25 g m^2 (300 g per 12.5 m^2 plot) 2 wk after transplanting. Renard and Brun (1980) successfully used a similar technique to inoculate canola cultivar trials.

Plant stems and leaves were examined individually for the presence of blackleg lesions. Leaf lesions were counted at approximately two-weekly intervals on six occasions. Only active lesions were counted; i.e., lesions surrounded by viable leaf tissue as opposed to lesions present on necrotic or senescing tissue. Lesion numbers fluctuated as older leaves were shed at the bases of plants. Stem lesions were also counted at approximately 14 d intervals. The stem of each plant was examined individually and the presence or absence of a typical blackleg lesion recorded. The size of each lesion was not quantified, an approach proposed by van den Berg and Rimmer (1992).

Statistical analysis was conducted on an Apple IIE microcomputer using *ANOVA II* from Human Systems Dynamics. A cube root transformation was applied to the leaf lesion data. Separation of means was by Fisher's LSD. Correlation coefficients and linear regressions were both calculated using either *Stats with Daisy* from Rainbow Computing, Inc., on an Apple IIE, or with Statsgraphics on an 80386 IBM PC clone.

5.2.3 Results

The results are presented in Tables 5.2.3.A-B and Fig. 5.2.3.A.

Table 5.2.3.A: Cultivar Trial 1: Stem and Leaf Blackleg Lesion Data

Cultivar cabbage = 1 cauliflower = 2		% of Stems Infected with *L.maculans*	√ Arc Sine of % Stem Lesions *		Foliar Lesion Count	³√ of Foliar Lesion Count *	
Sunlander	1	15.8	23.3	a	105.0	4.7	cd
Rotan	1	16.7	23.9	ab	126.0	5.0	de
Enkhuisen	1	18.3	25.2	abc	81.5	4.3	bc
Coronet	1	20.0	26.5	abc	160.0	5.4	e
Gloria F1	1	21.7	27.6	bc	77.3	4.2	b
Grandslam	1	31.7	34.2	d	138.3	5.2	de
Hercules	1	34.2	35.8	d	113.0	4.8	d
Gloria Osena	1	43.3	41.2	e	109.8	4.7	bcd
Igea	2	55.8	48.4	f	43.3	3.4	a
Snowcap	2	79.2	62.9	g	54.8	3.7	a
STATS		F = 119.6 *** CV% = 11.2 LSD (0.05) = 6.32	F = 88.0 *** CV% = 7.8 LSD (0.05) = 4.58		F = 5.1 ** CV% = 32.3	F = 5.72 ** CV% = 11.5 LSD (0.05) = 0.478	

* Figures with the same letter do not differ significantly at the level, P = 0.05, using Fisher's LSD Test.

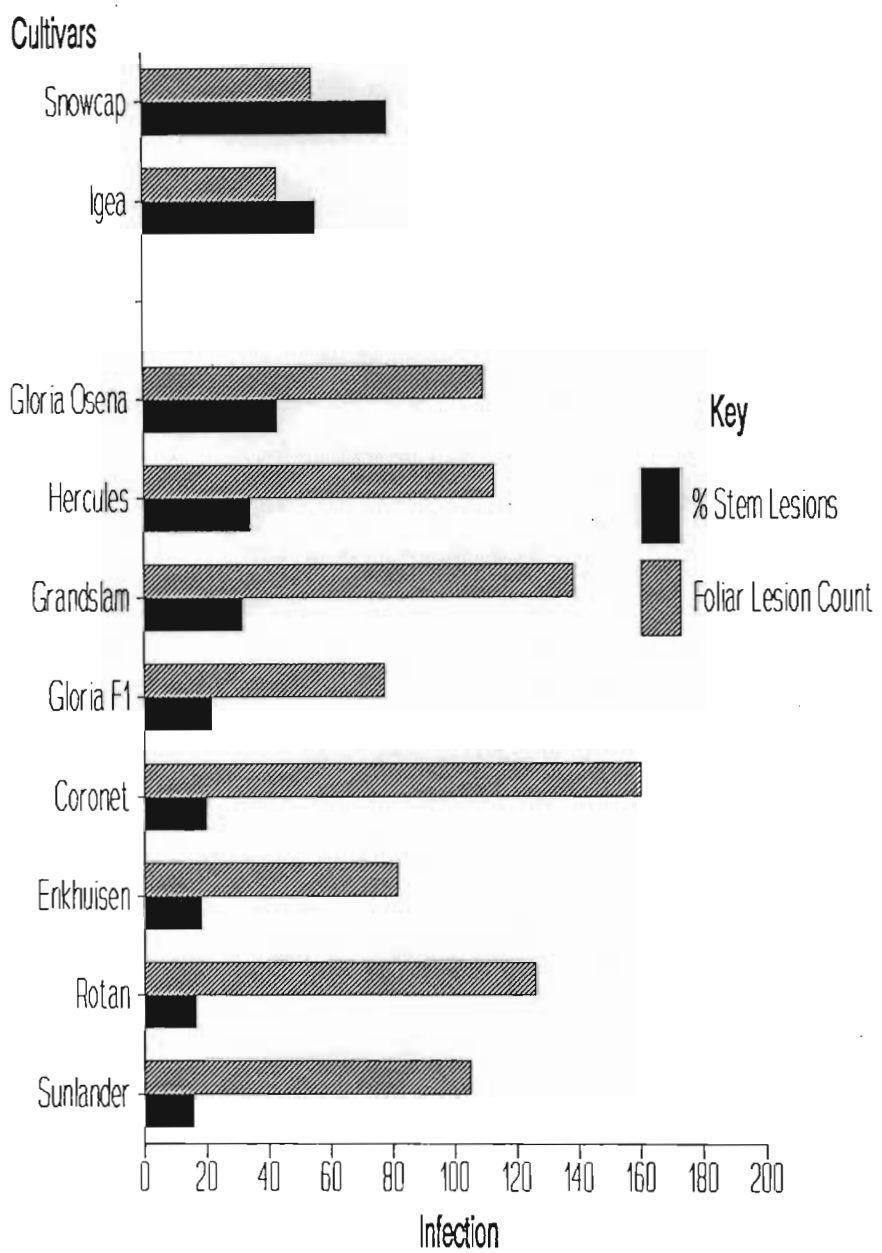

Fig 5.2.3.A Cultivar Trial 1: Susceptibility of Cabbage and Cauliflower Cultivars to Leptosphaeria maculans

The severity of foliar infection and the incidence of stem infections might be linked. If the primary inoculum for stem infection arose from pycnidiospores released from leaf lesions, then there should be a strong correlation between the two data sets. Correlation coefficients and linear regressions were therefore calculated between pairs of means of the two parameters.

Another possible link was between disease incidence and severity, with days-to-harvest of each cultivar. Correlation coefficients and linear regressions were therefore calculated between stem lesion incidence and foliar lesion severity, and each cultivar's average days-to-harvest.

The following results were produced by these analyses:

Table 5.2.3.B: Cultivar Trial 1: Correlation and Regression Relationships

Subject	Correlation Coefficient	R^2	Standard Error Estimate	F Value
Stem Lesion Incidence : Foliage Lesion Counts	0.069	0.0074	7.03	0.023 NS
Stem Lesion Incidence: Days-to-harvest	-1.43	0.123	6.98	0.126 NS
Leaf Lesion Counts: Days-to-harvest	0.205	0.069	0.42	0.264 NS

The correlation coefficients and linear regression values were non-significant for all of the relationships tested.

In order to clarify the Within Cultivar variation in blackleg susceptibility, the results of the two Gloria Osena seedlots are abstracted and presented in Table 5.2.3.C and Fig. 5.2.3.B.

Table 5.2.3.C: Cultivar Trial 1: Differences in Blackleg Susceptibility Between Two Seedlots of the Cabbage Cultivar Gloria Osena

Cultivar	% Stem Lesions	√ Arc Sine of % Stem Lesions	Foliar Lesion Count	^{3}√ of Foliar Lesion Count
Gloria F1 McDonalds 8A1235E	21,67 a	27,57 a	77,25	4,22 a
Gloria Osena Mayfords EZI5247	43,33 b	41,16 b	109,75	4,68 b
F Test	F = 119,6 ***	F = 88,0 ***	F = 5,1 **	F = 5,72 **
LSD (P < 0,05)	6,32	4,58	5,7	0,478
CV%	11,2%	7,8%	32,3%	11,5%

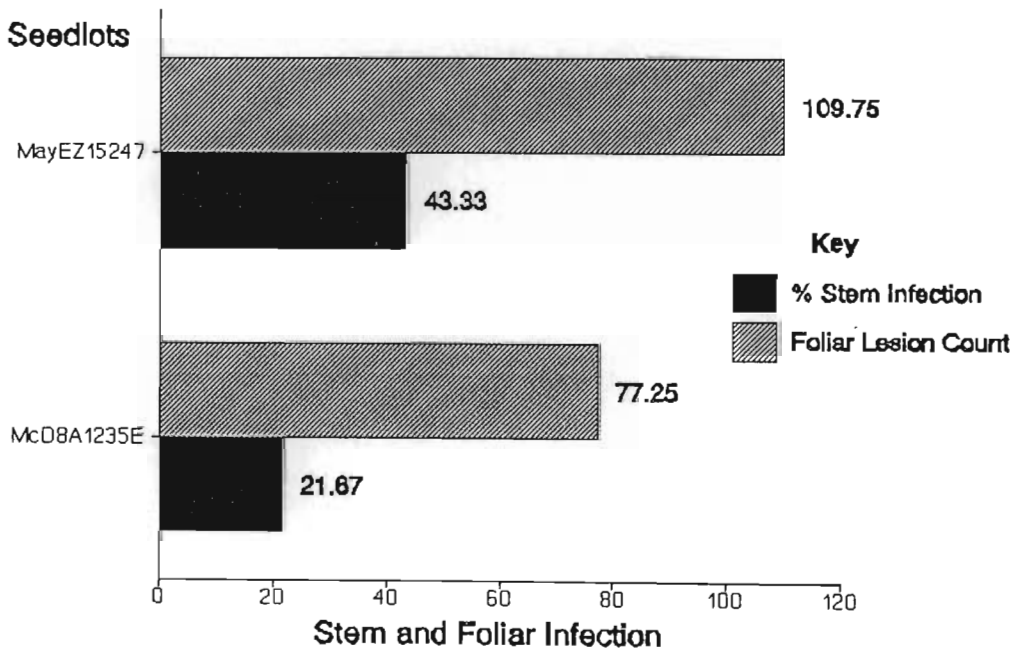

Fig. 5.2.3.B Within Cultivar Trial 1 Blackleg Incidence in Gloria Osena

5.2.4 Discussion

5.2.4.1 Between Cultivar Variation

Variation in the susceptibility of cabbage cultivars to blackleg is evident in Table 5.2.3.A and Fig. 5.2.3.A. Susceptibilities, as measured by the percentage of stems with one or more lesions caused by *L. maculans*, ranged from 16% for the cabbage cultivar Sunlander to 43% for Gloria Osena. Both the cauliflower cultivars tested were much more susceptible than any of the cabbage cultivars. Almost 80% of the stems of the Australian cauliflower hybrid, Snowcap, were infected with *L. maculans*.

The artificial inoculation technique used in this study parallels cabbage farming practices in KwaZulu-Natal. The usual harvesting procedure is for labour to avoid cutting undersized heads, thereby leaving diseased plants in the field. This ensures that blackleg-infected stems are left in the production fields which is likely to lead to levels of inoculum similar to those artificially created at the start of this experiment. By mimicking the natural pattern of inoculum continuity, the artificial inoculation used in this study clearly established the significant role of infected debris in the epidemiology of blackleg. Despite the careful use of clean seed and healthy seedlings, the most susceptible cauliflower cultivar, Snowcap, developed a terminal disease incidence of 80%.

Within the cabbage group, the wide range in the levels of susceptibility appear to contradict Van Marrewijk's (1974) conclusion that there is no useful resistance to blackleg in the *B. oleracea* group. It is apparent from the context of Van Marrewijk's (1974) discussion that he was looking for high levels of qualitative resistance, as is customary among plant breeders committed to first finding a "good source of resistance" for use in VR breeding programmes. Alternatively, if the emphasis was placed on HR, then quantitative variation in levels of susceptibility, as revealed in Table 5.2.3.A, might not only be adequate for immediate protection, but could also provide parent material for a HR breeding programme.

Starting with susceptible parents, effective levels of HR can be accumulated due to transgressive segregation. Vanderplank (1984) discussed the role of transgressive segregation in HR breeding programmes. The strategy of starting with susceptible parents, also advocated by Robinson (1987), has been used successfully by many, including Krupinsky and Sharp (1979), Sharp (1983), Beek (1983; 1984), de Milliano (1982; 1983), Van Der Graaff and Pieters (1983) and Van der Graaff (1983; 1985). Thus, the validity of a breeding strategy based not on spectacular, qualitative VR but on effective, quantitative resistance has been established. The variations in quantitative resistance measured in this study could, therefore, serve as a basis for a HR breeding programme. As a family, the crucifers are eminently suited to the technique of HR breeding because outcrossing is the norm and in many cases inbreeding is difficult, due to incompatibility phenomena. A further advantage is that the production of doubled haploids or dihaploids in crucifers is possible (MacDonald and Ingram, 1984). This would facilitate the rapid development of homozygous "inbred" parents, taken from a heterozygous, random polycrossed parent population, from which to produce hybrids. This technique is already being used in a Canadian canola breeding programme (Bansal *et al.*, 1994).

The quantitative nature of the resistance detected in the cabbage cultivars tested in KwaZulu-Natal is not clear evidence that the resistance is Horizontal. It may be quantitative VR. Until there is evidence for the existence of different pathotypes of *L. maculans* in KwaZulu-Natal, all that can be said is that the resistance detected here was quantitative. If cabbage breeding were to be undertaken, then the possibility of Vertical pathotypes of *L. maculans* existing in KwaZulu-Natal would need to be investigated. If present, such pathotypes should be used to detect possible differential interactions (*sensu* Vanderplank, 1963; 1984) in the cultivars evaluated here. Such Vertical pathotypes of *L. maculans* have been detected in other pathosystems (Thurling and Venn, 1977; Williams and Delwiche, 1979; Cargeeg and Thurling, 1980a; Bonman *et al.*, 1981; Pang and Halloran, 1995). However, Robinson (1987) reported the various breeding techniques available to eliminate the influence of vertical pathotypes and vertical resistance genes from a HR breeding programme. The easiest, and most consistent of these is the Single-Pathotype Technique, one which could easily be applied to the blackleg pathosystem.

Thus, although it is feasible to breed for HR in crucifers, this has not happened here or elsewhere. This is probably because farmers do not demand such host resistance in cabbage and other vegetable crucifers, with the result that seed companies are not prepared to initiate or fund such a breeding programme.

The extreme susceptibility of the stems of the two cauliflower compared to the stems of the cabbage cultivars was disconcerting. The increased susceptibility might have been due to physiological factors controlling resistance expression. However, the difference might also have been due to morphological differences between cauliflower and cabbages. The upright growth habit of cauliflowers, which leaves the lower stem very exposed, contrasts with the flattened growth habit of cabbages, where the stem is more-or-less covered with leaves. It was therefore postulated that less water-splashed inoculum would be deposited on cabbage stems than on cauliflower stems. This suggestion was also supported by the field observation that within a single cabbage cultivar, plants with multiple heads developed more stem infections than single headed plants (Laing, unpublished). Blackleg infection was frequently observed at the juncture where a stem divides; it has the shape to "catch" and hold water dripping off leaves. The stem of a plant with multiple heads is also more exposed, as the several heads grow away from each other, leaving it exposed.

The matter of klendusity (*sensu* Grau, *et al.*, 1982) remains an unresolved dilemma in disease resistance breeding. Not only should it be considered when levels of resistance are evaluated, but also cognizance should be taken that quick laboratory screening techniques may not reflect this phenotypic characteristic at all. Indeed, it is probable that klendusity has a confounding effect on all field screening procedures, but despite this, it is largely ignored.

Although there is a difference of approximately 2 wk in the cropping periods of the cauliflower cultivars, Igea 65 and Snowcap, their morphological habits are similar. Thus the difference of 24% in their levels of stem infections (56% and 80% respectively) is probably due to a real difference in physiological resistance. The difference in cropping period alluded to above is unlikely to be responsible for this

difference in stem infection because the majority of the recorded infections had occurred before the end of the cropping season. Although this argument addresses the difference in blackleg susceptibility between cauliflower varieties, it does not clarify the extent to which klendusity may have affected the disease ratings obtained.

Consider next the cumulative leaf lesion counts which reflect the totals of assessments made at bi-weekly intervals. Attempts were made to establish epidemiological rate parameters from the leaf lesion data. However, the determinate nature of *L. maculans* lesions, their confinement to older lower leaves, as well as the frequent loss of such senescing leaves, makes it difficult to measure disease activity in terms of the apparent infection rate or Progeny-to-Parent lesion ratio. Furthermore, the cabbage cultivars evaluated have significantly different leaf growth rates and morphologies, which would have affected the apparent infection rates.

More importantly, calculation of a correlation coefficient and a linear regression revealed that there was no correlation between foliar disease severity with stem disease incidence (measured as cumulative leaf lesion count) and percentage stem infection. This has an important implication for plant breeders: screening methods based on leaf lesions alone may not be accurate indicators of stem resistance, which must be the paramount disease parameter to be tested because stem lesions are more debilitating than leaf lesions. Thus any rapid assay of blackleg resistance using cotyledonary or foliar infection assessments must be validated with field assessments of stem susceptibility (Wood and Barbetti, 1977; Helms and Cruikshank, 1979; Cargeeg and Thurling, 1980b).

From the lack of correlation between each cultivar's infection data and its days-to-harvest, it would appear that there is no link between blackleg susceptibility and the rate of growth of the cultivar. This is surprising in that cultivars which spend a longer time in the soil would be expected to have a higher blackleg incidence. Alternatively, fast-growing cultivars might develop nutrient stress if their heads filled extremely rapidly and, therefore, would be more disease-susceptible (Vanderplank, 1984), a theory investigated later.

5.2.4.2 Within Cultivar Variation

In Table 5.2.3.A two seedlots of the same cultivar are labelled Gloria F1 and Gloria Osena, with 22% and 43% stem infections respectively. The difference was highly significant ($P < 0.001$). This particular trial result can be explained in one of two ways:

EITHER

1. The trial result was, in statistical terms, an Error Type II; i.e., a false positive result, where a significant difference is apparently detected, but which does not actually exist. The measured F value was large, and the corresponding P value was vanishingly small. However, there remains a small possibility that such an error occurred here.

   OR, more probably,

2. A real differences between the different seedlots of a single cultivar was detected.

This latter, unexpected discovery lead to two subsequent Within Cultivar Trials. The primary implication of such a result was that disease resistance and other genetic characters in cultivars could vary from seedlot to seedlot. This brought into question the validity of cultivar trials.

It also raised the question as to the genetic constitution of the two seedlots tested: how did they differ ? However, attempts to identify the genetic nature of the two seed lots of Gloria Osena tested here were unsuccessful as the seed companies involved were not prepared to divulge any specific information. It was pieced together that the cultivar originated from Japanese parent lines. The commercial seed was produced either in Italy or the USA, and a Dutch company marketed it world-wide. However, the international seed trade is a very secretive business, and it became apparent that there was no chance of getting any detailed information about seedlots, their inbred parents, where seed production took place, etc., of this or other seedlots.

5.3 Cultivar Trial 2

5.3.1 Introduction

A number of questions emerged from the first cultivar trial, including the variability of within cultivar susceptibility. Other commercial and experimental cultivars were included in this follow-up trial. Given the high susceptibility of the two cultivars tested in Cultivar Trial 1, several commercial cauliflower cultivars were tested to see whether the high levels of susceptibility observed previously were common to other cauliflower cultivars. Several other cruciferous vegetables are grown in KwaZulu-Natal and these also were tested for their levels of blackleg susceptibility. Multiple seedlots of several cabbage and cauliflower cultivars were tested to see if blackleg susceptibility varied between seedlots of the same cultivars.

The second trial site was at Baynesfield Estates, Thornville Junction, a large farming operation left in trust to South Africa by the original owner, Thomas Baynes. The farm lies 25 km south-west of Pietermaritzburg, and is run as a mixed pig, beef, crops and horticultural operation. The horticultural section, which depended largely upon cabbage production for income, had experienced two successive outbreaks of blackleg in preceding cabbage crops, and therefore agreed to the trial being conducted on their lands.

5.3.2 Materials and Methods

A randomized complete block design was used with three replicates, 36 plants per plot, spaced at 0.5 m x 0.5 m, with a 1 m border between plots.

Fifteen cabbage cultivars were tested, and three within-cultivar variation assessments were made. Eight seed sources of the cultivar Gloria Osena (Starke-Ayres 3057/J; McDonald's 8A1235E, 8B1024; Mayford's EZI5247, 11210,30401; Ohlsen Enke A1, A2), five of Green Coronet (McDonald's 1006, 8C427D, 8B233; Starke-Ayres 2019/A/OJC; Mayford's 5749), and three of Hercules (Starke-Ayres, 19281; Mayford's

L1720, 5659) were tested to determine the extent of within-cultivar variation in blackleg susceptibility. Five experimental cultivars from Starke-Ayres (1749-1753) and the cultivars Rotan (McDonald's 8A123A)), Green Star (Starke-Ayres 3194), Sunlander (Starke-Ayres 29781), Grandslam (Mayfords 5672), Glory of Enkhuisen (Starke-Ayres 3057/B), Bonanza (Starke-Ayres 3131/C) and Dynasty (Mayfords 5680) were also tested in the cabbage trial.

Four different cauliflower cultivars were tested: Igea 65 (Starke-Ayres 0234); Couchamp 3 (1054); a locally bred open-pollinated variety from Weenen, (called Hojem after the breeder); and Snowcap. Three different seedlots of the cultivar Snowcap were tested, two from Australia (Starke-Ayres 0110B; Starke-Ayres 1239A); and one from Oudtshoorn, South Africa (Gellman 213/80).

Other crucifers tested in the trial were grown from a single seed sample each: broccoli (McDonald's 31264); kohl rabi (McDonald's 20313); and Brussels sprout (McDonald's 21195).

In the absence of enough land for a complete trial, the following single plot, observational trials were planted with cruciferous fodder and vegetable crops: swedes, turnips, tyfon (*B. napus* L.), Japanese radish, chou moulier (marrow-stemmed kale) (all from McDonald's Seeds) and a red cabbage (cultivar Red Rock, from Starke-Ayres).

All seedlings were grown in a commercial CGS nursery and transplanted after approximately 6 wk.

The field used has a Hutton soil with >35% clay content, and was fertilized with calcitic lime and 2.3.2 (28) at the rate of 1000 kg ha^{-1} preplant, disced into the soil. Application of topdressing and micronutrients, and insect and weed control was as described for the Cultivar Trial 1, Section 5.2.2.

The inoculation procedure with infected debris was much the same as in the previous trial (Section 5.2.2), the infected cabbage stems having been collected from previous blackleg epidemics at Haliwell Farm, Howick. A minor difference was that the infected stem debris used as inoculum in this trial was prepared in a Black and Decker compost-maker, a garden device which chopped the cabbage stems into lengths of 30 mm or less.

Every plant was evaluated for four parameters:

1. Presence of blackleg lesions on the stem
2. Death of plant
3. Length of stem
4. Yield (head mass).

It was hoped that if disease susceptibility could not reliably be measured as a function of the incidence of disease, then disease severity would be an alternative, using a count of dead plants. Head mass of each cabbage was measured because some plants might have exhibited disease tolerance (*sensu* Robinson, 1979), and although infected, still produce large, hard heads. In view of earlier observations in Cultivar Trial 1, on the possible role of morphology on disease susceptibility, stem lengths were measured, to determine any correlation between this parameter and blackleg susceptibility. The mean mass of the heaviest 33% of the heads of each plot was used to calculate a mean mass for each cultivar.

ANOVA and correlation coefficients of the data were conducted on the disease data using the same statistical packages as discussed in Section 5.2.2 above.

5.3.3 Results

The results are presented in Tables 5.3.3.A-C and Figs 5.3.3.A-B.

Table 5.3.3.A: Cultivar Trial 2: Incidence of Blackleg Stem Infection and Other Parameters in Cabbage Cultivars

Cabbage Cultivars	% of Stems Infected by *L. maculans* *	% Killed by *L. maculans*	Stem Length (mm)	Mean Mass: All Heads (g)	Mean Mass: Top 33% of Heads (g)
Rotan	50.7 a	0	121	1683	2603
Green Star	72.2 cde	0.9	204	1570	2217
Sunlander	77.8 cdefghij	15.7	177	1456	2214
Grandslam	88.0 klmn	9.3	197	947	2667
G.Enkhuisen	53.1 a	0.9	158	1200	2012
Bonanza	80.1 defghij	3.0	224	1958	2337
Dynasty	95.4 n	14.3	183	1538	2602
Experimental 2	85.2 hijk	1.9	208	1870	2689
Experimental 3	60.2 ab	2.8	134	1172	1710
Experimental 4	68.1 cde	0.9	165	994	1977
Experimental 5	68.5 bc	4.6	164	1069	1765
Experimental 6	70.8 cd	5.6	195	1473	2185
Hercules SA19281	91.3 klmn	17.2	193	1448	2677
Hercules MayL1720	89.8 jklm	19.3	196	1709	2666
Hercules May 5659	86.1 hijklm	18.5	193	1722	2480
Green Coronet McD1006	93.5 lmn	6.5	181	1772	2837
Green Coronet McD8C427D	83.0 efghijk	1.9	192	2148	3198
Green Coronet McD8B233	91.5 efghij	4.7	180	2017	3204
Green Coronet SA2019/A/OJ	77.8 cdefgh	5.6	192	1884	2807
Green Coronet May5749	84.7 fghijk	4.6	190	2386	2888
Gloria SA3057/J	86.1 hijkl	0	160	1317	1989
Gloria McD8A1235E	67.6 cd	0.9	180	1426	2355
Gloria McD8B1024	78.7 ghijkl	0	176	1742	2673
Gloria MayEZ15247	83.9 ghijkl	0.9	169	1154	2329
Gloria May11210	91.0 hijk	0.9	167	1492	2331
Gloria May30401	74.1 cdefg	0.9	158	1263	2265
Gloria OE A1	73.6 cdef	1.9	177	1791	2492
Gloria OE A2	80.0 defghi	0	176	1635	2529
F value	3.3 **	1.4 NS			
LSD (P=0.05)	10.0				
CV %	13.5%	23%			

* Figures with the same letter do not differ significantly at the level, P = 0.05., using Fisher's LSD Test.

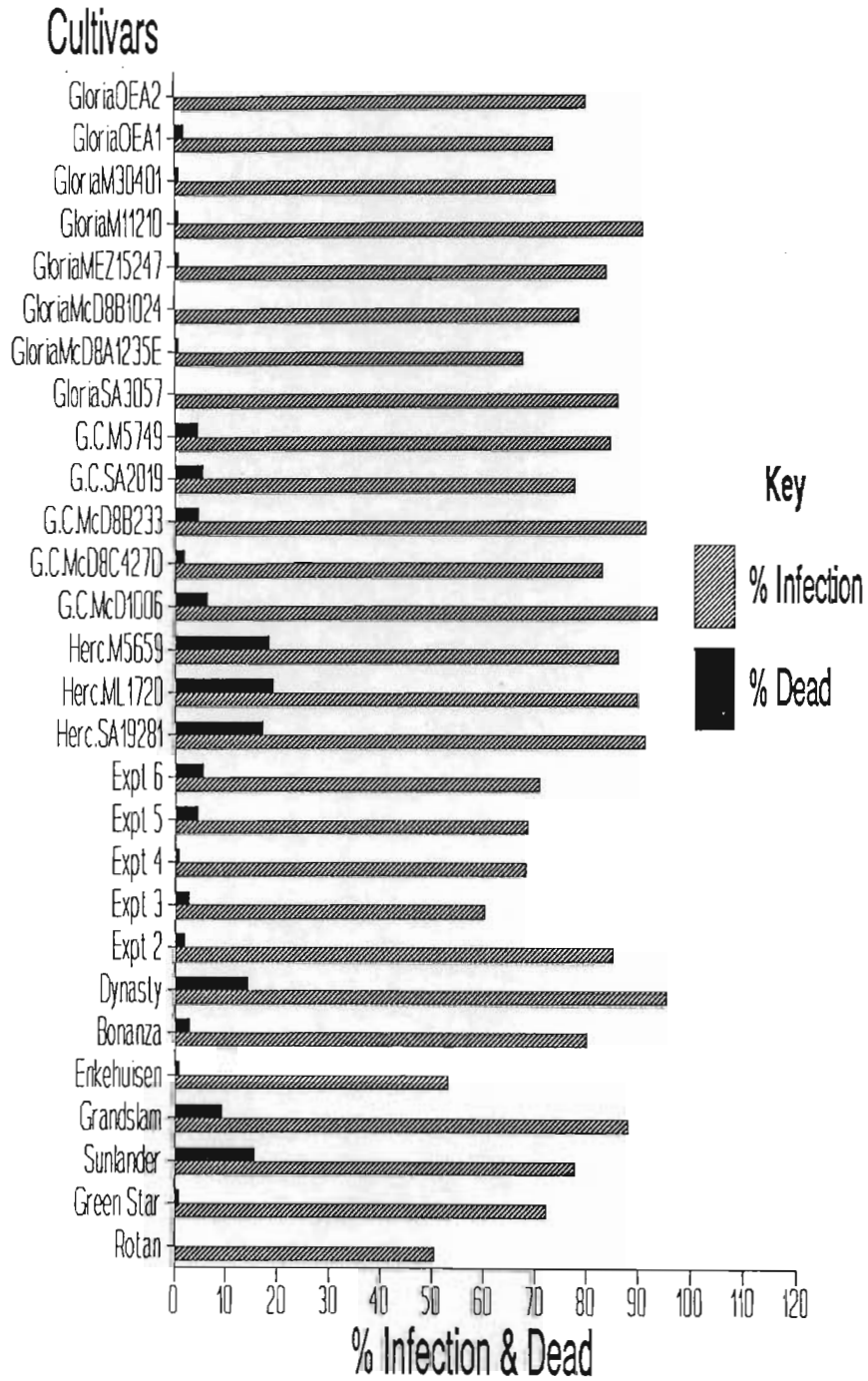

Fig. 5.3.3.A Cultivar Trial 2: Susceptibility of Cabbage Cultivars to Leptosphaeria maculans

Ranked in terms of decreasing resistance to stem infection by *L. maculans*, the cabbage cultivars and seedlots tested fall into the following order (the LSD grouping is given in brackets):

1. Rotan	(a)
2. Glory of Enkhuisen	(a)
3. Experimental 3	(ab)
4. Experimental 5	(bc)
5. Gloria Osena 2	(cd)
6. Experimental 6	(cd)
7. Green Star	(cde)
8. Experimental 4	(cde)
9. Gloria 7	(cdef)
10. Gloria 6	(cdefg)
11. Green Coronet 4	(cdefgh)
12. Sunlander	(cdefghi)
13. Bonanza	(defghij)
14. Gloria 8	(defghij)
15. Green Coronet 3	(efghij)
16. Green Coronet 2	(efghijk)
17. Green Coronet 5	(fghijk)
18. Gloria 3	(ghijkkl)
19. Gloria 4	(ghijkl)
20. Gloria 1	(hijkl)
21. Hercules 3	(hijklm)
22. Experimental 2	(hijk)
23. Gloria 5	(ijklm)
24. Hercules 2	(jklm)
25. Grand Slam	(klmn)
26. Hercules 1	(klmn)
27. Green Coronet 1	(lmn)
28. Dynasty	(n)

Table 5.3.3.B: Cultivar Trial 2: Incidence of Blackleg Stem Infection and Other Parameters in Cauliflower Cultivars and Other Crucifers

Cultivars	% of Stems Infected with *L. maculans* *		% Killed by *L. maculans*	Stem Length (mm)	Total Head Mass (g)	33% Head Mass (g)
Brussels Sprouts	90.1	b	14.5	404	—	—
Broccoli	94.3	b	20.7	259	—	—
Hojem	89.9	b	0	310	—	—
Couchamp	92.1	b	12.6	235	—	—
Igea 65	64.4	a	2.0	295	—	—
Snowcap SA011B	96.6	b	34.4	320	—	—
Snowcap Gel213/80	95.3	b	39.0	320	—	—
Snowcap SA1239A	95.7	b	26.0	297	—	—
Snowcap SA1239T1	95.1	b	16.0		1502	2796
Snowcap SA1239T2	90.9	b	14.7		1588	3110
Snowcap SA1239T3	64.2	a	2.5		1848	3430
F value	17.02	***	2.4	NS		
LSD (P=0.05)	7.0					
CV %	4.73%		19%			

* Figures with the same letter do not differ significantly at the level, P = 0.05., using Fisher's LSD Test.

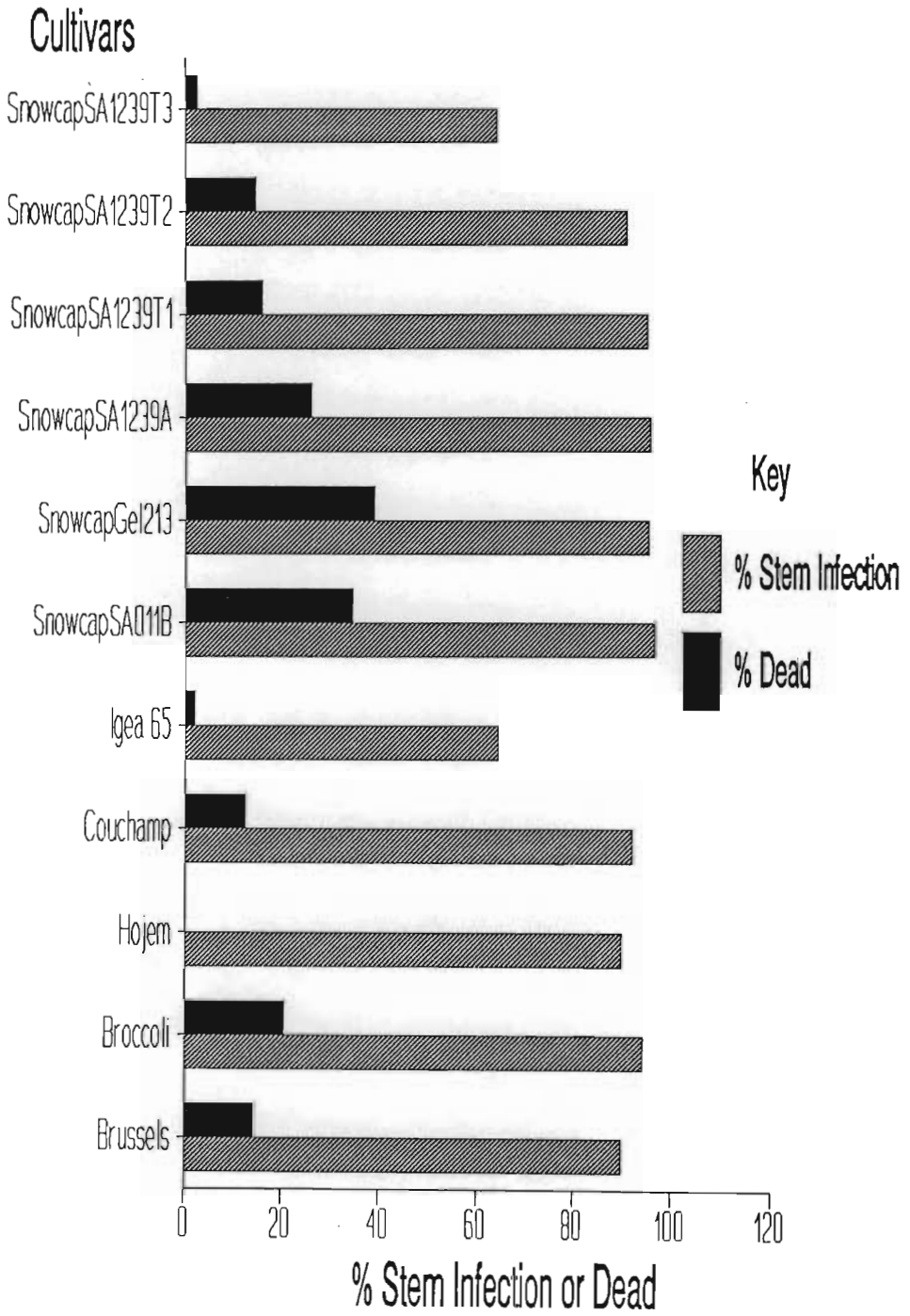

Fig. 5.3.3.B Cultivar Trial 2: Susceptibility of Cauliflower and Other Crucifers to Leptosphaeria maculans

Table 5.3.3.C: Cultivar Trial 2: Blackleg Susceptibility of Selected Crucifers in Unreplicated Plots

Crucifer	Blackleg Susceptibility
Red cabbage	very susceptible
Chou moulier	very susceptible
Swedes	very susceptible
turnips	no blackleg
Tyfon	no blackleg

Many seedlots of the same cultivars were included in Cultivar Trial 2. For the sake of clarity, these results were extracted from the above composite tables and are presented in Tables 5.3.3.D-G and Figs 5.3.3.C-F, which allows for easy comparison of Within Cultivar results.

Table 5.3.3.D: Cultivar Trial 2: Incidence of Blackleg Stem Infection and Other Parameters Within Multiple Seedlots of the Cabbage Cultivar, Gloria Osena

Seedlot	% of Stems Infected with *L. maculans* *	% Killed by *L. maculans*	Stem Length (mm) *	Total Head Mass (g) *	33% Head Mass (g) *
Gloria SA3057/J	86.1 d	0	160 a	1317 ab	1989 a
Gloria McD8A1235E	67.6 a	0.9	180 c	1426 b	2355 b
Gloria McD8B1024	78.7 bc	0	176 c	1742 c	2673 c
Gloria MayEZ15247	83.9 c	0.9	169 b	1154 a	2329 b
Gloria May11210	91.0 d	0.9	167 b	1492 b	2331 b
Gloria May30401	74.1 b	0.9	158 a	1263 a	2265 b
Gloria OE A1	73.6 b	1.9	177 c	1791 c	2492 b
Gloria OE A2	80.0 c	0	176 c	1635 c	2529 bc
F Test	14.5 ***	1.2 NS	6.3 **	4.5 *	6.9 **
LSD (P=0.05)	5.9	NA	4.6	209	127
CV %	12.2	32.2	11.4	25.9	13.4

* Figures with the same letter do not differ significantly at the level, P = 0.05., using Fisher's LSD Test.

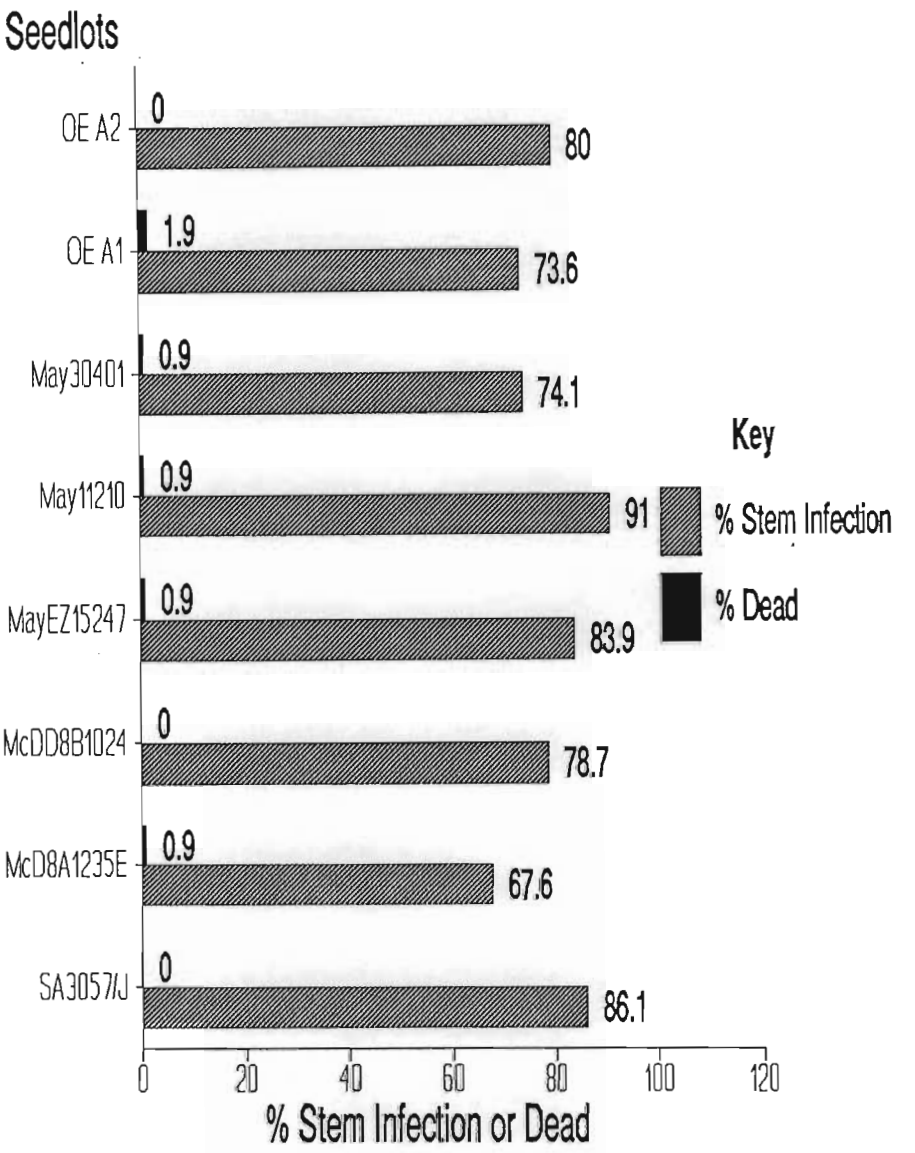

Fig. 5.3.3.C Cultivar Trial 2: Susceptibility of Different Seedlots of Gloria Osena Cabbage to Leptosphaeria maculans

Table 5.3.3.E: Cultivar Trial 2: Incidence of Blackleg Stem Infection and Other Parameters Within Multiple Seedlots of the Cabbage Cultivar, Green Coronet

Seedlot	% of Stems Infected with *L. maculans*	% Plants Killed by *L. maculans*	Stem Length (mm)	Mean Head Mass (g)	Mean of Biggest 33% Head Mass (g)
Green Coronet McD1006	93.5 c	6.5 c	181 a	1772 a	2837 a
Green Coronet McD8C427D	83.0 b	1.9 a	192 b	2148 b	3198 b
Green Coronet McD8B233	91.5 c	4.7 b	180 a	2017 b	3204 b
Green Coronet SA2019/A/OJ	77.8 a	5.6 b	192 b	1884 a	2807 a
Green Coronet May5749	84.7 b	4.6 b	190 b	2386 b	2888 a
F Test	16.9 ***	4.9 **	9.5 **	6.4 **	12.9 **
LSD (P=0.05)	4.5	0.9	8.2	382	82.0
CV %	9.8	17.3	14.7	32.1	13.3

* Figures with the same letter do not differ significantly at the level, P = 0.05., using Fisher's LSD Test.

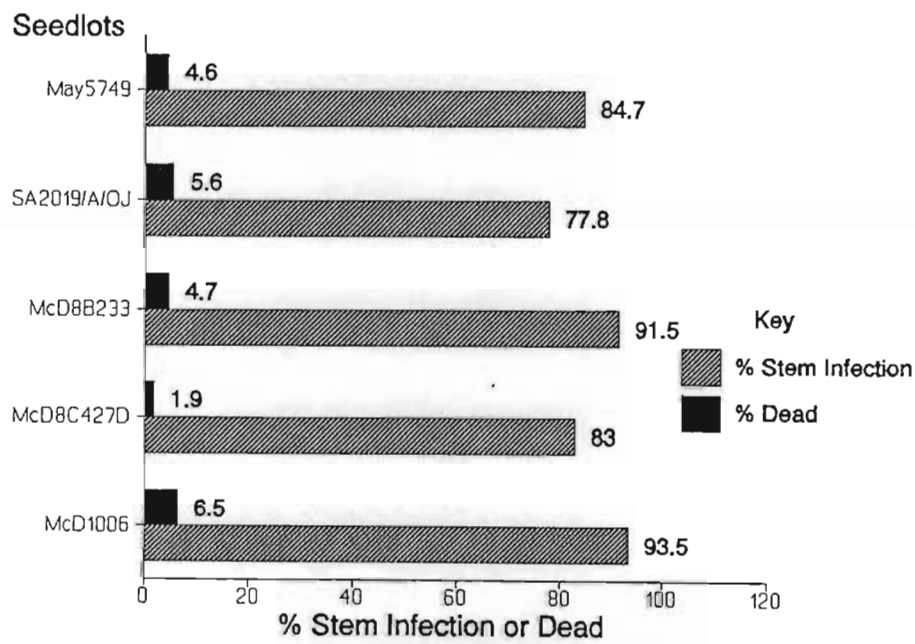

Fig. 5.3.3.D Cultivar Trial 2: Susceptibility of Different Seedlots of Green Coronet Cabbage to Leptosphaeria maculans

Table 5.3.3.F: Cultivar Trial 2: Incidence of Blackleg Stem Infection and Other Parameters Within Multiple Seedlots of the Cabbage Cultivar, Hercules

Seedlot	% Stem Infection	% Killed by *L. maculans*	Stem Length (mm)	Total Head Mass (g)	33% Head Mass (g)
Hercules SA19281	91.3	17.2	193	1448	2677
Hercules MayL1720	89.8	19.3	196	1709	2666
Hercules May 5659	86.1	18.5	193	1722	2480
F Test LSD (P=0.05) CV %	1.8 NS 6.4 8.4	2.2 NS 3.8 23.3	4.7 NS 6.3 12.2	1.3 NS NA 35.6	4.3 NS 247.2 18.9

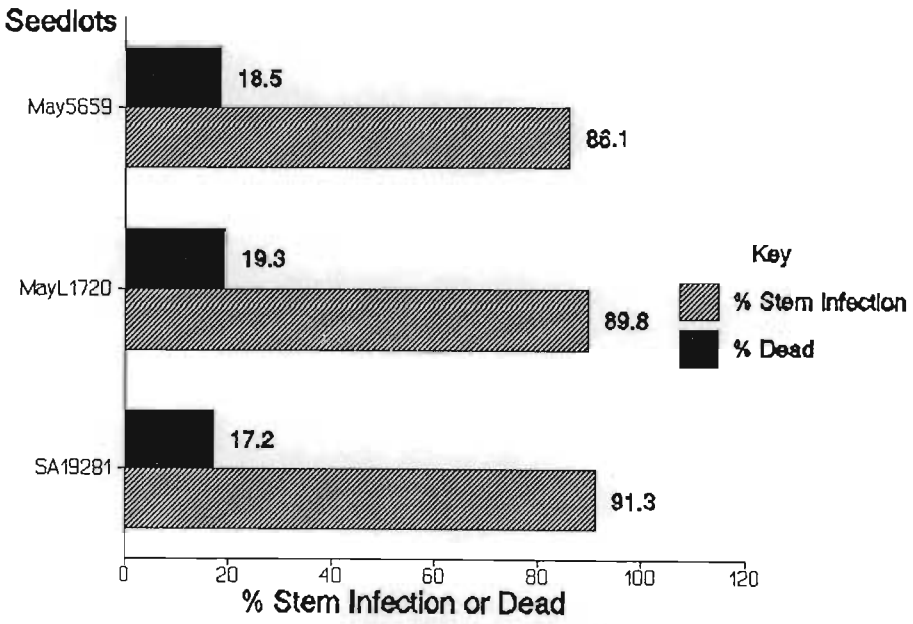

Fig. 5.3.3.E Cultivar Trial 2: Susceptibility of Different Seedlots of Hercules Cabbage to Leptosphaeria maculans

Table 5.3.3.G: Cultivar Trial 2: Incidence of Blackleg Stem Infection and Other Parameters Within Multiple Seedlots of the Cauliflower Cultivar, Snowcap

Seedlot	% of Stems Infected with *L.maculans* *	% of Plants Killed by *L. maculans**	Mean Stem Length (mm)	Mean Head Mass (g) *	Mean of Biggest 33% Head Mass (g) *
Snowcap SA011B	96.6 b	34.4 d	320	—	—
Snowcap Gel213/80	95.3 b	39.0 d	320	—	—
Snowcap SA1239A	95.7 b	26.0 c	297	—	—
Snowcap SA1239T1	95.1 b	16.0 b		1502 a	2796 a
Snowcap SA1239T2	90.9 b	14.7 b		1588 a	3110 b
Snowcap SA1239T3	64.2 a	2.5 a		1848 b	3430 c
F Test	23.2 ***	6.7 **		4.3 **	12.8 **
LSD (P=0.05)	12.7	7.6		210.3	259.4
CV %	14.3	24.3		18.2	16.5

* Figures with the same letter do not differ significantly at the level, P = 0.05., using Fisher's LSD Test.

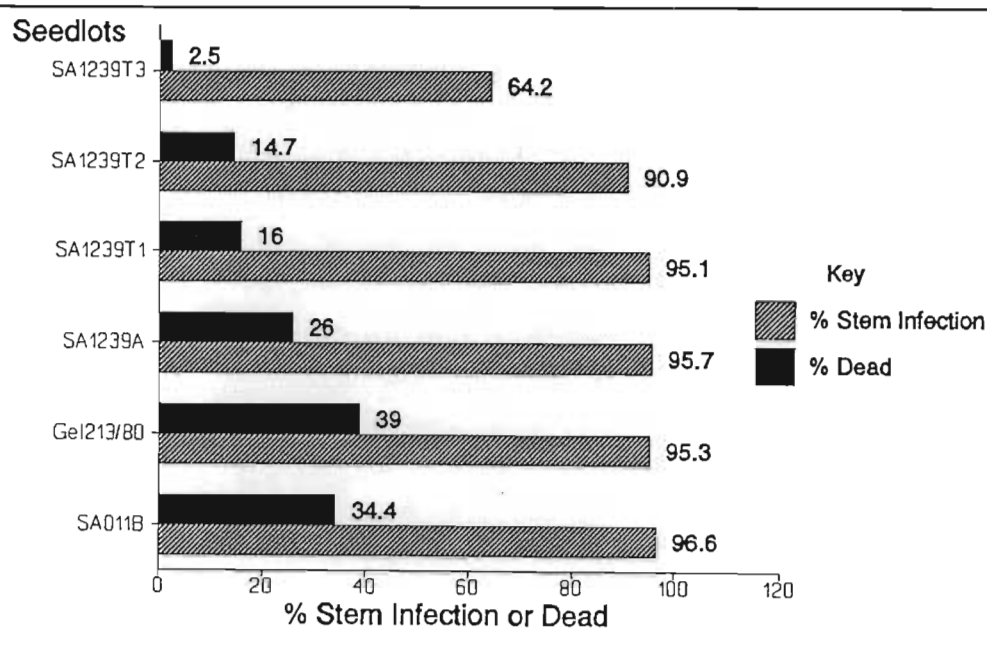

Fig. 5.3.3.F Cultivar Trial 2: Susceptibility of Different Seedlots of Snowcap Cauliflower to Leptosphaeria maculans

5.3.4 Discussion

5.3.4.1 Between Cultivar Variation

Disease incidence, measured as the percentage of plants with blackleg stem infection, was a selection parameter which successfully quantified relative susceptibilities to *L. maculans* of the different cultivars, and seedlots. Significant quantitative differences were found, both between and within cabbage cultivars, in terms of differential susceptibility to blackleg ($F=3.337$, $P<0.001$), confirming the basic findings of Cultivar Trial 1.

There was a much higher incidence of infection in this trial than in Cultivar Trial 1, the lowest disease incidence in this case being 50.6% compared to 15.8% in Cultivar Trial 1. This can be ascribed to a number of causes, such as differences in the two environments, irrigation practices, days-to-harvest, weather experienced, etc.

The rankings of cabbage cultivars for disease incidence determined here corresponded closely with those obtained in Cultivar Trial 1, but with notable exceptions. The cultivars Sunlander and Green Coronet were found to be relatively resistant in the Cultivar Trial 1 but relatively susceptible in this trial. In contrast, the cultivars Rotan and Glory of Enkhuisen gave consistently good performances in both trials. The cultivar Dynasty displayed the highest level of disease incidence in this trial (95.27%).

As discussed above, in Section 5.2.4, there was clear evidence of a pool of quantitative resistance in the cabbage cultivars tested. Again the point can be made that this material should be quite adequate to start an HR breeding programme. A positive feature is that there appeared to be no VR cultivars of cabbage (unless it is Quantitative VR and this would be eliminated in a breeding programme using a single pathotype). It would be important to monitor resistance gains and ensure that they are quantitative and gradual.

Significant variation in blackleg susceptibility existed between the cauliflower cultivars tested. Some of them displayed extreme susceptibility, the one sample of Snowcap (SA011B), developing a 96.6% incidence of stem infection, with 34.4% of the plants being killed by blackleg. However, Igea 65 and Snowcap SA1239T3 showed far greater resistance to stem infection by blackleg than the other cauliflower cultivars tested, which suggested that breeding for HR to blackleg in cauliflower would also be feasible with existing genetic resources.

The broccoli, Brussels sprouts and kohl rabi cultivars tested were all highly susceptible to blackleg. The morphology of kohl rabi is interesting in that a "cup" forms at the juncture of each leaf petiole and the swollen stem, where dew or irrigation water readily collects. Blackleg lesions almost invariably developed in this "cup". Kohl rabi is also notable in that the crop grows to maturity very rapidly and the development of blackleg is more apparent and rapid than with other crucifer vegetables. It could therefore be most useful as a test plant in pathogenicity trials. Broccoli flowers and produces seed more easily than other crucifers. It could therefore be of particular use in studies of seedborne crucifer diseases, such as blackleg or black rot. It is easy to infect mature crops of broccoli with a pathogen, and collect infected seed for subsequent research.

Disease Severity was estimated by counting the number of plant deaths. However, it provided no useful information. The parameter was not statistically significant. Surprisingly, it was not correlated with Disease Incidence (r^2 = 0.099). It therefore does not appear to be possible to measure Disease Severity in this way. An alternative would have been to visually rate the size of stem lesions on live plants (Anon., 1984; van den Berg and Rimmer, 1992).

Mean head mass was measured in order to identify blackleg tolerance, if it existed in the crucifers tested. However, the parameter was confounded between genetic potential and the effect of blackleg: a low overall mean could reflect a normally small crucifer, or it could mean that blackleg caused severe stunting.

Masses of the heaviest 33% of the heads from each plot were used to estimate the yield potential of each cultivar. However, this parameter was disrupted by the effect of blackleg when blackleg incidence was higher than 66%. A more accurate approach would have been to run a parallel trial, without the presence of blackleg, to measure horticultural parameters. However, this would have been expensive and would have required more land which was not available at the time. The approach adopted here did, however, allow for an estimate of this parameter.

No significant correlation was found between stem length and disease incidence ($r = 0.45$, $r^2 = 0.20$). This is of significance because in Cultivar Trial 1, cauliflower cultivars developed significantly more blackleg than cabbage. It was therefore speculated that the morphology of the stem, and stem length in particular, predisposed crucifers to blackleg infection. The evidence from this trial indicated that this theory is probably incorrect, and the consistently high level of susceptibility of cauliflowers is a product of a high level of genetic susceptibility, rather than as a result of klendusity.

From the unreplicated, observational trials of blackleg susceptibility conducted, the following comments could be made:

1. The red cabbage cultivar tested, Red Rock, was exceptionally susceptible and the plants developed more lesions and died sooner than any other crucifer tested. This extreme susceptibility is worth studying further as it may provide a greater insight into the physiological factors leading to blackleg susceptibility.
2. Chou moulier was highly susceptible and developed large lesions on both stems and leaves. This has important implications for dairy farmers using this crop as winter fodder crop, and for neighbours growing cruciferous vegetables because of its potential to be an alternate crop for *L. maculans*.
3. Swedes, as reported in the literature (Henderson, 1918), were highly susceptible and developed large black stem cankers covered with pycnidia. However, swedes are virtually unknown in South Africa, and do not play a significant role as either a vegetable or a fodder crop. If they were to be introduced into KwaZulu-Natal, it is likely that blackleg epidemics would occur since blackleg

inoculum is present locally, and environmental conditions are suitable for *L. maculans*.

4. The turnips tested were symptomless, a result reflecting previously published results (Chupp and Sherf, 1960; Petrie, 1969).
5. Tyfon was symptomless, a surprising result given Tyfon's close relationship with canola, and the cross-infectivity of canola and cabbage biotypes of *L. maculans* (Humpherson-Jones, 1985). This is an important finding as Tyfon is a fodder crop growing in popularity in KwaZulu-Natal (Findlay, pers. comm.).
6. Japanese radish was also symptomless, again a surprising result because *R. raphanistrum* is a recognized host of *L. maculans*, and *L. maculans* has been found in Japanese radish seed in South Africa (Holtzhausen, 1978). One can only assume that there was differentiation between the *Brassica* strain used in this trial, and the *Raphanus* strain isolated by Holtzhausen. A question that arises then is whether the different strains can mate, and if so, then what would the pathogenicity of the subsequent strains be. The finding is also important because Japanese radish is the dominant winter fodder crop in KwaZulu-Natal, as discussed in Section 1.2.6.

With regards the fodder crucifers, it is apparent that KwaZulu-Natal farmers should avoid growing swedes or chou moulier, particularly if they are in mistbelt areas with a history of blackleg and, most especially, if they also crop cruciferous vegetables. Japanese radish should remain the fodder crop of choice.

5.3.4.2 Within Cultivar Variation

In this trial, there was significant variation in blackleg incidence within cultivars, between seedlots, a confirmation of the discovery in Cultivar Trial 1 of within-cultivar variation in blackleg resistance. The within-cultivar variation in Cultivar Trial 2 was in several cultivars, and was in both cabbages and cauliflowers.

Considering the percentage of plants per plot with stem infection by *L. maculans*, within seedlots of the cabbage cultivar Gloria Osena, two distinct susceptibility groups appeared to exist:

A. McD8A1235E (67.6%), May30401 (74.1%) and OE A1 (73.6%)

B. SA3057/J (85.0%), McD8B1024 (78.7%), MayEZ15247 (83.9%), May11210 (91.0%) and OE A2 (80.0%).

Within the seedlots of the cabbage cultivar Green Coronet which were tested, two susceptibility groups also appeared to exist:

1. McD8C427D (83.0%), SA2019/A/OJ (77.8%) and May5749 (84.7%)
2. McD8B233 (91.5%) and McD1006 (93.5%).

Within the seedlots of the cabbage cultivar Hercules tested, no significant differences were apparent; the cultivar appeared to be homogenous for its resistance to blackleg in the seedlots tested.

Within the seedlots tested of the cauliflower cultivar Snowcap, significant variation in blackleg susceptibility existed, two groups being obvious:

1. SA011B (96.6%), Gel213/80 (95.3%), SA1239A (95.7%), SA1239T1 (95.1%) SA1239T2 (90.9%)
2. SA1239T3 (64.2%).

The consistent finding of variable susceptibility to blackleg **WITHIN** single hybrids calls into question the meaning of the term "cultivar". It is a remarkable discovery because the cultivars tested were all F1 hybrids, which ostensibly should have been produced from the same inbred, homogenous parents, with no genetic differences between seedlots. However, the classification of the hybrids into distinct groups suggests that different genetic groups have developed within individual cultivars for characteristics not deliberately selected for in the parent lines.

5.4 Cultivar Trial 3

5.4.1 Introduction

In both Cultivar Trials 1 and 2, evidence was found for within-cultivar variation in blackleg susceptibility. However, in these trials only four and three replications, respectively, were used. This may have been too few to separate out small differences, especially under field conditions. In order to clarify the prior findings of within cultivar variation, a third cultivar trial was conducted. Ten replications were used to distinguish clearly between cultivars and to reduce experimental error to a minimum.

The trial site was at Cedara Agricultural Development Institute (CADI) in the Plant Pathology Section. This site was chosen as a consistent supply of trained labour was available to plant out trials, accidental infection from commercial crops would not occur as no crucifers were being grown at CADI, and CADI is situated in a mistbelt region of KwaZulu-Natal and is, therefore, an ideal site for the development of cabbage diseases.

5.4.2 Materials and Methods

The trial design was a randomized complete blocks, with four treatments, each with 10 replicates, 36 plants per plot, spaced at 0.5 m x 0.5 m, with a 1 m border between plots.

The cultivar Hercules was chosen as different seedlots of the cultivar were available, and seedlots of the cultivar had appeared to be homogenous in the previous trial. Four different seed lots were obtained (McDonald's 5950, 5952, 5862, 6107) and seedlings were grown in a commercial nursery.

The soil was disced and 1000 kg ha^{-1} 2.3.2 (28) fertilizer was rotovated in. Topdressing and micronutrient applications were applied as in the previous trials. Immediately prior to planting, Gramoxone® (paraquat, FBC) and Lasso® (alachlor, Monsanto) were applied for weed control. Some hand weeding was needed during the growing season.

growing season. Insect control was by a single application of Curaterr® (carbofuran, Bayer) at transplanting. The same inoculation procedure as used in previous trials was followed, using infected debris. Every plant was evaluated for infection of the stem by *L. maculans*, a parameter which worked effectively in previous trials.

ANOVA was conducted on the disease data using the same statistical packages, as reported in Section 5.2.2. In view of the high CV% of the original analysis (36.2%), an angular transformation was applied to the data before a second ANOVA was conducted, which resulted in an improved CV% (18.4%).

5.4.3 Results

The results are presented in Table 5.4.3.A and Figure 5.4.3.A.

Table 5.4.3.A: Cultivar Trial 3: Variation of Percentage Blackleg Stem Infection Within Multiple Seedlots of the Cabbage Cultivar Hercules

Seedlot	% of Stems Infected with *L. maculans*	% of Stems Infected with *L. maculans* (Transformed) *
Hercules McD5952	7.8	15.8 a
Hercules McD6107	9.7	17.9 ab
Hercules McD5950	13.1	20.8 bc
Hercules McD5862	16.2	23.4 c
F Test	7.76 **	8.5 **
LSD (P=0.05)	N/A	3.3
CV %	36.2	18.4

* Figures with the same letter do not differ significantly at the level, P = 0.05., using Fisher's LSD Test.

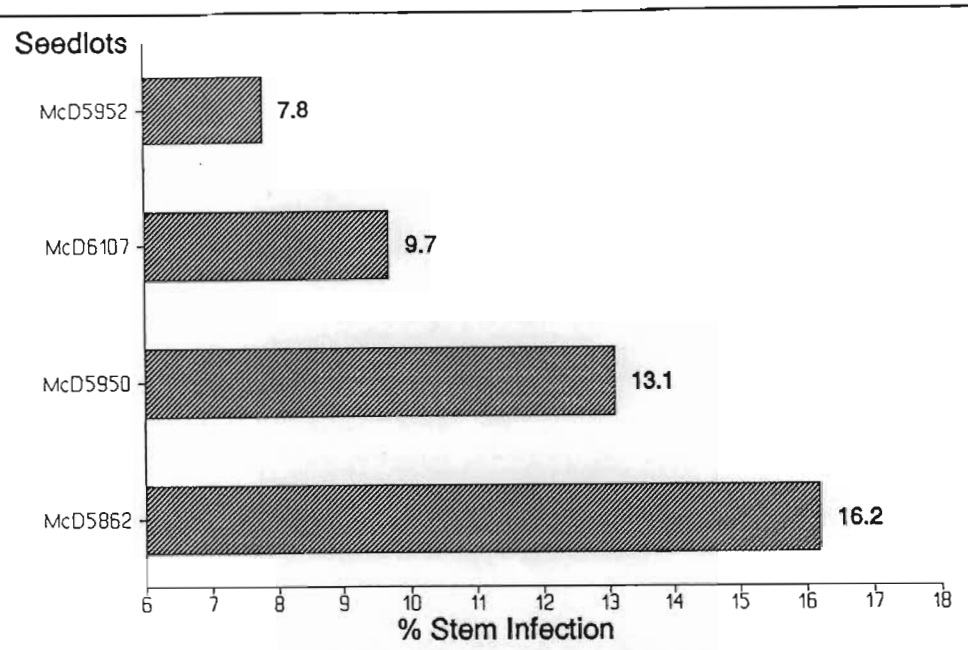

Fig. 5.4.3.A Cultivar Trial 3: Susceptibility of Different Seedlots of Hercules Cabbage to Leptosphaeria maculans

5.4.4 Discussion

ANOVA of both the original and transformed data again indicated that significant differences in susceptibility to blackleg existed within one cultivar ($P < 0.001$). Using an LSD set at the 1% level, distinct differences between the seedlots appeared to exist, Seedlot 5951 showing the least disease, Seedlots 6107 and 5950 fitting the mid-range and Seedlot 5862 being the most susceptible.

Despite 10 replicates, the trial displayed a high CV%, even when the data was transformed (18.4%). However, the F test was strongly positive, and the most susceptible seedlots showed more than twice the infection levels of the most resistant seedlot. The results of this trial therefore confirm the findings of the two previous Within Cultivar trials.

A hypothesis that blackleg stem resistance may vary significantly within single hybrid cultivars of cabbage and cauliflower is therefore proposed.

As discussed in Sections 5.2.4 and 5.3.4, this finding has profound implications for seed companies and for scientists evaluating cultivars:

> *the levels of disease resistance expressed by cultivars cannot be assumed to be genetically-fixed within those cultivars, even if they are F1 hybrids; resistance expression of a single cultivar may vary between different seedlots.*

From the individual scientist's point of view, it is important to obtain a large quantity of each seedlot so that continuity in trials may be maintained between different seasons, and that observed differences in disease levels can safely be assumed to reflect changes in treatments or in environmental condition, and are not due to genetic variation within the cultivar itself.

The discovery of within-cultivar variations raises some important issues as to the nature and validity of the term "cultivar". In most cases, the genetic constituents of a cultivar are usually considered to remain stable between generations. It is this assumption that underlies cultivar resistance trials. However, this assumption may not be valid. Firstly, genetic drift introduces significant variations between generations (Mayo, 1980; Simmonds, 1979). Allard (1960) explained the problem quite clearly:

> "aside from random fluctuations associated with small population size (genetic drift), random mating has poor powers of fixation of genes, with or without selection".

Secondly, if stock seed is produced in several different seasons or in different locations, even if only in different fields, then natural selection will introduce variations in those fitness characteristics required to cope with various selection pressures. This will be particularly apparent when strong selection pressures, such as blackleg, are active. Such variations within a cultivar would not be detected by seed

producers unless these genetic variations resulted in morphological changes, or specific tests were conducted to detect non-morphological variation. Natural selection for disease resistance would be in response to the shifting spectrum of diseases affecting the seed crops, and the effect on seed production would vary from site to site, and season to season.

A cogent explanation for the variations observed between seedlots is that inbreds may not be completely homozygous. In particular, when inbreds start to lose their vigour owing to inbreeding, breeders may use backcross recovery to rejuvenate the inbreds. Although genetically different from their parents, these inbreds are treated as though unaltered and the hybrids they are used to produce are not renamed (Shanahan, pers. comm.).

This theory has profound implications for cultivar resistance testing: there can be no certainty that a named cultivar's tested resistance will be consistent between seedlots, unless each test for resistance becomes a standard assay conducted on each seed lot to ensure its resistance level is equivalent to its parent line. This would be a logistical nightmare for seed producers. However, if such assays are not conducted, then a situation may arise where the disease resistance expressed by different seedlots of the same cultivar were not the same, as detected in the above three trials.

Furthermore, the findings call into question the validity of widespread cultivar testing, such as is reported in the APS annual publication, Cultural and Biological Tests, unless it is known that the resistance evaluation criteria being applied are included in the breeding selection criteria.

5.5 References

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CHAPTER 6. THE EFFECT OF BENOMYL ON BLACKLEG INCIDENCE

Chemicals may act either to reduce, remove or eliminate inoculum at the source (eradication); to prevent plant diseases (protection); or to cure them (therapy). The great majority of chemical control measures involve the principle of protection; this requires preventing inoculum from entering the host and starting an infection. To accomplish this, chemicals may be used to prevent growth or sporulation of microorganisms, or to kill or inactivate the inoculum at the source, in transit, or in the court of infection.

While therapy involves control of the pathogen after it has entered a host, chemotherapeutants may be applied to plants either before or after infection.

Anon., 1968

Abstract

The literature on field application of fungicides to crucifers for the control of *L. maculans* is reviewed.

A field trial was conducted using benomyl, applied either at the seedling stage only, or at the seedling stage, followed by field applications every 14 d. The latter treatment resulted in a 33% reduction in stem infection relative to the untreated control, a ten-fold reduction in plants killed by blackleg and a 50% reduction in non-harvestable heads, all relative to an untreated control. The plants only treated as seedlings had a lower infection level, a lower mortality rate and a greater mean head mass than the untreated control plants. However, these differences were not statistically significant.

6.1 Introduction

Tables 6.1.A-B summarize most of the available literature on the use of fungicides for the control of blackleg of crucifers in the field. The summary is necessarily crude in that the more subtle variations in dose and timing have been left out, and the overall results have been summarized into only three categories: *good, partial* and *none.*

Table 6.1.A: Field Fungicide Trials on Cabbage: Protectant Fungicides

Chemical	Group	Efficacy	Author
lime	calcium carbonate	partial	Manns, 1911
quintozene	chlorinated hydrocarbon	partial	Lambe *et al.*, 1978
Bordeaux mixture	copper sulphate / lime	partial partial	Gregory, 1925 Cunningham, 1939
copper oxychloride	copper	partial	Van Bakel, 1968
mercuric chloride	mercury	partial	Van Bakel, 1968
colloidal S	sulphur	partial	Antonov, 1978

Key to Effectiveness Classes:

none: no useful control of blackleg stem infection

partial: only partial control of blackleg stem infection

good: excellent control of blackleg stem infection

Table 6.1.B: Field Fungicide Trials on Canola: Systemic Fungicides [4]

Chemical	Group	Efficacy	Author
benomyl	benzimidazole	good partial poor poor complete good good good good none partial	Brunin, 1972. Cruger, *et al.*, 1974 Barbetti, 1975b Brown *et al.*, 1976 Lambe 1973; 1978 [c] Rothamstead, 1979 Rawlinson and Muthyalu, 1979 Daebeler *et al.*, 1981 Anon., 1981a; 1981b Seidel *et al.*, 1984 Rawlinson *et al.*, 1984
carbendazim	benzimidazole	poor partial	Barbetti, 1975b Paul and Beineke, 1993
thiophanate-methyl	benzimidazole	good	Sansford and Hardwick, 1992
iprodione	dicarboximide	partial partial good	Kruger, 1991a Kharbanda, 1992 Sansford and Hardwick, 1992 Paul and Beineke, 1993
procymidone	dicarboximide	partial	Ballinger, *et al.*, 1988a
vinclozolin	dicarboximide	partial	Garbe, 1993
imazalil	imidazole	good	Rothamstead, 1981
prochloraz	imidazole	good partial partial phytotoxic partial good partial good partial	Rothamstead, 1981 Rawlinson *et al.*, 1984 Kruger, 1991a Kruger, 1991b Kharbanda, 1992 Sansford and Hardwick, 1992 Schramm and Hoffmann, 1992 Badawy, 1994 Garbe, 1993
prochloraz + carbendazim	imidazole + carbendazim	good good	Church and Fitt, 1993 Paul and Beineke, 1993
diconazole	triazole	good	Rempel and Hall, 1995
difenoconazole	triazole	good	Kirk *et al.*, 1993
diniconazole	triazole	none	Ballinger *et al.*, 1988a; 1988b
difenoconazole	triazole	good	Kirk *et al.*, 1993
diniconazole	triazole	none	Ballinger *et al.*, 1988a; 1988b
flutriafol	triazole triazole	good partial	Ballinger *et al.*, 1988a; 1988b Xi *et al.*, 1991
flusilazole + carbendazim	triazole + benzimidazole	good	Chisholm and Williams, 1993

[4] *Continued on next page*

Chemical	Group	Efficacy	Author
propiconazole	triazole	partial	Badawy, 1994
tebuconazole	triazole	good partial partial partial	Bolton and Adam, 1992 Schramm and Hoffmann, 1992 Paul and Beineke, 1993 Badawy, 1994
triadimefon	triazole	partial none partial partial	Rothamstead, 1981 Rawlinson *et al.*, 1984 Ballinger *et al.*, 1988a; 1988b Rempel and Hall, 1995
uniconazole	triazole (PGR)	good	Rempel and Hall, 1995

Key:

1. All trials conducted on canola, except those marked with **C** = cabbage
2. Key to Effectiveness Classes:

none no useful control of blackleg stem infection

partial only partial control of blackleg stem infection

good excellent control of blackleg stem infection

Early attempts at chemical control of blackleg of crucifers include the use of lime to control soilborne seedbed inoculum (Cockayne, 1918), copper sprays in the seedbed (Manns, 1911) or Bordeaux mixture in the field (Gregory, 1925; Cunningham, 1939), and copper and mercury sprays on cabbage seed plants to delay disease progress (Van Bakel, 1968). Benomyl (Benlate®, Du Pont) has been regularly investigated, its high *in vitro* activity against *L. maculans* and its systemic nature giving it a possible curative role, particularly against *L. maculans* on canola. French workers (Chancogne *et al.*, 1970a; Chancogne *et al.*, 1970b; Brunin, 1972; Pierre *et al.*, 1972) reported the successful protection of seedlings for at least 6 wk by heavy applications of benomyl to seed (3-20 g kg^{-1} seed). This period is the canola seedling's most susceptible stage (Alabouvette, 1970; Brunin and Lacoste, 1970) and may be the growth stage when most infections occur, although lesions only appear after a long latent period (Nathaniels and Taylor, 1983). In Australia, summer canola is grown, which is a short season crop (100 d), and late blackleg infections appear to be significant (Barbetti, 1975a; 1975b). Therefore fungicidal sprays ought to be able to protect it. However, the use of benomyl and carbendazim, both on seed and as back-up sprays (110 g and 280 g ha^{-1} at 1 and 2 wk, for both fungicides) failed to increase yields, probably because their protective effect did not last long enough. However, it was not cost effective to spray a canola crop more frequently (Barbetti, 1975b). Also in Australia,

Brown *et al.* (1976) found that the effect of benomyl seed treatments carried through to the crop only under glasshouse conditions, and not in the field. In further trials, they found that field sprays of benomyl + oil at 2, 4 and 6 wk after germination failed to give consistent control, giving significant, but not cost effective, yield increases in only three of eight trials. English workers, Rawlinson and Muthyalu (1979), reported complex results following benomyl seed and field treatments of canola. Light leaf spot (*Pyrenopeziza brassicae* Sut. and Rawl.), downy mildew (*Peronospora parasitica* Pers; Fr.), grey mould (*Botrytis cinerea* Pers. Fr.) and canker (*L. maculans*) all attacked the crop under study at all three trial sites. Yield data presented for one experiment are perplexing: seed treatments reduced yields in two of three trials conducted. Furthermore, multiple regression analysis showed yield losses to canker to be statistically insignificant. Whilst benomyl was shown here to successfully control light leaf spot, no consistent control of canker was observed. These authors point out some limitations to the regular use of benomyl sprays:

i) *B. cinerea*, the causal organism of grey mould on canola, is an important pathogen of many crops, and frequently has developed resistance to benzimidazole fungicides. It would be undesirable to increase the selection pressures for benzimidazole-resistant strains of *B. cinerea* by applying routine field sprays of canola with benomyl.

ii) They observed an increase in the incidence of downy mildew, a non-target fungus unaffected by benomyl. The incidence of infection by non-target *Alternaria* spp. would probably also increase, due to the same forces of competition (in this case, the removal of competition) acting to increase their relative populations, as occurs in other pathosystems (Putter, 1980).

In another British paper (Rothamstead, 1979), the successful use of benomyl as a seed treatment followed by one or two foliar applications was reported, the combination of which reduced both incidence and severity of canker in canola. Subsequent British papers (Anon., 1981a; 1981b) report using an autumn and spring spray of benomyl, prochloraz and imazalil, all of which more than halved the incidence of summer stem cankers.

Lambe (1973) used benomyl (1 g a.i. $100\ell^{-1}$ water) to treat transplants, the treatment providing 100% control of *L. maculans*, whereas the untreated control showed 38% infection. As research on inoculum in Chapter 7 reveals, the dissemination of *L. maculans* pycnidiospores during the transplanting process is a very significant stage of disease spread, when the effective dissemination of pycnidiospores is maximized. Lambe's transplant treatment should therefore control the fungus at its most vulnerable stage. Furthermore, efficient application of the fungicide onto plants occurs with little off-target loss of the chemical.

The efficacy of benomyl may be improved by using various adjuvants to enhance the uptake of the fungicide. Adjuvants are commonly added to fungicides to decrease foliar surface tension. In some cases, this has significantly increased the absorption of benomyl (Booth and Rawlins, 1970; Buchenauer and Erwin, 1972; Smith and Crosby, 1972; Wicks, 1973), whereas in others, it has proved ineffective (Hawthorne, 1979). The efficacy of benomyl may also be significantly enhanced in acidic solutions because acid hydrolysis produces methyl 2-benzimidazole carbamate and butylcarbamic acid. Both these products have greater water solubility than benomyl and hence penetrate cuticular waxes more rapidly. The lowered pH of the solution may also increase the speed of fungicide uptake, the hydrogen ion concentration affecting membrane permeability by controlling the rate of reactions of active transport (Buchenauer and Erwin, 1972).

Triazoles, imidazoles and dicarboximide fungicides have been tested against *L. maculans* on canola in field fungicide trials since 1981 (Table 6.1.B). The results were mixed, with several fungicides working in some situations but not others. However, the triazole fungicide, flutriafol, consistently appeared to control *L. maculans*. Trials of Rempel and Hall (1995) showed that application of three triazole fungicides can significantly reduce disease incidence and severity, reduce plant height and lodging and increase vigour and seed yield. However, timing of fungicide application was found to be critical.

There have been no publications on the control of blackleg of cabbage in the field (as opposed to canola) using modern fungicides despite Gabrielson's *et al.*'s (1973) success at controlling *Sclerotinia sclerotiorum* (Lib.) de Bary in cabbage seed fields with benomyl sprays. He reported that 1 or 3 sprays at a rate of 5.4 kg ha^{-1} and 10.8 kg ha^{-1} increased yields by up to 17 %.

One reason for the variable results of fungicide trials for the control of *L. maculans* on canola is probably owing to variation in the timing of application of fungicide sprays. A wide range of spray timings have been reported, from applications to seed at planting, to post-flowering sprays (Rawlinson and Muthyalu, 1979; Rawlinson *et al.*, 1984; Kruger, 1991; Bolton and Adam, 1992; Sansford and Hardwick, 1992; Schramm and Hoffmann, 1992). Overall, it would appear that foliar fungicides failed if they were applied too soon, to seed or young seedlings (with the exception of flutriafol). This failure probably related to a limited half-life of the systemic fungicides in the crop. However, fungicides only applied post-flowering also failed to control the pathogen. The critical period where continuous protection by fungicides is needed is during the pre-and post-flowering phases, with multiple sprays being needed to provide adequate protection for the required period.

Research on the control of *Rhizoctonia solani* has shown that benomyl decomposes after about 10 d in cabbage plants (Osborne and Laing, 1987). Based on this, a hypothesis is proposed here that benomyl sprays need to be timed at approximately 10-14 d intervals if complete control of *L. maculans* is to be maintained.

When the fungus becomes well established in a host plant, lignification of the stem lesion occurs (Hammond and Lewis, 1986). Effectively, the fungus becomes encased in a protective, tough shell of lignin. Furthermore, the host transport system becomes severely compromised, and the movement of systemic fungicides into the lignified zone will be limited. A second hypothesis is therefore proposed, that late sprays of systemic fungicides are ineffective because the fungicides cannot reach the target area of the plant where the fungus is located.

From the literature it appeared that it might be possible to develop an eradicant fungicidal spray to control cabbage blackleg under local conditions. A trial with two different applications was therefore designed to investigate the efficacy of benomyl:

1. Application to seedlings prior to planting out, as a parallel to Lambe's (1973) approach of drenching transplants with benomyl, as discussed above;
2. Regular applications from seedling tray to harvest.

6.2 Materials and Methods

The trial was run at Baynesfield Estates, Thornville Junction, KwaZulu-Natal. The trial design was a randomised complete blocks trial with three replications and three treatments. Thirty six CGS were transplanted at 0.5 m x 0.5 m spacings into plots of 3.5 x 3.5 m, with 1 m borders between plots. Because of its high susceptibility to blackleg, the cauliflower cultivar Snowcap was used in this trial (Section 5.2.3).

At transplanting the plots were inoculated with blackleg inoculum, as described in Section 5.3.2, using chopped-up, infected cabbage stems. Field preparation was also as described in Section 5.3.2.

The three treatments were:

1. Control; unsprayed.
2. Shadehouse-treated CGS. These were sprayed twice, at 4 and 6 wk post-planting, with benomyl (Benlate, Du Pont Chemicals) plus a wetter (Agrowett, a polyethylene glycol, non-ionic wetter) at rates of 1 g ℓ^{-1} and 0.5 ml ℓ^{-1}, respectively, applied with a CP3 backpack sprayer to runoff. CGS were transplanted immediately after their second spray.
3. Field-treated plants. Shadehouse-treated CGS (Treatment 2) were further protected by spraying biweekly in the field till harvest, using the same fungicide and adjuvant and at the same rates as in Treatment 2; i.e., field application of an additional four sprays.

Disease evaluation was based on four criteria:

1. the presence or absence of one or more typical blackleg stem lesions;
2. death or severe wilting of the plant;
3. total head mass harvested;
4. percentage of non-harvestable heads.

ANOVA was conducted on the above four parameters. Fisher's LSD test was used for means separation.

6.3 Results

Table 6.3.A contains the data relating to this experiment, and Fig 6.3.A depicts a histogram of the means of the treatments, in terms of the parameters evaluated.

Table 6.3.A: Field Fungicide Trial 1: the Effect of Benomyl Treatments on Blackleg Incidence and Other Related Parameters

Treatment	% of Stems Infected with *L. maculans* [1]	% Plants Killed by *L. maculans*[1]	Mean Head Mass (g)	% Non-Harvestable Heads [1]
Control	95.1 a	21.6 a	1502	22.9 a
CGS Treated	90.9 a	14.7 b	1753	26.6 a
CGS & Field Treatment	62.0 b	2.7 c	1848	12.63 b
F Test	8.7 **	7.9 **	1.3 NS	7.8 **
CV %	14.3%	12.2%	22.1%	16.7%
LSD (5%)	13.6	4.5		5.1

[1] Figures with the same letter do not differ significantly at the level, P = 0.05, using Fisher's LSD Test.

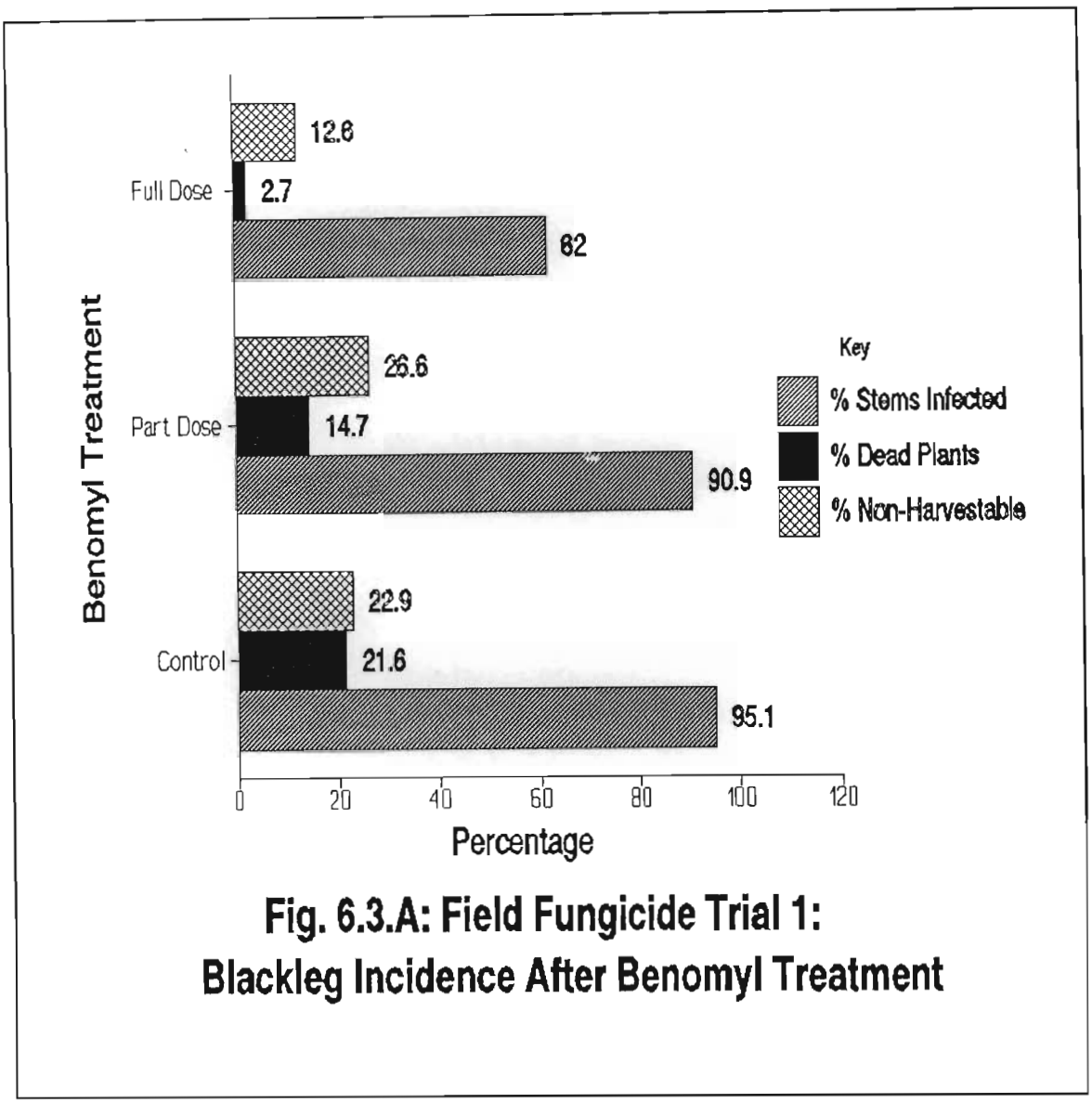

Fig. 6.3.A: Field Fungicide Trial 1: Blackleg Incidence After Benomyl Treatment

6.4 Discussion

Examination of the data show that the field treatment of the cauliflowers was successful in controlling blackleg. The best treatment resulted in a 33% reduction of infection relative to the untreated control. There was a ten-fold reduction in cauliflower death and a 50% reduction in the proportion of non-harvestable heads. The CGS-treated plants had lower infection levels, lower mortality rate and a greater mean head mass than the untreated control plants.

Thus it appears that benomyl applied regularly has sufficient activity against this fungus to warrant consideration as a treatment to minimize crop damage in the event of a blackleg epidemic. However, the fungicide needs to be applied preventatively.

The question then is whether it is economically viable to spray crucifer crops with benomyl. Table 6.3.B is based on market prices for 1995 (Anon, 1995a), and yield figures from Table 1.2.3.3 (cabbage) or government data (cauliflower and broccoli) Anon, 1995b). The cost for benomyl sprays is based on four sprays of 600g ha^{-1} with a price of R60 kg^{-1} (4 x R36 = R144).

Table 6.3.B: Field Fungicide Trial 1: Economics of Benomyl Application

Crop	Yield ha^{-1} ($t\ ha^{-1}$)	Value ha^{-1} (Rands)	Cost ha^{-1} (Rands)	Net Return (Rands)	Benomyl Costs as % of Costs	Benomyl Cost per 30kg Bag (cents)
Cabbage [1]	52.3	14 794	13 210	1 584	1.09	8.3
Cabbage [2]	82.5	20 350	13 210	7 140	1.09	5.2
Cabbage [3]	99.0	24 420	13 210	12 210	1.09	4.4
Cauliflower [1]	7	8 225	4 059	4 166	3.5	61.7
Broccoli [1]	7	14 150	4 059	10 090	3.5	61.7

1 Anon, 1995b

2 Own estimate, 75% harvest

3. Own estimate, 90% harvest

Table 6.3.B shows that it would have been economic to apply benomyl to cabbage, cauliflower and broccoli crops, even with conservative production estimates. In particular, four benomyl sprays would have added 1% and 3.5% to costs of cabbage, and broccoli or cauliflower production, respectively, and 4-8 cents per cabbage bag and 62 cents per 30kg box of cauliflower or broccoli.

Given the longer half-life of powerful sterol-biosynthesis inhibitor fungicides of the triazole, imidazole and morpholine groups, their strong systemicity and their powerful curative action (Ward *et al.*, 1997), they should provide better control of *L. maculans* in crucifer vegetables than benomyl. One spray prior to heading and concurrently, increased blackleg susceptibility, should eliminate all latent infections (Hammond *et al.* 1985). Whether further sprays would be necessary would depend on the source of inoculum: if it was from debris in the field, or airborne ascospores, then additional sprays would be needed. However, if the infections occurred in the seedbed and the disease was essentially monocyclic, then a single spray should suffice to control blackleg.

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CHAPTER 7. THE Rôle OF DEBRIS IN THE PROPAGATION OF CRUCIFER BLACKLEG IN KwaZulu-NATAL

The plant diseases caused by pathogens are contagious. In order for more plants in a field to become diseased, particles of the pathogen must reach healthy plants and establish new infections. These particles are called inoculum (sic).

Anonymous, 1968

Abstract

The role, significance and survival period of crucifer debris in the lifecycle of *L. maculans* is reviewed. A trial was conducted to investigate the persistence of cabbage stems infected by *L. maculans*, either buried or left on the soil surface. More than 90% of the buried debris had decomposed after 2.5 yr, whereas only 80% of the surface debris had decomposed.

In another trial the susceptibilities of seedbed transplants (SBT) and container-grown seedlings (CGS) were compared using different forms of *L. maculans* inoculum. "Dunk" inoculation of SBT into a pycnidiosporial suspension resulted in a stem infection level of 50% greater than an uninoculated control. Contamination of seedbeds resulted in an infection level of 46%. "Dunk" inoculation of CGS resulted in infection of 22%. When CGS were grown in contaminated trays an infection level of 33.4% resulted. Interplot interference in the form of inoculum dispersal over a 1 m border was low (1.8 and 2.7% for SBT and CGS, respectively).

In a further trial examining the relationship of inoculum level and blackleg, a strong interaction was found between inoculation technique and inoculum level. Inoculation of field plots with infected debris was a more efficient technique than dipping seedlings into a pycnidiospore suspension prior to transplanting.

7.1 Introduction

L. maculans is a poorly competitive soil saprophyte, surviving less than 2 mo in the soil (Hughes, 1933; Cunningham, 1939). However, it is able to survive in infected crop debris until the debris is decomposed by saprophytic organisms. *L. maculans*'s saprophytic survival period depends on several factors:

1) The nature of the debris involved, roots and stems decomposing more slowly than leaves.
2) The level of natural biological activity in the area, insects being the initial colonizers of stem and root debris, and fungi and bacteria decomposing the remains. The activities of all of these organisms are affected by climatic conditions, while microbial activity is also affected by soil fertility.
3) Cultural practices such as deep ploughing leave little debris on the soil surface. Conversely, rotovating and discing leave much debris on the soil surface, where it often mummifies and remains undecomposed for long periods.

L. maculans, therefore, depends on the survival of host debris to maintain high inoculum levels between crops and, not surprisingly, the fungus has a sophisticated evolutionary stable strategy (ESS) (Maynard-Smith, 1974) in its pathogenesis, using both host and self-generated biochemical protection to increase the survival period of host debris. Firstly, it induces a host lignification process in the fungus-affected area. Brunin (1972) observed that *L. maculans* induced intensive irregular lignification in infected host stems, resulting in necrotic islands and constriction of xylem vessels. The lignification process appears to be outside the host's control, for it effectively debilitates, or destroys the host's vascular system, maiming or killing the plant. The advantages to the fungus of this lignification is a greatly increased structural and chemical integrity of the cabbage stem since lignin is a tough structural material of plants, degraded by few microbes. Alexander (1961) commented, "the outstanding microbiological characteristic of lignin is its resistance to enzymatic degradation; decomposition of lignin is characteristically less than that observed for cellulose, hemicellulose, and other carbohydrates". And further, "lignin protects associated carbonaceous compounds (cellulose and hemicelluloses) from degradation. For

example, hemicelluloses in plants with a high lignin content are less susceptible to microbial degradation. This probably results from a physical or physico-chemical barrier set up by the close interlinkage between the lignins and the hemicelluloses and cellulose of the plant cell walls, possibly by means of a lignin encrustation which mechanically separates the micro-organism from the carbohydrate".

Secondly, the debris is protected from saprophytes by a number of toxins secreted by *L. maculans*; e.g., sirodesmin PL, deacetylsirodesmin PL, sirodesmins H, J, and K and phomalirazine have been isolated from infected stem tissue (Soledade *et al.*, 1992). These toxins are related to a family of antiviral epipolythiodioxopiperazines, the sirodesmins. The toxins are also antibacterial, mycotoxic and phytotoxic (Boudart and Lacoste, 1972; Ferezou *et al.*, 1977; 1980; Poiret *et al.*, 1985; Boudart, 1989), inhibiting the growth of turnip canola roots at concentrations of 3 ppm (Bousquet *et al.*, 1977). Salicylic acid is also released into the plant tissues (Dixelius, 1994).

The combination of heavy lignification, the presence of potent biocides in the host tissues and mummification make a dried, blackleg-infected crucifer stem an effective survival vessel, a "suspended-animation capsule". In addition to making evolutionary sense, personal observation of specific debris provided subjective evidence that in the field, the fungus-affected zone of cabbage stems degrade much more slowly than uninfected tissues.

Brunin (1972) took a different viewpoint of the lignification process, considering the aberrant lignification only in terms of a host resistance response, and found that resistant canola plants underwent early differentiation of the xylem at the infection collar, which appeared to inhibit the further progress of *L. maculans*. However, this may be a limited perspective: the lignification process of susceptible cultivars certainly assists the survival of the fungus.

Safely ensconced in the debris, the fungus develops both pycnidia and pseudothecia, inside which pycnidiospores and ascospores are produced, to be released under favourable climatic conditions. *L. maculans* continues to produce fresh pycnidia and

pseudothecia in repeated cycles in response to moisture, light and temperature (20^0C) (Alabouvette *et al.*, 1970; Bokor, 1972), similar in this aspect to *L. nodorum* Mull. (*Septoria nodorum* Berk.) (Harrower, 1974). The spores produced infect seedlings, secondary pycnidia are formed and pycnidiospores are released which infect other seedlings, particularly in the transplanting process, generating the standard cabbage epidemic, and creating infectious debris capable of repeating the disease cycle. In France, Alabouvette and Brunin (1970) found *L. maculans* to survive in the xylem vessels of canola which were 3, 4 and 5 yr old, having survived on the soil surface or having been unearthed by ploughing 2, 3 or 4 yr later. Clayton (1927) and Chupp and Sherf (1960) considered the fungus to survive at least 3 yr of external weathering under American conditions. Cunningham (1939) found the fungus to survive at least 2 yr of weathering under natural conditions in New Zealand.

Information in the literature on the survival of *L. maculans* is summarized in Table 7.1.A.

Table 7.1.A: Reported Survival of *L. maculans* in the Soil and in Debris

Crop	Observation	Author
cabbage	*L. maculans* survived >3 yr in debris	Clayton, 1927; Chupp and Sherf, 1960
swedes	*L. maculans* survived less than 2 mo in the soil, outside crop residues	Hughes, 1933; Cunningham, 1939
swedes	*L. maculans* survived >2 yr in debris	Cunningham, 1939
canola	*L. maculans* survived in xylem vessels 3, 4 and 5 yr old, on surface or buried.	Alabouvette and Brunin (1970)
canola	ascospore discharge from canola debris is drastically reduced after 12 mo. weathering	McGee, 1977
canola	surface debris survived >18 mo; shallow burial ineffective; deep burial effective after 18 mo	MacNish, 1979b
canola	sprays of triarimol, fenarimol, ethylmercury phosphate, dinoseb, diquat, paraquat, Manoxol OT, Manoxol N, Bradasol, Cetrimide, Deciquam and 5% urea resulted in 97-100% inhibition of pseudothecia formation	Humpherson-Jones *et al.*, 1980 Humpherson-Jones and Burchill, 1982
canola	3 yr rotation reduces blackleg incidence	George *et al.*, 1985
canola	6-8 yr more effective than 3 yr rotation	Daebeler *et al.*, 1987
canola	In Kentucky, a break of 15 mo. may be adequate; rotations >26 mo. considered optimal	Hershman and Perkins, 1995
canola	In semi-arid Saskatchewan, 90% of canola stubble decomposed after 2 yr; however, ascospores discharged from debris for 5-7 yr;	Petrie, 1995a
canola	application of benomyl, chlorothalonil, imazalil, nuarimol, prochloraz, propiconazole, triadimenol and vinclozolin (fungicides), Agral-90 and Triton X-100 (wetters), urea (fertilizer) and glyphosate (herbicide) to canola debris reduced ascospore discharges; application of trifluralin (herbicide) increased ascospore discharges	Petrie, 1995c

Within the local cabbage blackleg pathosystem two situations, one natural and one unnatural which appear to contribute significantly to the volume of infectious material left on the soil after harvest of cabbages:

1. Cabbages infected with blackleg early in the season are stunted and therefore the plant is left unharvested, and the whole plant is left undisturbed in the soil to die and mummify (Fig. 7.1.A);

2. The local harvesting technique is to cut the cabbage heads off with a machete (Fig. 7.1.B), leaving a large root in the soil and a thick stem protruding into the air (Fig. 7.1.C - D). Following death, the stem section is in an ideal situation to mummify. However, the stem often ratoon, and the plant will live on until the land is ploughed. This extends the period of protection of any colonizing pathogen against saprophytes.

The combination of these two factors, and the absence of crop rotation and post-harvest sanitation, provide excellent opportunities for *L. maculans* to survive its saprophytic stage in debris (Fig. 7.1.E - F). The common postharvest soil treatment is tillage by either rotovation or disc ploughing, both practices which leave much of the crop debris on the soil surface (Fig. 7.1.G - H). Deep ploughing with a mould-board plough is more efficient in burying debris (Fig. 7.1.I) but is not practised widely because it is expensive and damages soil structure.

It is safe to graze ruminants on infected debris, since it has been shown that *L. maculans* spores are killed during passage through the gut of these animals (MacNish, 1979a). However, this common practice is dangerous because it usually leaves a substantial quantity of debris on the land, and ensilage or composting would be a far safer treatment of the debris.

Fig. 7.1.A
A mummified cabbage stem. Note the cracks resulting from blackleg.

Fig. 7.1.B
Cabbage harvesting in South Africa: cutting cabbage heads with a machete.

Fig. 7.1.C
A cabbage stem left in the soil after the head has been harvested.

Fig. 7.1.D
A cabbage stem left in the soil after the head has been harvested. Note the cracks in the stem caused by blackleg infection.

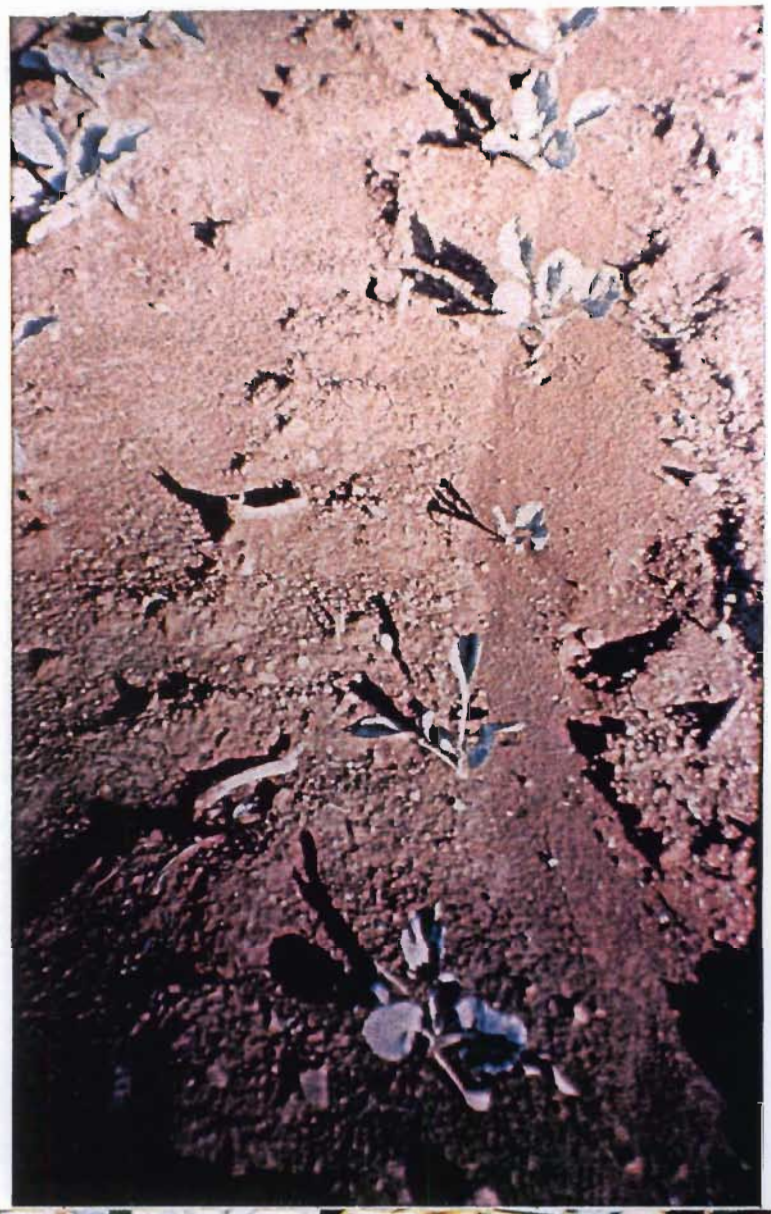

Fig. 7.1.E Monoculture of cabbage: Weathered cabbage stems from a previous crop present in the current cabbage crop.

Fig. 7.1.F
A mature cauliflower wilting from blackleg. Note the crucifer debris in the foreground.

Fig. 7.1.G
Rotovating the soil after a broccoli crop. Note the debris left on the soil surface.

Fig. 7.1.H
A closeup of a broccoli land after rotovation. Note all the debris present.

Fig. 7.1.I
Deep-ploughing using a mould-board plough to inter crop debris. A powerful four-wheel drive tractor is needed for this operation, which also results in damage to soil structure and loss of soil moisture.

7.2 Inoculum Trial 1: Survival of Cabbage Debris under KwaZulu-Natal Conditions

After the death of the host or following the trashing of crop residues, most pathogens are able to survive until at least the next crop by means of a variety of desiccation- resistant structures.
All of these intercrop survival structures constitute an important inoculum potential, compounded of inoculum density, endogenous and exogenous energy, the genetic virulence of the propagules, and the environment limiting their invasive force. It is important to be able to recognize this potential and eradicate the surviving inoculum.

Jarvis, 1992

7.2.1 Introduction

Given that the survival of debris determines the survival of the fungus, and its ongoing opportunity to initiate infection, a trial was set up to determine the survival of cabbage debris under KwaZulu-Natal conditions.

7.2.2 Materials and Methods

This trial was conducted on a farm in the Dargle district of KwaZulu-Natal. The previous cabbage crop had suffered a severe blackleg epidemic in spring and therefore there was an abundance of infected cabbage stems. A small cabbage land was dedicated to the trial, because the soil would approximate the tilth and biological activity of production lands. The soil concerned was a Hutton soil with >30% clay content. In mid-summer (December), twenty five porous "cabbage" string bags (the standard plastic fibre bags used to carry 30 kg of cabbage heads to market) were each filled with 1 kg of freshly-harvested cabbage stems infected with *L. maculans*. Twenty of these were buried separately at a depth of 350 mm. Five were left on the soil

surface. Both the surface and buried bags were secured by attaching them to a tent peg. Every 3 mo two of the buried bags were uncovered, and together with the surface bags, were examined with a hand lens for the presence of pycnidia and pseudothecia. After examination the bags of debris were then returned to the laboratory, and dried in an oven at 50^0C for 24 hr. The surviving debris were then weighed, the data being used to construct half-life graphs of the debris.

7.2.3 Results

The results of this trial are summarized in Table 7.2.3.A, and are presented graphically in Fig. 7.2.3.A.

Table 7.2.3.A: Inoculum Trial 1: Breakdown of Buried and Surface Cabbage Debris as a Function of Time

Period of Exposure (months)	BURIED: Mean Mass of Debris (g)	BURIED: *Visible Pycnidia	SURFACE: Mean Mass of Debris (g)	SURFACE: *Visible Pycnidia
0	1000	+	1000	+
3	782	+	558	+
6	424	+	391	+
9	385	-	305	+
12	256	-	280	+
15	220	-	237	+
18	185	-	215	+
21	150	-	192	+
24	105	-	183	+
27	95	-	190	+
30	78	-	163	+

* Viability of pycnidia was not determined.

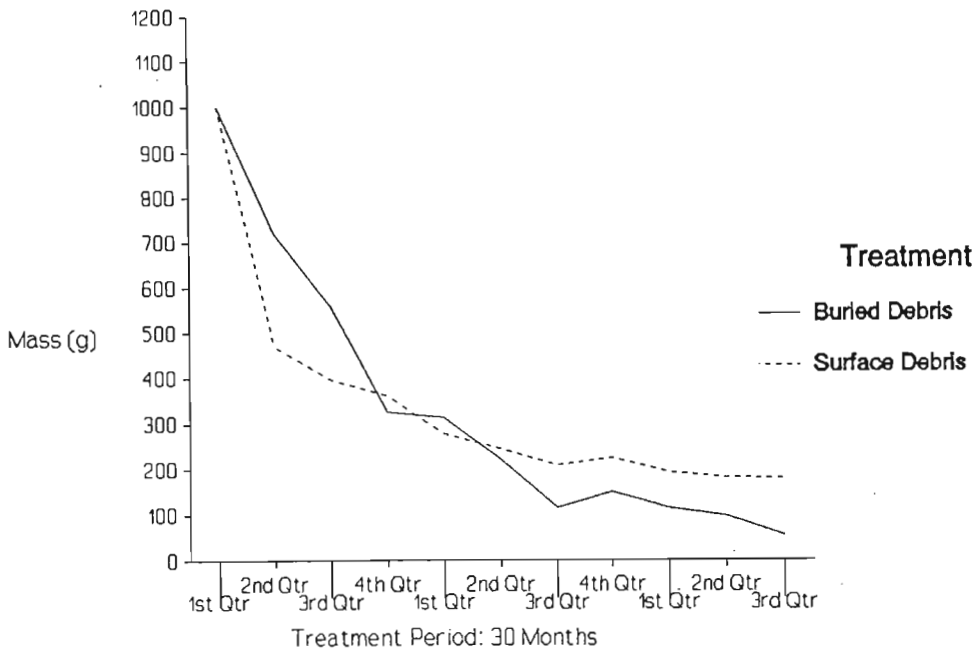

Fig. 7.2.3.A Cabbage Stem Decomposition
As a Function of Treatment and Time

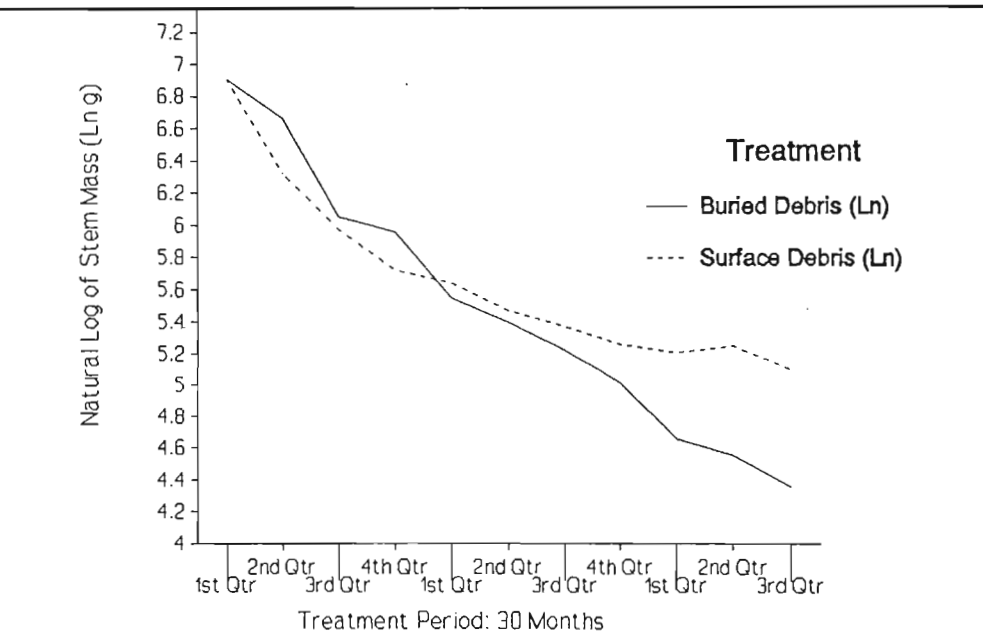

Fig. 7.2.3.B Natural Log of Cabbage Stem Decomposition
As a Function of Treatment and Time

Despite the limitations in the buried-bags technique used for this trial, and the fact that it was an observational trial, the results are nonetheless fairly clear:

1. Burial of cabbage stem debris in biologically active topsoil is a relatively effective way of decomposing it, and most of the debris buried had effectively decomposed after 2.5 yr;
2. Cabbage stem debris left on the soil surface appeared to dry out initially and then to undergo slow degradation. After 2.5 yr at least twice as much debris survived on the soil surface as survived after burial.
3. Pycnidia were visible on the surface stem debris for much longer than on the buried debris. However, testing pycnidia for viability was logistically impossible, and the presence of visible pycnidia did not guarantee that they would produce viable pycnidiospores.

7.2.4 Discussion

The implications of this trial are that, in KwaZulu-Natal, stems left in the field after harvest will pose a threat to subsequent crops for at least 2 yr, especially if the debris is left on the soil surface. The results also raise the possibility that, if surface debris can survive for several years, then subsequent seedbeds could be located on top of debris left over from previous crops. It is clear also that active removal of surface debris would reduce the effective rotation period needed in crucifer fields.

These results partially concur with those of MacNish (1979b), who found that, under Australian conditions, canola debris on the soil surface, or buried deeply (100 mm), decomposed more rapidly than material just under the soil surface. These results also agree with French, (Alabouvette and Brunin, 1970), New Zealand (Cunningham, 1939) and American research (Clayton, 1927; Chupp and Sherf, 1960), all of which indicated that *L. maculans* can survive for at least 2 yr inside crucifer debris, either when buried or on the soil surface.

A number of factors would affect the life of plant debris on or under the soil: the season, and hence the weather conditions; the particular soil composition; the animal and microbiological status of the soil; soil nutrition [especially nitrogen level

(Humpherson-Jones *et al.*, 1980; Humpherson-Jones and Burchill, 1982; Petrie, 1995c) and farming activity.

A combination of these factors occur under real conditions, in that soil preparation practices such as ploughing, and herbicide, fertilizer and lime application, all affect the biological activity of the soil, and therefore would affect the degradation of infected cabbage stems. For example, stems lying on the soil surface may be buried by ploughing, or buried stems could be brought to the soil surface. Ploughing also affects the moisture content of the soil and the microbiological composition of the soil. A rotovator may play a different role, cutting the stems into smaller chunks of material which are more readily degraded, but also killing much of the larger soil fauna, such as earthworms. Herbicides, lime and fertilizer affect the biological activity of the soil, and hence will impact on the degradation rate of cabbage stems. The experimental problem is that these interactions are very complex and difficult to quantify. It was not possible to measure these effects on stem degradation in this trial (a problem most debris degradation studies face) where samples of debris were buried in porous bags in the soil.

A further complication is that if physical treatments of the soil are allowed, they destroy the bag itself. So the technique used here, and in the other studies discussed above, creates a highly artificial situation of an enforced fallow period. I suggest that most farming activities mentioned above will accelerate debris degradation. It is therefore likely that, under normal farming conditions, debris degradation will in fact be faster than the time scales measured here or in other studies, which have produced conservative estimates of the rotation period required for debris decomposition to have occurred. This may be safer, but also may discourage farmers from using even short rotation periods, which may be far more effective than is realized. As Humpherson-Jones and Burchill (1982) showed in a seminal paper, with accelerated biodegradation or the chemical elimination of pseudothecia in debris, short rotations can be effective for the control of *L. maculans* inoculum on canola debris. Petrie (1995c) found similar results, after applying a similar range of chemicals to naturally-infected canola debris.

7.3 Inoculum Trial 2: Disease Incidence as a Function of Inoculum Form and Seedling Type

7.3.1 Introduction

As discussed in Section 1.2.4, both SBT and CGS seedlings were in common use in KwaZulu-Natal in the early 1980s. However, examination of blackleg epidemics revealed that the majority of blackleg epidemics were associated with the use of SBT or transplanted DDP; in the four cases where a blackleg epidemic had been observed in a crop grown from CGS, the lands had been monocropped to cabbage for 4, 8, 12 and 14 successive crops, and there was a considerable accumulation of infected debris in the fields. Therefore, it was considered important to investigate the relative susceptibilities of crops grown from seedlings and from speedlings.

A theory was developed that **the major** transfer of inoculum takes place at transplanting, when seedbeds are "pulled". In this procedure, the seedbed is first heavily watered, then the seedlings are pulled out of the ground and packed, bunch upon bunch, into a wooden or plastic crate. This dripping crate is then moved to the field and the seedlings are transplanted. If one seedling sitting on top of the others is infected with blackleg, then pycnidiospores will be released from active lesions, and will contaminate the other plants. In this way, large numbers of healthy seedlings may be rapidly infected. Furthermore, a distinctive pattern of blackleg incidence will occur in the field: infection developing mostly in runs down the planting lines, with little spread sideways. The issue to be investigated, therefore, was whether a major transfer of *L. maculans* pycnidiospore inoculum could be simulated during the transplanting process.

The other major inoculum source of blackleg is from debris already in the field; i.e., infected material from the previous season. The relative contributions of these two forms of *L. maculans* inoculum, transplant infection versus field inoculum, were also investigated.

7.3.2 Materials and Methods

The trial site was at the Plant Pathology Section of the Agriculture Development Institute (CADI), KwaZulu-Natal. The trial design was a duplicated factorial (3 x 2), randomized complete blocks design with 4 replicates, 36 plants per plot, spaced at 0.5 m x 0.5 m with 1 m borders.

The cabbage cultivar Gloria Osena (seedlot McDonald's 8B1024) was used for this trial.

The treatments were:

Factor 1: Plant Type

Level 1: SBT

Level 2: CGS

Both sets of seedlings were grown at the Univ. of Natal using normal cultural practices.

Factor 2: Contamination of Seedbed or Speedling trays

Level 1: No inoculation

Level 2: Inoculation at 3 wk with 25 g m^2 of chopped, infected cabbage stems.

At 6 wk after planting, the seedlings were transplanted at CADI.

Factor 3: Transplant inoculation

Level 1: Dip seedlings into water.

Level 2: "Dunk" seedlings into a suspension of 200 g chopped, infected cabbage stems in 2 ℓ water, for 2 min.

As a fourth, but unreplicated factor, one of the duplicated fields was first inoculated with 25 g per m^2 of chopped, infected cabbage stems to simulate a field contaminated with infectious debris left over from the previous crop. The infected cabbage stems were from a previous blackleg trial and were 6 mo old. Pycnidia were present on the stems, in the center of the blackleg lesions.

Plot preparation was the same as for Cultivar Trial 3 (Section 5.4.2).

Every plant was evaluated for infection of the stem by *L. maculans*.

ANOVA of the data was conducted on the infection incidence only, using the same statistical packages as discussed previously (Section 5.2.2).

7.3.3 Results

The results from this trial are presented below in Tables 7.3.3.A-C, and Fig. 7.3.3.A-F.

Table 7.3.3.A: Inoculum Trial 2, Field A:
Blackleg Inoculum ABSENT From the Field
Percent Incidence of Blackleg on Cabbage Stems

Seedling Source: Seedbed or Container	Seedbed/Tray Contamination	CONTROL: [1] Water Only	[2] Seedlings "Dunk" Inoculated
Seedbed Transplants (SBT)	Clean Seedbed	1.8	52.7
	[3] Contaminated Seedbed	46.4	62.7
Container-Grown Seedlings (CGS)	Clean Seedling Tray	2.7	21.8
	[4] Contaminated Seedling Tray	33.4	57.3

[1] : Control seedlings were dipped into water only.

[2] : "Dunk" treated seedlings were given a 2 min dip into a pycnidiospore suspension.

[3] : Seedbed contaminated with chopped, infected stem debris at 25 g per m^2

[4] : Seedling tray contaminated with chopped, infected stem debris at 25 g per m^2

A number of conclusions can be drawn from this table:

1. When clean SBT were dunked into clean water only 1.8% of plants became infected. However, the equivalent "dunk" inoculation resulted in a stem infection level of 52.7%, a difference of 50%.
2. When SBT were taken from a contaminated seedbed and dunked into clean water, an infection level of 46.4% occurred. When also dunked into a

pycnidiosporial suspension, an infection level of 62.7% developed, an increase of 16%.

3. When clean CGS were dunked into clean water, an infection level of 2.7% resulted, whereas when dunked into a pycnidiosporial suspension a level of 21.8% resulted, a difference of 20%.
4. When CGS were taken from contaminated trays and dunked into clean water an infection level of 33.4% resulted, whereas when they were also "dunk" inoculated an infection level of 57.3% occurred, a difference of 24%.
5. Interplot interference in the form of inoculum dispersal over a 1 m border pathway was low (1.8 and 2.7% for SBT and CGS, respectively).
6. Overall, CGS consistently showed a lower incidence of blackleg infection than SBT inoculated with the same levels of inoculum.

Table 7.3.3.B: Inoculum Trial 2, Field B:
Blackleg Inoculum PRESENT in the Field
(Percent Incidence of Blackleg on Cabbage Stems)

Seedling Source: Seedbed or Container	Seedbed/Tray Contamination	[1] CONTROL: Water only	[2] Seedlings "Dunk" Inoculated
Seedbed Transplants (SBT)	Clean Seedbed	82.5	86.4
	[3] Contaminated Seedbed	93.9	86.9
Container-Grown Seedlings (CGS)	clean seedbed	70.2	67.1
	[4] Contaminated Seedling Tray	80.8	84.9

[1] : Control seedlings were dipped into water only.

[2] : "Dunk" treated seedlings were given a 2 min dip into a pycnidiospore suspension.

[3] : Seedbed contaminated with chopped, infected stem debris at 25 g per m^2

[4] : Seedling tray contaminated with chopped, infected stem debris at 25 g per m^2

Results in this table indicate that in Field B, which was inoculated with blackleg-infected cabbage debris:

1. Clean SBT dunked in water still developed an 82% infection level from field inoculum. This could only have come from the plot inoculation. When Clean SBT were also dunked into a pycnidiosporial suspension before planting, only 4% more blackleg occurred than the water only treatment (86.4%).
2. When SBT were taken from a contaminated seedbed were dunked into clean water before planting, a 93.9% infection level occurred. When contaminated SBT were also inoculated with pycnidiospores, an infection level of 86.9% occurred. The relative level of infection, a difference of 7%, is minimal, although the lower incidence of blackleg was surprising, given the greater inoculum of the latter treatment.
3. When clean CGS were dunked into clean water an infection level 70.2% resulted. A very similar level of 67.1% occurred when the seedlings were dunked into a pycnidiosporial suspension. The difference of only 3% in blackleg infection is insignificant.
4. When CGS were taken from contaminated trays and dunked into clean water, an infection level of 80.8% occurred. Additional inoculum from a dunk in a pycnidiosporial suspension increased the infection level marginally to 84.9%, a difference of 4%.
5. Overall, the contamination of seedbeds or trays raised infection levels of both the water dunked and contaminated-water dunked SBT and CGS by about 10%.
6. Overall, CGS consistently developed an incidence of blackleg infection about 10% lower than SBT inoculated with the same levels of inoculum.

Table 7.3.3.C: Inoculum Trial 2: Results of ANOVA

Main Effects		Field A, No Plot Inoculum		Field B, Plot Inoculum	
A:	**SBT vs CGS**	F=16.7	*	F=25.72	**
B:	**Seedbed inoculum vs control**	F=461.9	***	F=11.64	*
C:	**"dunking" inoculation vs water control**	F=32.7	**	F=1.02	NS
Interaction Effects					
AxB:	Seedling type x seedbed inoculation	F=0.29	NS	F=0.021	NS
AxC:	Seedling type x "dunking"	F=3.47	NS	F=0.37	NS
BxC:	Seedbed inoculation x "dunking	F=5.51	NS	F=0.163	NS
AxBxC:	Seedling type x seedbed inoculation x "dunking"	F=37.45	**	F=1.9	NS
		CV% = 13.11%		CV% = 8.66%	

One interaction effect was significant, the **AxBxC** interaction between all treatments in Field A, the uninoculated field: seedling type x seedbed inoculation x "dunking" inoculation.

The main effects were all significant in both Field A and Field B, except for the "dunking" inoculation in Field B.

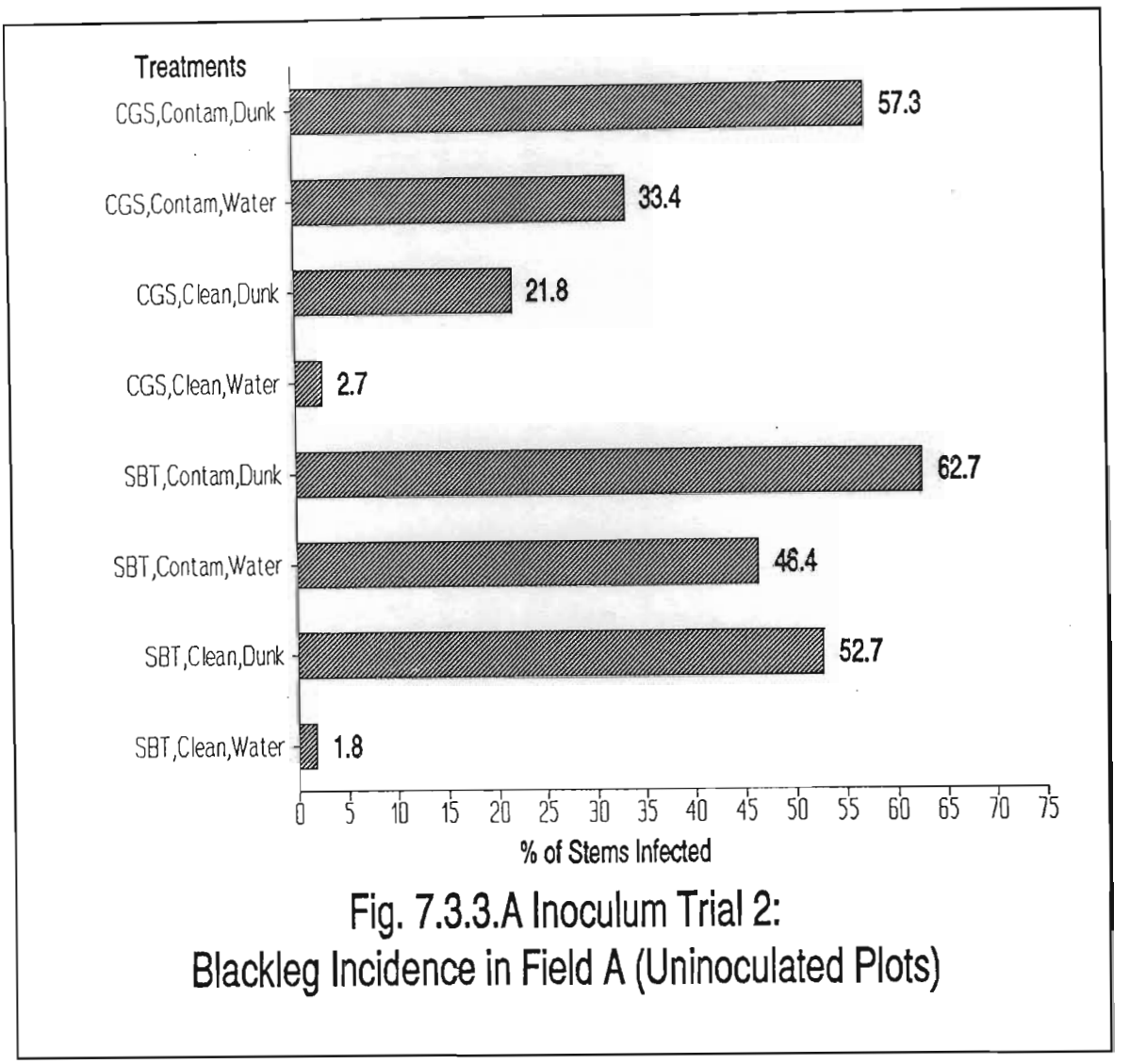

Fig. 7.3.3.A Inoculum Trial 2:
Blackleg Incidence in Field A (Uninoculated Plots)

Key to Treatments:

Field A: The field plots were NOT inoculated with infected debris

CGS = Container-grown seedlings

SBT = Seedbed transplants

Contam = Seedbed or seedling tray contaminated with chopped, infected stem debris at 25 g per m^2

Clean = Seedbed or seedling tray remained uninoculated

Dunk = Seedlings were given a two minute dip into a pycnidiospore suspension

Water = Seedlings were dipped into water only

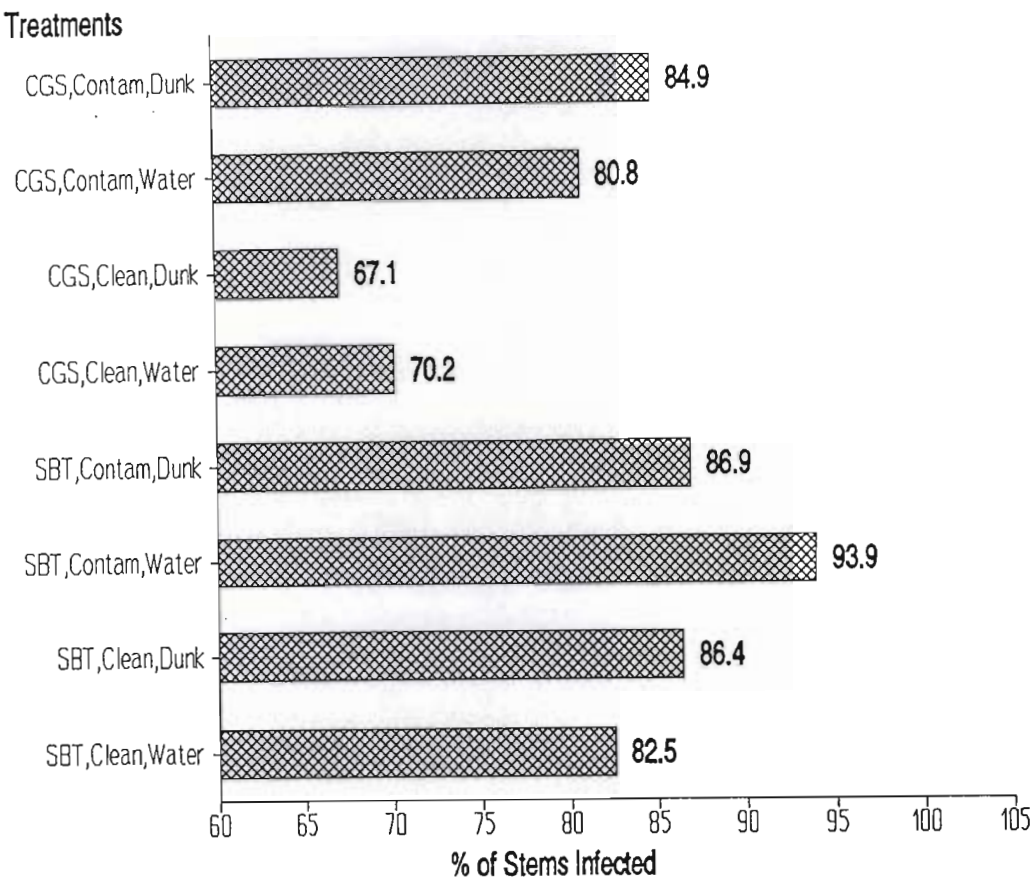

Fig. 7.3.3.B Inoculum Trial 2:
Blackleg Incidence in Field B (Inoculated Plots)

Key to Treatments:

Field B: The field plots were inoculated with infected debris at a rate of 25 g per m^2 at transplanting

CGS = Container-grown seedlings

SBT = Seedbed transplants

Contam = Seedbed or seedling tray contaminated with chopped, infected stem debris at 25 g per m^2

Clean = Seedbed or seedling tray remained uninoculated

Dunk = Seedlings were given a two minute dip into a pycnidiospore suspension

Water = Seedlings were dipped into water only

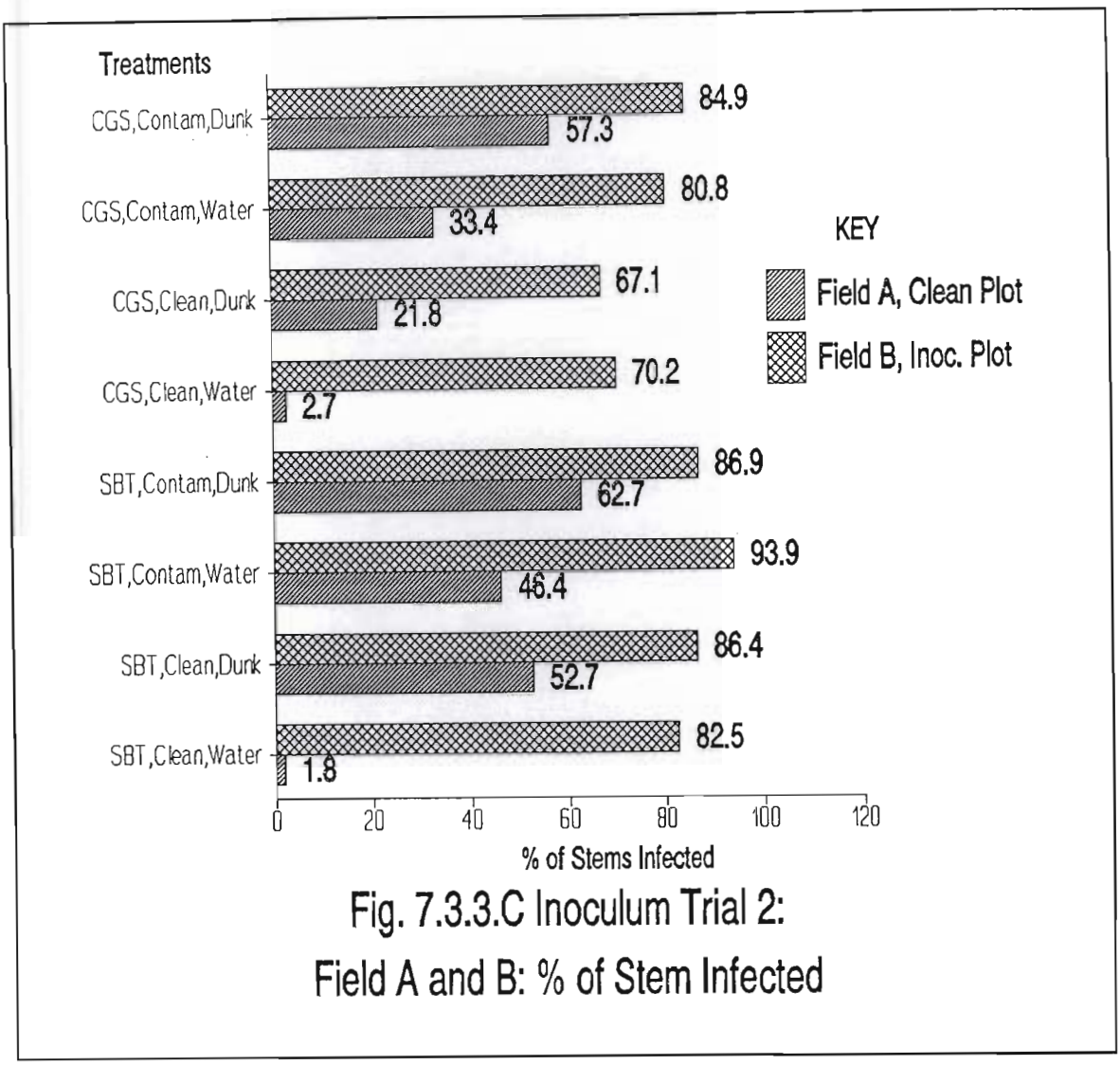

Fig. 7.3.3.C Inoculum Trial 2:
Field A and B: % of Stem Infected

Key to Treatments:

Field A: The field plots were NOT inoculated with infected debris

Field B: The field plots were inoculated with infected debris at a rate of 25 g per m^2 at transplanting

CGS = Container-grown seedlings

SBT = Seedbed transplants

Contam = Seedbed or seedling tray contaminated with chopped, infected stem debris at 25 g per m^2

Clean = Seedbed or seedling tray remained uninoculated

Dunk = Seedlings were given a two minute dip into a pycnidiospore suspension

Water = Seedlings were dipped into water only

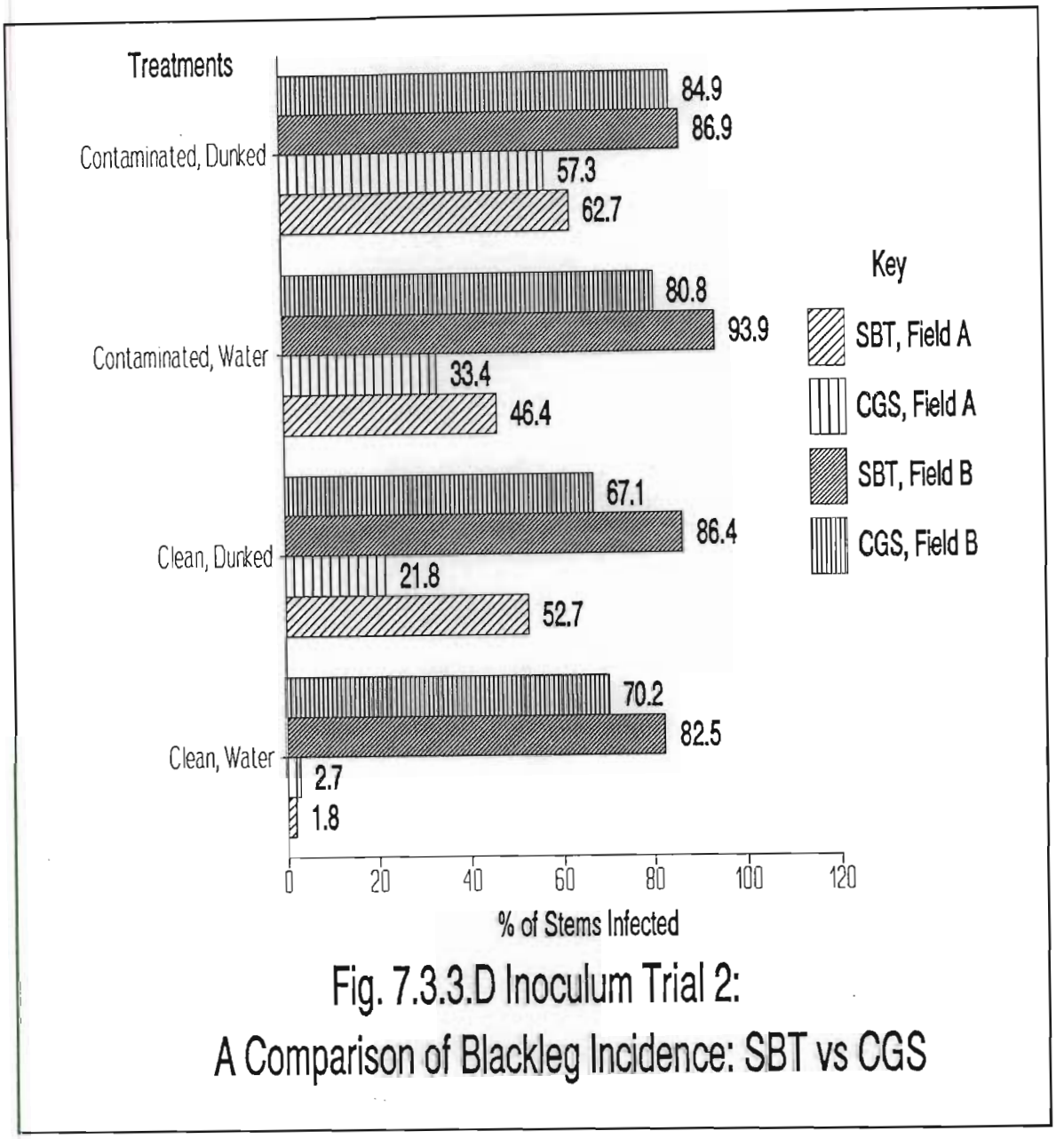

Fig. 7.3.3.D Inoculum Trial 2:
A Comparison of Blackleg Incidence: SBT vs CGS

Key to Treatments:

Field A: The field plots were NOT inoculated with infected debris
Field B: The field plots were inoculated with infected debris at a rate of 25 g per m^2 at transplanting
CGS = Container-grown seedlings
SBT = Seedbed transplants
Contam = Seedbed or seedling tray contaminated with chopped, infected stem debris at 25 g per m^2
Clean = Seedbed or seedling tray remained uninoculated
Dunk = Seedlings were given a two minute dip into a pycnidiospore suspension
Water = Seedlings were dipped into water only

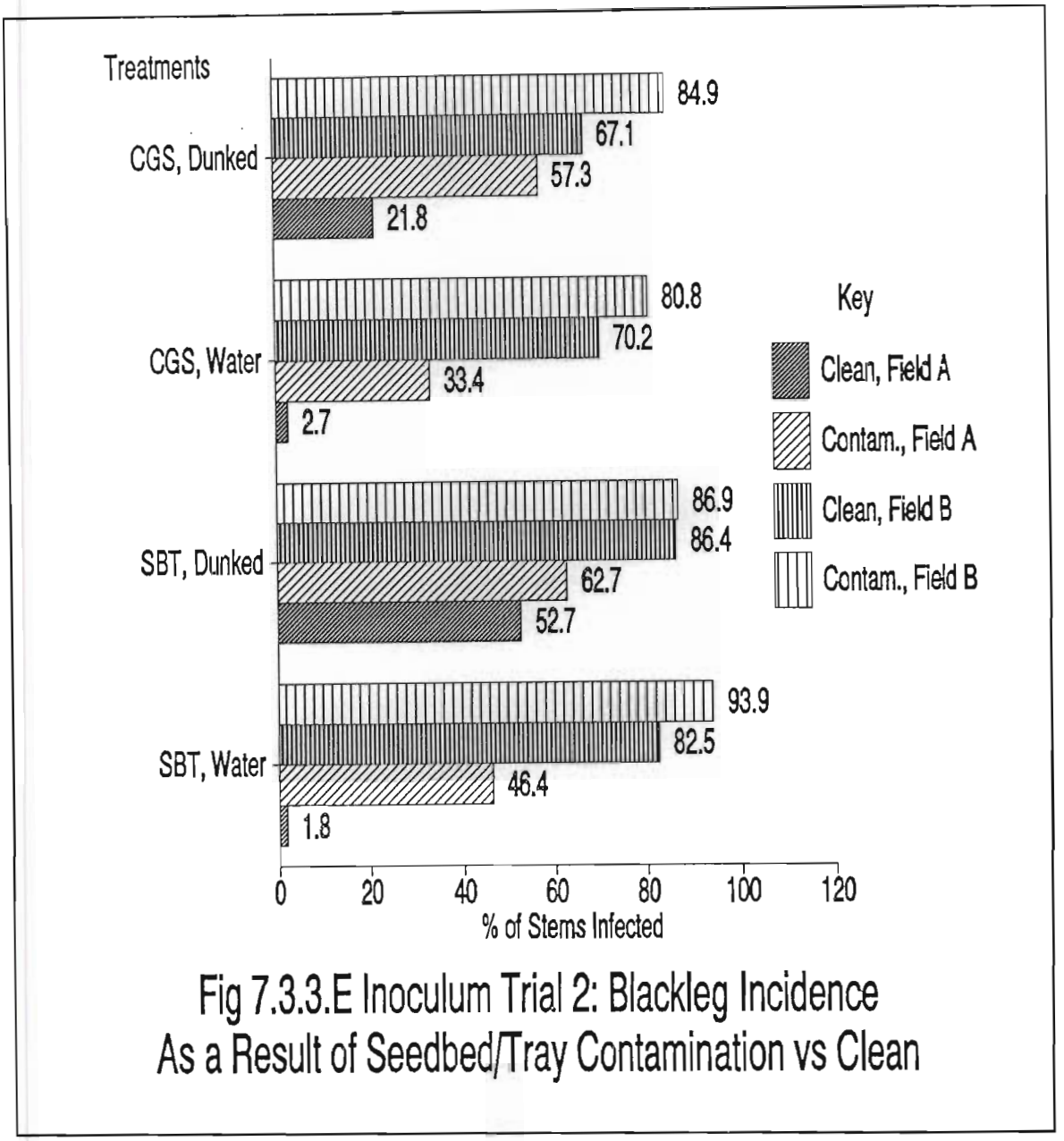

Fig 7.3.3.E Inoculum Trial 2: Blackleg Incidence As a Result of Seedbed/Tray Contamination vs Clean

Key to Treatments:

Field A: The field plots were NOT inoculated with infected debris

Field B: The field plots were inoculated with infected debris at a rate of 25 g per m^2 at transplanting

CGS = Container-grown seedlings

SBT = Seedbed transplants

Contam = Seedbed or seedling tray contaminated with chopped, infected stem debris at 25 g per m^2

Clean = Seedbed or seedling tray remained uninoculated

Dunk = Seedlings were given a two minute dip into a pycnidiospore suspension

Water = Seedlings were dipped into water only

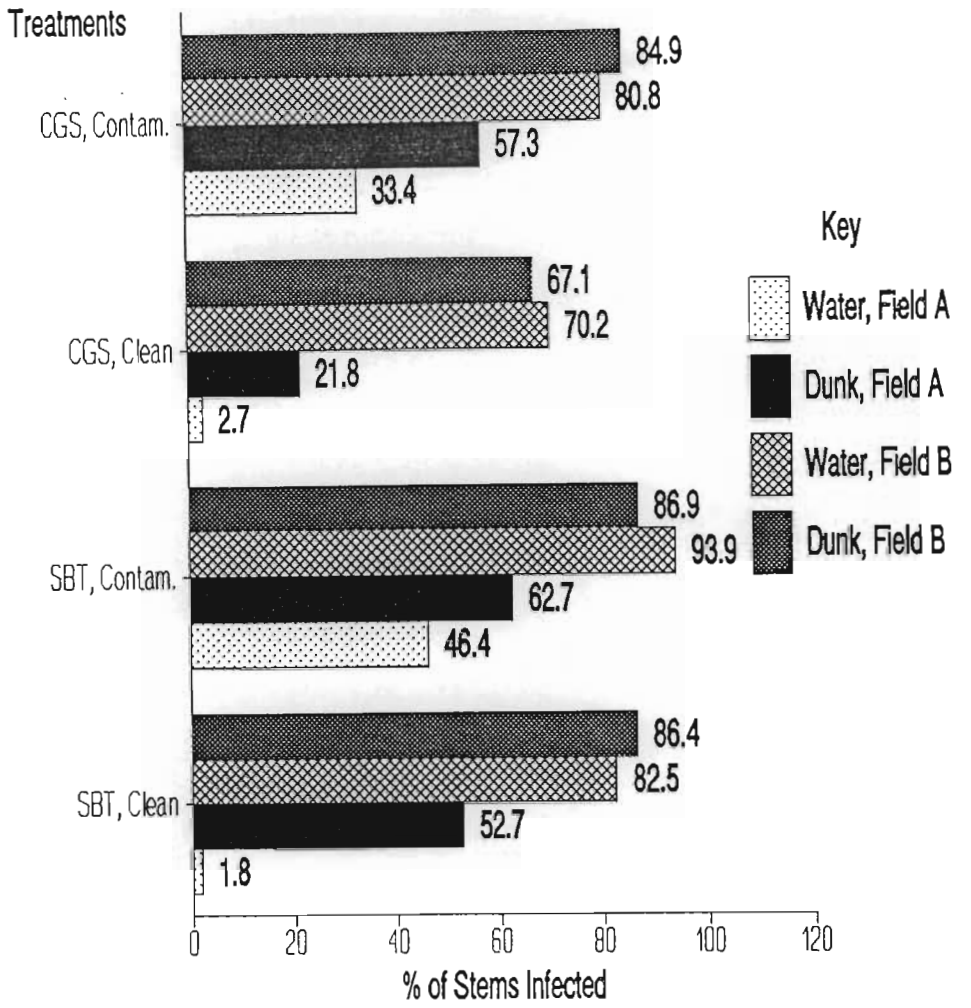

Fig. 7.3.3.F Inoculum Trial 2: Blackleg Incidence As a Result of "Dunk" Inoculation vs Clean Seedlings

Key to Treatments:

Field A: The field plots were NOT inoculated with infected debris

Field B: The field plots were inoculated with infected debris at a rate of 25 g per m^2 at transplanting

CGS = Container-grown seedlings

SBT = Seedbed transplants

Contam = Seedbed or seedling tray contaminated with chopped, infected stem debris at 25 g per m^2

Clean = Seedbed or seedling tray remained uninoculated

Dunk = Seedlings were given a two minute dip into a pycnidiospore suspension

Water = Seedlings were dipped into water only

7.3.4 Discussion

This trial examined three factors and their interactions using a factorial design, plus the effect of in-field inoculum. The factorial design successfully identified both interaction and main effects.

Fig. 7.3.3.A reflects the pattern of inoculum efficacy. The two controls (SBT,Clean,Water and CGS,Clean,Water) were effective in identifying the level of interplot interference present in the trial. It was limited to 1.8% and 2.7% infection, respectively,, an important discovery. This indicated that:

1. Ascospores played little or no role in this trial, despite the presence of infectious debris in Field B, located immediately adjacent to Field A. This trial was conducted in April, which is autumn in KwaZulu-Natal, providing cool conditions suitable for *L. maculans* to sporulate. Rain, heavy dew and overhead irrigation were regular events providing free water, the other environmental parameter necessary for ascospore release.
2. The majority of pycnidiospores released from infected plants did not cross the 1 m border between plots, which provided an accurate measure of their limited epidemiological competence to initiate secondary spread by splash dispersal. Under the conditions of this trial, blackleg was clearly shown to be a monocyclic disease following seedling infection. Hammond and Lewis (1986) also found *L. maculans* to be monocyclic in the UK.
3. For CGS, the "dunk" inoculation was less effective in causing blackleg than the contamination of seedling trays with infected debris. As could be expected, the combination of both inoculations resulted in a higher incidence of stem infection than caused by either inoculation by itself.
4. In contrast to Point 3, the inoculation of SBT by the "dunk" treatment was highly effective. Indeed, it was more effective than seedbed inoculation alone. This results indicates that SBT are substantially more susceptible to pycnidiospore infection at transplanting than are CGS. This confirms the theory presented in Section 1.2.4, that the transfer of pycnidiospores during the transplanting process is the dominant means of pycnidiospore dissemination in

SBT. Again, the combination of both seedbed and "dunk" inoculations caused a higher level of infection than the application of either by themselves.

A comparison of the different incidence of infection with parallel treatments in Field A and B clarifies the effect of inoculum (Fig. 7.3.3.C). In-field inoculum applied in Field B resulted in substantially higher levels of blackleg infection, over all treatments. This suggests that the trial was conducted with a relatively low level of inoculum, and that the other sources of inoculum (seedbed/tray contamination; "dunk" inoculation) were supplemented by the field inoculum placed in Field B. A useful comparison is to look at the two controls (clean seedbeds and water) in Field A where infection levels were 1.8% and 2.7% infection for SBT and CGS respectively. In Field B the same treatments caused infection levels of 82.5% and 70.2%, respectively. The level of inoculum applied to Field B, 25 g per m^2 of chopped, infected cabbage stems, could cause high levels of blackleg incidence in susceptible cabbage crops if the inoculum was well distributed throughout the field. Combination of all three inoculum sources added about 10% more infection, and the maximal infection level appeared to be in the region of 90% under the conditions of this trial.

Factorial analysis reveals the relative contributions of various treatments, and the interaction effects between treatments. Statistical protocol determines that interaction effects are more important than main effects, and must be analysed first.

Examination of the interaction effects, shown in Table 7.3.3.C, reveals that the first three interactions, **AxB**, **AxC** and **BxC**, were not significant. This means that the three main effects (A: SBT versus CGS; B: Seedbed Inoculation versus None; C: "Dunking" Inoculation versus None) were all unaffected by interaction effects; i.e., when the treatments were combined in pairs, they were neutral, acting additively in each paired combination. However, the final interaction of **AxBxC** was highly significant ($F = 37.45^{**}$). This indicates that the two forms of inoculum (Seedbed/tray contamination and "dunk" inoculation) interacted synergistically with the high susceptibility of SBT, resulting in greater levels of disease than one would expect from the primary effects by themselves, or combined with another treatment. This result fits the theory

presented in Section 1.2.4, that most blackleg epidemics in KwaZulu-Natal have resulted from the contamination of SBT at transplanting, arising from seedbed contamination with infected debris.

The main effect of Plant Type (CGS compared with SBT) in Field A was significant ($F = 16.7^{*}$). This result is illustrated in Fig. 7.3.3.D, where in all cases (except for the Control), SBT had higher levels of infection than CGS. Interestingly, this result is more pronounced in Field B ($F = 25.7^{**}$), suggesting that SBT are very vulnerable to *L. maculans* infection when transplanted into an infected field. This is an important result, confirming the theory advanced in Section 1.2.4, that SBT are physiologically more susceptible to blackleg than are CGS. There are several possible reasons for this. Firstly, seedbed seedlings lose large numbers of roots in the transplanting process. This is likely to increase disease incidence because of the injury to the plants (as discussed in Section 1.2.4.2). Secondly, since plants would be under considerable stress at the time of transplanting and if (as is suspected) blackleg is a low-sugar disease (*sensu* Horsefall and Dimond, 1957; Vanderplank, 1984), then this would result in a high incidence of disease. In contrast, CGS suffer little or no transplanting shock as their root plug is left virtually undisturbed in the transplanting process.

The main effect of Seedbed/Tray Contamination (Contaminated seedbed/tray compared with Clean seedbed/tray) was significant in Field A and Field B ($F = 461.9^{**}$; $F = 11.6^{*}$, respectively. The substantial difference in Field A ($F = 462^{***}$) is not surprising: uniform distribution of inoculum in the seedbed should have infected the seedlings evenly, resulting in high levels of disease in the field. In Field B, there was still a significant difference caused by the two treatments, although the field inoculum partially masked the effect of seedbed/tray inoculation. Examination of Fig. 7.3.3.E reveals that in every comparison, inoculation of seedbeds/trays resulted in a higher level of stem infection at harvest than the untreated control. It is, therefore, vital that seedbeds for SBT production, or growing media for CGS, are free of *L. maculans* propagules if blackleg epidemics are to eliminated. The pathogen-free nature of composted pine bark in South Africa (Kemp and Wingfield, 1994), and the

difficulty and expense of sterilizing seedbeds, provide additional reasons for crucifer farmers to switch from SBT to CGS.

The third main effect was Seedling "Dunk" Inoculation (dipping seedlings at transplanting into clean water compared with dipping into a pycnidiosporial suspension). The difference between treatments was highly significant in Field A ($F = 32.7^{**}$), but was not significant in Field B ($F = 1.02$, NS). In Field A, the controls (SBT or CGS, CLEAN, WATER ONLY) developed 1.8% and 2.7% stem infection respectively, whereas the two treatments (SBT or CGS, CLEAN, "DUNK" INOCULATED) developed 52.7% and 21.8% stem infection, respectively. Clearly SBT are more than twice as susceptible than CGS to inoculation in this manner. The implication is that infection at this stage alone would be enough to initiate substantial infection in the field. Obviously prior infection would have had to occur for this to happen, meaning that there would have to be a primary source of inoculum, such as seed-borne inoculum, or seedbed contamination, which would boost the overall level of infection, as occurred in this trial where different forms of inoculum acted at least additively, and synergistically when all three treatments were combined.

Examination of Fig. 7.3.3.F reveals that in Field A, "dunk" inoculation with pycnidiospores consistently resulted in a higher level of infection than the clean water control. In Field B, the presence of inoculum already in the field masked this effect. The implications of this result are again an indictment of the SBT system which provides an ideal opportunity for this kind of "dunk" inoculation to occur, and can therefore initiate significant levels of infection in the field.

Overall, these results confirm observations made in the field survey discussed in Chapter 8. They also go a long way to explaining the observation that in KwaZulu-Natal, crucifer blackleg is no longer the problem that it used to be; indeed, it has virtually disappeared from all major production areas. The disappearance of the disease in KwaZulu-Natal coincides with the emergence of a strong and efficient CGS nursery trade in KwaZulu-Natal, and the almost universal adoption of CGS by commercial cabbage farmers.

It is postulated that the finding that seedbed infections may be a crucial starting point of epidemics (with the exception of fields heavily contaminated with inoculum) applies to many diseases, especially soil-borne, monocyclic diseases such as crucifer clubroot and tomato bacterial wilt. It will not apply to polycyclic diseases, such as crucifer downy mildew or black rot, because these diseases are able to develop an epidemic from very low levels of inoculum without special means of transport, given their greater efficiency in inoculum dissemination.

7.4 Inoculum Trial 3: Disease Incidence as a Function of Inoculum Level

What is the quantitative relation between the amount of inoculum and the amount of disease it produces, that is, between inoculum dose and disease response ?

Vanderplank, 1975

7.4.1 Introduction

Using Vanderplank's (1963) Logarithmic Equation,

$$X_1 = X_0 \cdot e^{rt} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 1$$

where
X_1 = final level of disease
X_0 = initial level of disease; i.e., initial inoculum
r = logarithmic infection rate
t = time between initial infection and final evaluation,

it is clear that the final level of disease is a direct function of the level of initial inoculum. Vanderplank (1963) showed that the significance of the initial inoculum level is inversely related to *rt*; i.e., the faster a pathogen multiplies, and the longer the growing season, the less significant is the effect of initial inoculum level on the final disease level, and hence, the less valuable is sanitation as disease management option.

The converse, however, applies to blackleg where the disease is monocyclic, and develops on a crop in the ground for only 100 d.

For monocyclic diseases,

$$^{dx}/_{dt} = QR\ (1\text{-}X) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ 4$$

where $^{dx}/_{dt}$ = a constant infection rate
Q = initial inoculum
R = relative infection rate
1-X = proportion of crop left uninfected

(Vanderplank, 1963).

The initial inoculum is an even more significant parameter therefore in determining the final level of disease in a monocyclic disease; time no longer plays a role, and the infection rate is no longer a power function. Thus, the level of initial inoculum, and conversely sanitation, are absolutely critical with monocyclic diseases. Their significance is reduced as a function of the number of disease cycles per season. Given that blackleg develops one or two cycles per season in cabbages in KwaZulu-Natal, the initial inoculum level is theoretically critical to its development.

The quantity of inoculum and the point of inoculation (during transplanting or in the field) are primary factors which were considered to affect the levels of blackleg experienced in cabbage fields. A factorial trial was therefore conducted to test these hypotheses.

7.4.2 Materials and Methods

The trial site was at the Plant Pathology Section of Cedara Agricultural Development Institute, as discussed above in Section 7.3.2. The trial had a 2 x 6 factorial design, using a randomized complete blocks layout. Plots were prepared as for Inoculum Trial 2. The cultivar Gloria Osena was used. Seedlings were grown in a commercial speedling nursery. Thirty six plants were planted in each plot, with 500 x 500 mm spacings between plants, 1 m borders between plots and four replications per treatment.

The treatments were:

Factor 1: Inoculation Procedure

Level 1: Inoculate plot with infected debris at the determined rate

Level 2: Inoculate seedlings by dipping them into an inoculum suspension of chopped, infected cabbage stems left for 10 min in 2 ℓ water. This was to simulate the planting of seedlings contaminated in the seedbed.

Factor 2: Inoculum Quantity

Levels 1-6: 40 g, 80 g, 120 g, 160 g, 200 g, 240 g of chopped infected cabbage stems applied per plot or 2 ℓ of inoculation water.

All plants, whether healthy, dead or severely wilted plants were evaluated for blackleg stem infection (the presence of a typical blackleg lesion on the stem) to provide a measure of disease incidence. Percentage of Dead Plants was also evaluated as an alternative measurement of disease, approximating disease severity. The limitation of this parameter was its extreme variability, which introduced a large experimental error in all trials in which it was evaluated.

Statistical analysis was as for Inoculum Trial 2. ANOVA was run on % Infected Plants and % Dead Plants. In addition, linear regressions were run to determine the relationship between these parameters and inoculum levels using the linear regression function in the software package Statsgraphics 5.0.

7.4.3 Results

The results are presented in Tables 7.4.3.A-D and Fig. 7.4.3.A-D.

Table 7.4.3.A: Inoculum Trial 3: Inoculum Levels and Methods of Inoculation

Inoculum Level	% of Stems Infected by *L. maculans*		% of Plants Killed by *L. maculans*	
	[1] Dunked	[2] Plot	Dunked	Plot
40 g	42.85	92.73	5.85	15.58
80 g	66.23	99.08	5.00	25.05
120 g	70.18	95.68	7.53	31.70
160 g	78.18	97.73	22.50	27.23
200 g	74.30	100	13.40	45.13
240 g	84.10	100	12.38	37.03

Treatment Key

[1] **Dunked** = plots inoculated with infected debris at the determined rate
[2] **Plot** = seedlings inoculated by dipping them into an inoculum suspension of chopped, infected cabbage stems left for 10 min in 2 ℓ water.

Table 7.4.3.B: Inoculum Trial 3: Results of ANOVA

Main Effects		% of Stems Infected with *L. maculans*	% Killed by *L. maculans*
A:	[1] Dunk inoculation vs [2] Plot inoculation	F = 420 ***	F = 194 ***
B:	[3] Quantity of inoculum	F = 8.5 **	F = 3.2 *
INTERACTION EFFECTS			
AxB:	Type of inoculation x Quantity of inoculum	F = 3.6 *	F = 2.1 NS
CV%:		CV% = 10.27	CV% = 48.72

Treatment Key

[1] **Dunked** = plots inoculated with infected debris at the determined rate
[2] **Plot** = seedlings inoculated by dipping them into an inoculum suspension of chopped, infected cabbage stems left for 10 min in 2 ℓ water.
[3] **Inoculum Quantity** = 40 g, 80 g, 120 g, 160 g, 200 g, 240 g of chopped infected cabbage stems applied per plot or 2 ℓ of inoculation water.

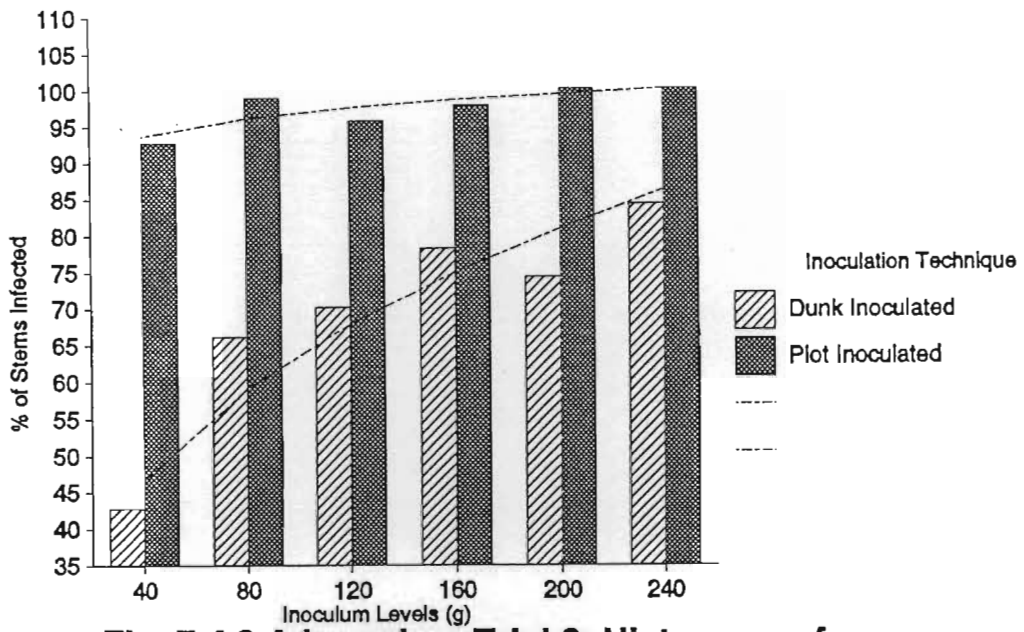

Fig. 7.4.3.A Inoculum Trial 3: Histogram of Blackleg Incidence as a Function of Inoculum Level

Regression of % Stem Infection resulting from increasing levels of inoculum follows a power function for both "dunk" and plot inoculation.

Table 7.4.3.C: Inoculum Trial 3: Results of Linear Regression of % of Stems Infected with Blackleg on Inoculum Levels

	Dunk	Plot
Equation	$Y = 3.84 X^{0.342}$	$4.54 X^{0.036}$
r	0.945	0.810
r^2	0.893	0.656

Treatment Key

[1] Dunked	=	plots inoculated with infected debris at the determined rate
[2] Plot	=	seedlings inoculated by dipping them into an inoculum suspension of chopped, infected cabbage stems left for 10 min in 2 ℓ water.
[3] Inoculum Quantity	=	40 g, 80 g, 120 g, 160 g, 200 g, 240 g of chopped infected cabbage stems applied per plot or 2 ℓ of inoculation water.

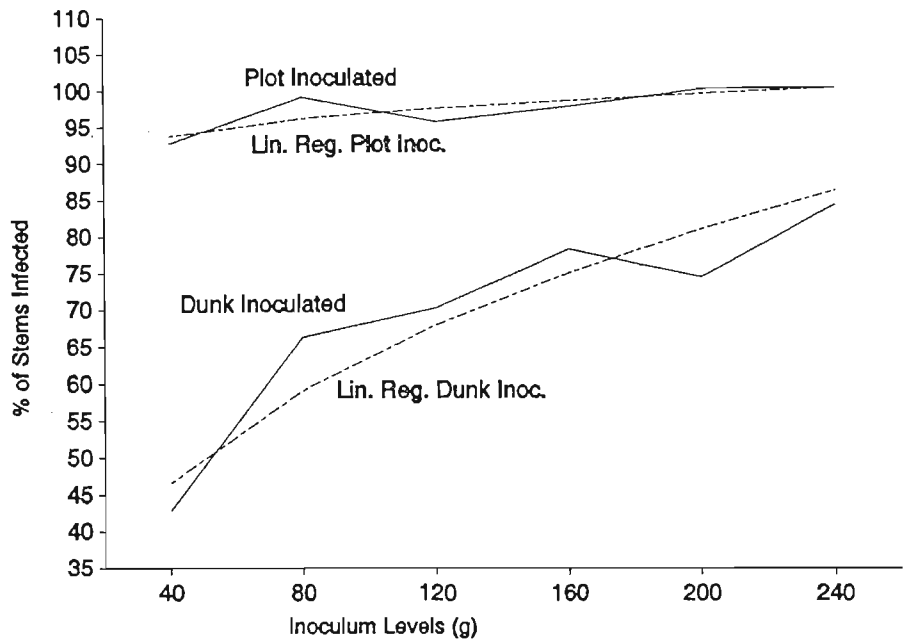

Fig. 7.4.3.B Inoculum Trial 3:
Blackleg Incidence as a Function of Inoculum Level

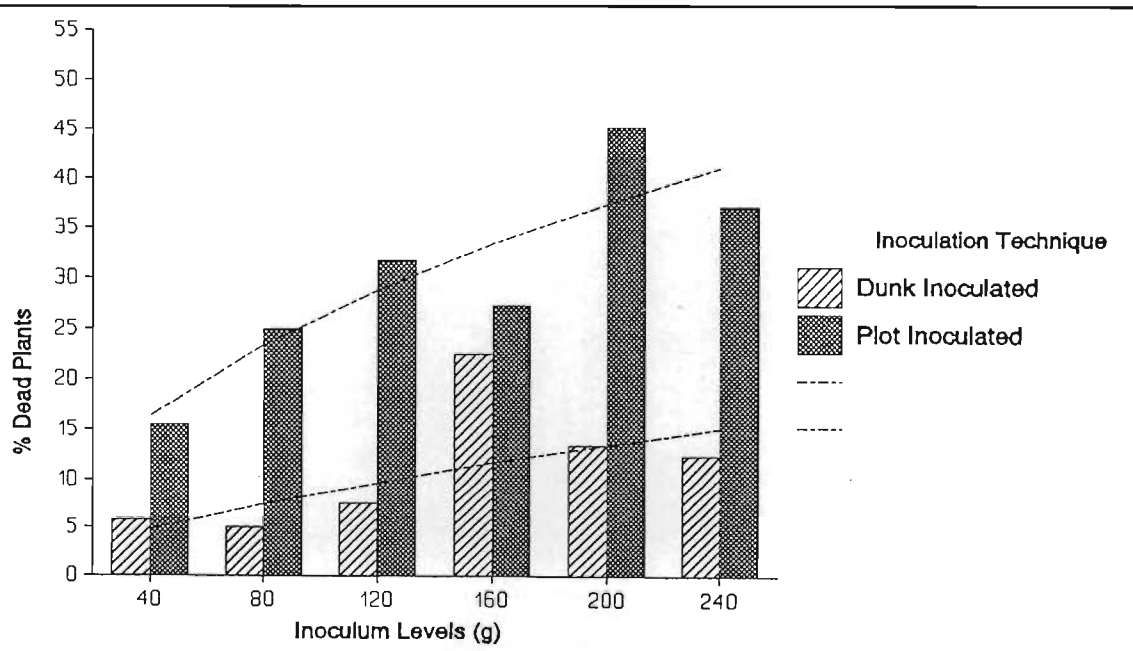

Fig 7.4.3.C Inoculum Trial 3:
% Dead Plants as a Function of Inoculum Levels

The regression of % Dead Plants resulting from increasing levels of inoculum also follows a power function for both "dunk" and plot inoculation.

Table 7.4.3.D: Inoculum Trial 3: Results of Linear Regression of % Dead Plants on Levels of Inoculum

	Dunk	Plot
Equation	$Y = 1.57\ X^{0.64}$	$2.80\ X^{0.51}$
r	0.74	0.92
r^2	0.66	0.85

Treatment Key

1 Dunked = plots inoculated with infected debris at the determined rate

2 Plot = seedlings inoculated by dipping them into an inoculum suspension of chopped, infected cabbage stems left for 10 min in 2 ℓ water.

3 Inoculum Quantity = 40 g, 80 g, 120 g, 160 g, 200 g, 240 g of chopped infected cabbage stems applied per plot or 2 ℓ of inoculation water.

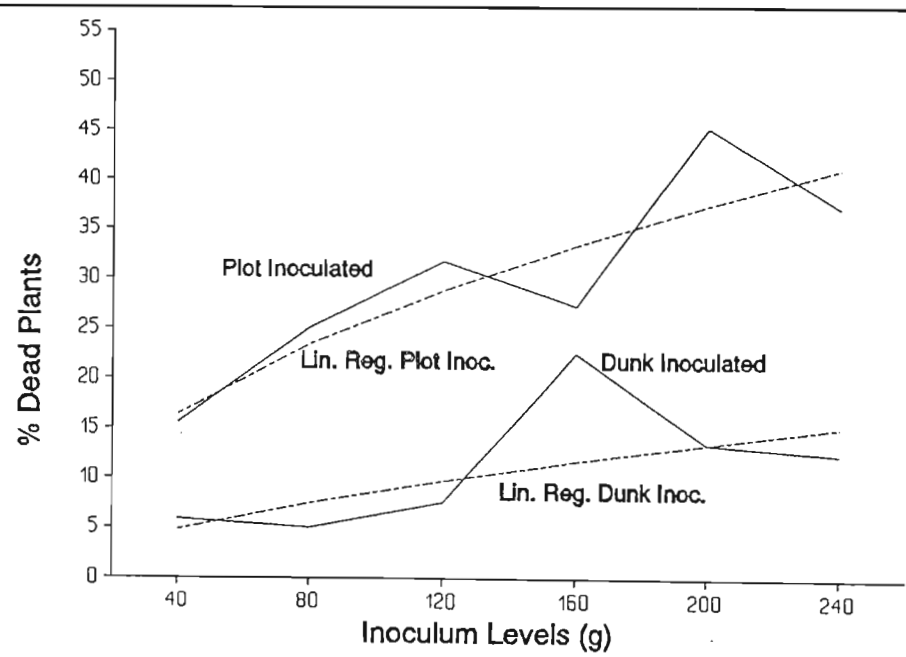

Fig 7.4.3.D Inoculum Trial 3: % Dead Plants as a Function of Inoculum Levels

7.4.4 Discussion

There was a significant interaction effect between the Inoculation Type and Inoculation Quantity. This was because the different levels of inoculum had different effects on the two forms of inoculation. In both cases, each 40 g increment of inoculum caused proportionately less disease than the previous 40 g. This can be seen in the power functions of both types of inoculation being less than 1 ($Y = aX^b$ where **b = 0.34 and 0.036**, respectively). However, the degree to which this occurred differed markedly between inoculation techniques. The regressions in Fig. 7.4.3.A display this clearly. The technique of "dunking" of seedlings had a low level of efficiency. Forty grams of inoculum resulted in a 40% infection level, 160 g of inoculum caused 78% infection, and 240 g resulted in the maximum infection level of 84%. In contrast, plot application of inoculum was extremely efficient. Forty grams of inoculum resulted in a 93% infection level, 160 g of inoculum caused 98% infection, and 240 g resulted in an infection level of 100%.

The % Stem Infection data revealed that plot inoculation was more efficient than "dunk" inoculation in causing blackleg infection ($F = 419.6$, $P < 0.001$). This confirms the result obtained in Inoculum Trial 2, namely that evenly distributed, infected crucifer stems left in seedbeds or production lands are a highly efficient form of blackleg inoculum. However, given the limited field spread of the disease, a critical proviso is that the inoculum must be evenly spread to ensure widespread infection. In contrast, "dunking" or its natural equivalent is less efficient in causing infection. However, by the very nature of the process, where a few infected seedlings infect hundreds of others during the transplanting process, it will result in widespread distribution of infected plants. It must also be remembered that in Inoculum Trial 2, SBT were much more susceptible to "dunk" inoculation than were CGS, and therefore proportionately higher levels of infection would have occurred if the seedlings used had been SBT. This trial must therefore be seen as giving a conservative estimate of what used to occur in the field, where "dunk" inoculation of SBT rather than of CGS occurred.

As would be expected, a dose response to inoculum levels was evident, disease incidence increasing with inoculum levels ($F = 8.5^{**}$), as illustrated in Fig. 7.4.3.A. Little inoculum was required to cause high levels of infection. Indeed, a shortcoming of this trial was that with plot inoculation, the lowest level of 40 g per 2.5 m^2 plot still resulted in a high level of disease (93%). This relationship could be examined at an even lower set of inoculum levels, say rates of 80, 40, 20, 10, 5 and 0 g per plot to complete the picture with respect to the plot inoculation technique.

The CV% for the % Dead Plants was high, limiting its value. If the trial had been run for longer, more plants would have died and the results may have been more consistent. Despite the inconsistency found, the trend was the same as for the % Infected Plants; namely, that "Dunk" inoculation was less effective than Plot inoculation ($F = 194.3^{***}$) and that % Dead Plants increased as inoculum levels increased ($F = 3.15^{*}$). Fig. 7.4.3.B shows trends that parallel those seen so clearly in Fig. 7.4.3.A, i.e., that increased inoculum resulted in increased disease, and that Plot inoculation was more efficient than the "Dunk" method.

Overall, it is clear that there was a direct relationship between levels of inoculum, and disease incidence and severity. In field trials, inoculation of field plots with infected debris was a more efficient technique than dipping seedlings into a pycnidiospore suspension prior to transplanting. This indicated the role debris-borne inoculum can play in initiating a blackleg epidemic, if evenly distributed. However, inoculation of seedlings at transplanting was also successful as a means of causing widespread infection of cabbage crops. This technique simulated the epidemic scenario of seedbed seedlings being lifted wet from a seedbed, after which a few infected seedlings could contaminate entire consignments of seedlings and supports the findings of the previous trial, in Section 7.3.

An intriguing aspect of epidemiological studies of crucifer blackleg is how little work has focused on inoculum, and the saprophytic stage of *L. maculans*' life cycle (Williams, 1992). A notable exception is the work of Bonman in the 1980s on cabbage blackleg at Univ. of Madison-Wisconsin (Bonman and Gabrielson, 1981; Bonman *et al.*, 1981). Most of the epidemiological work has been conducted by Australian, Canadian and French researchers working on blackleg in canola (e.g., Alabouvette 1970; Alabouvette and Brunin 1970; Alabouvette *et al.*, 1974; Barbetti, 1975a; Barbetti, 1975b; Barbetti, 1976; Barbetti, 1978; Bokor, 1972; Bokor *et al.*, 1975; Brunin, 1972; Brunin and Lacoste, 1970; McGee, 1974; McGee, 1977; McGee and Petrie, 1978; McGee and Petrie, 1979; Petrie, 1994; 1995a; 1995b; 1995c). However, because the crop cycles are so different, it is unlikely that the epidemiology of *L. maculans* on canola will be the same as on cruciferous vegetables.

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CHAPTER 8. DISEASE SPREAD IN THE FIELD

To investigate the non-uniformity of disease, it is necessary to describe the patterns of the host and the pathogen populations in space, how these patterns are related, and how they are affected by spacial variation in the environment.

Jeger, 1990

Abstract

Twenty nine blackleg epidemics were surveyed over 11 yr. Lands affected had been monocropped to crucifers for 3-12 yr in succession. Seedbed transplants (SBT) were used in 83% of cases. Two cases (7%) involved direct drilled seedlings (DDS), where excess seedlings were transplanted, making them the functional equivalent of SBT. Three cases (10%) involved container-grown seedlings (CGS) grown on monocropped lands. Disease occurred in two patterns: disease in crops grown from SBT and DDS occurred down the lines, because disease spread was onto boxes of wet transplants. In all CGS cases, disease occurrence was randomly patterned. Diseased debris was found in seedbeds and production fields and is presumed to have provided the dominant initial inoculum. Disease spread was limited to two plants on either side of an initially infected plant, 1.3 m or less in all cases, suggesting that field infection resulted from splash dispersal of pycnidiospores. The disease cycle was mono- or oligocyclic but not polycyclic.

8.1 Introduction

The author surveyed 29 blackleg epidemics in KwaZulu-Natal over a period of 11 years. The fields were visited at the request of the farmers concerned, and do not represent the only outbreaks of blackleg that occurred in KwaZulu-Natal over this period. However, the survey represents a substantial proportion of those blackleg outbreaks which occurred over period concerned.

8.2 Materials and Methods

The affected fields were closely examined. Details looked for were:

1. The identity of the crop: cabbage, cauliflower, etc. Cultivar information was not readily available from farmers.
2. The source of seedlings; i.e., whether CGS, SBT or DDP.
3. The presence of infected debris in seed beds and field sites.
4. The pattern of disease spread in the production lands; i.e., was the pattern down rows, in circles (foci) or at random ? Counts of infection were also made so that doublet or runs analysis could be undertaken, if needed (Madden *et al.*, 1982).

Farmers were asked for details of field treatments and prior crop history of the affected lands.

8.3 Results

The results of the survey are presented in Table 8.3.A.

Table 8.3.A: An 11 Year Survey of Blackleg Infected Fields in KwaZulu-Natal and the Free State Provinces

Farmer	Site	Crop	Planting	Disease Pattern	Source of Inoculum
McCormick	Tala Valley	cabbage	SBT	down the rows	seedbed debris
Fey[1]	Kokstad	cabbage	SBT	random	ascospores ? Unknown
Hillier	Tala Valley	cabbage	SBT	down the rows	seedbed debris
McGrath	Tala Valley	cauliflower	SBT	down the rows	seedbed debris
Griffin	Dargle	cabbage	SBT	down the rows	seedbed debris
RNA	Tala Valley	cabbage	SBT	down the rows	seedbed debris
RNA	Tala Valley	cabbage	SBT	down the rows	seedbed debris
Hillier	Tala Valley	cabbage	SBT	down the rows	seedbed debris
Andrews	Howick	cabbage	SBT	down the rows	seedbed debris
Mattison	Howick	cabbage	SBT	down the rows	seedbed debris
Mattison	Howick	cabbage	SBT	down the rows	seedbed debris
Mattison	Howick	cabbage	SBT	down the rows	seedbed debris
RNA Farms	Tala Valley	cabbage	SBT	down the rows	seedbed debris
Baynesfield	Thornville	cabbage	SBT	down the row	seedbed debris
Main	Crammond	cabbage	SBT	down the row	seedbed debris
Egeland[2]	Umzimkulu	cabbage	DDP	down the rows	field debris
Egeland[3]	Umzimkulu	cabbage	DDP	down the rows	field debris
Cole[4]	Highflats	cabbage	CGS	random; localised	field debris
Bouverie	Harding	cabbage	SBT	down the rows	seedbed debris
Mathee	Harding	cabbage	SBT	down the rows	seedbed debris
Baynesfield	Thornville	cabbage	SBT	down the row	seedbed debris
Baynesfield	Thornville	cabbage	SBT	down the row	seedbed debris
Hillier	Tala Valley	cabbage	SBT	down the row	seedbed debris
Bouverie	Harding	cabbage	SBT	down the rows	seedbed debris
Mathee	Harding	cabbage	SBT	down the rows	seedbed debris
Strauss	Estcourt	cabbage	SBT	down the rows	seedbed debris
Cole[5]	Highflats	cabbage	CGS	random	field debris
Jackson	Vryheid	cabbage	SBT	down the rows	seedbed debris
Harrison[6]	Bloemfontein, Free State	cabbage	CGS	random	field debris

1 C.Fey had planted this field with three consecutive crops of cabbage. The site is extremely cold and wet throughout the year being in the foothills of the Drakensberg in land leased in the Transkei district.

2 O.Egeland used a combination of direct seeding and seedbed transplanting: he planted the affected field using direct seeding, followed up by transplanting of extra seedlings into unplanted areas.

3 O.Egeland used a combination of direct seeding and seedbed transplanting: he planted the affected field using direct seeding, followed up by transplanting of extra seedlings into unplanted areas.

4 D.Cole had planted this land with six consecutive crops of cabbage. His farm at Highflats is characteristically cool and wet relative to the rest of KwaZulu-Natal.

5 D.Cole had planted this land with 12 consecutive crops of cabbage.

6 I.Harrison had planted this land with six consecutive crops of cabbage. This epidemic was different in that the farm is in another province, the Free State, and is situated about 50 km from Bloemfontein. It is a site of extreme hot and cold conditions and relatively little rain.

In all cases, the farmers had been farming the same lands with cruciferous vegetables for at least 3 yr. In one case, the farmer had planted 12 successive crops of cabbage on the same land.

In 28 of 29 cases, the crop involved was cabbage. The exception was a single cauliflower crop.

SBT were involved in 24 of the 29 cases (83%). Two cases involved DDP (7%). However, in both these cases, it was effectively a combination of DDP and SBT: Every second row was planted with excess seedlings transplanted from the direct-seeded rows, effectively a seedbed transplant. These two cases should therefore be considered as falling into the SBT category (83% increases to 90%). Three cases involved CGS (10%). Each of these involved repeated planting of the same land to crucifer crops with no rotation, which would have allowed for a build up of blackleg infested debris over the seasons.

The pattern of disease observed in the field were distinct: in all but one case the pattern for crops derived from SBT and DDP were down the lines (Fig. 8.3.A - B). In all three of the blackleg cases involving CGS, the pattern of field infection was a random distribution.

In most cases, it was possible to find the likely source of initial inoculum in the form of infected debris from a previous crucifer crop situated either in the seedbed area (79%) or in the production field (17%). In one case, no clear source of debris was discovered. Notably, disease spread after initial infection was limited in all cases. Spread was limited to one or two plants on either side of the initially infected plant, a distance of 1.3 m or less in all cases.

8.4 Discussion

It was clear that some KwaZulu-Natal cabbage farmers did not practice crop rotation, and that blackleg only occurred on farms where successive crops of crucifers had been planted into the same lands. The implication was that major outbreaks of the disease only occurred after the pathogen had built up inoculum levels in the form of increasing quantities of contaminated debris, particularly in the cases involving CGS. In the case of SBT, the routine accumulation of cabbage debris would have increased the likelihood that an ex-production field, contaminated with *L. maculans*-infected debris, would be chosen as a seedbed site. The result would be infected seedlings initiating a blackleg epidemic, with a **down-the-row** pattern.

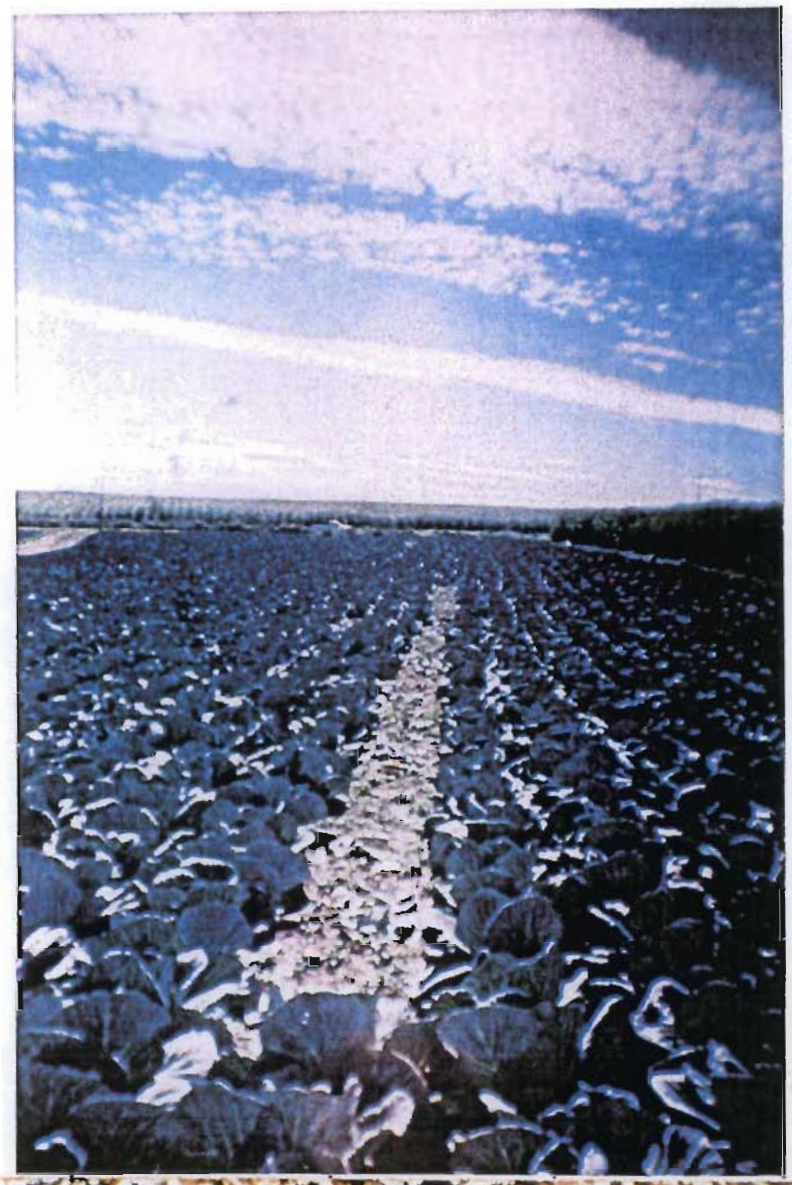

Fig. 8.3.A
Cabbage infection down the row, with infected plants removed or wilted. Seedbed transplant seedlings were used for production.

Fig. 8.3.B
Cabbage infection down the row, with infected plants wilted. Direct-drilled seedlings were used for production, the affected rows having been transplanted using excess seedlings from direct-drilled rows.

The majority of cases (83%) involved SBT. Both the DDP epidemics arose from the transplanting of excess seedlings produced by direct seeding; i.e., as with SBT, there was a transplanting step which served to efficiently disseminate *L. maculans*. Only three cases involved CGS, suggesting that blackleg was considerably more likely to occur where SBT rather than CGS were used.

The dominant pattern of disease infection for SBT and DDP crops was **along rows**, indicating seedling infection prior to transplanting. Observation of the SBT transplanting process, as discussed above in Section 1.2.4.2, suggested that this was a perfect situation for the slime spores of the pycnidial stage of *L. maculans* to be disseminated and for infection of the weakened cabbage transplants to occur, as discussed in Chapter 7.

The three cases involving CGS exhibited random disease patterns. This suggests that infections occurred after transplanting. Two scenarios can explain the pattern observed:

1. airborne ascospores arrived from a distant inoculum;
2. splash-dispersed pycnidiospore inoculum arose from debris evenly distributed throughout the field.

A scenario based on pycnidiospore infection is as follows: seed-borne inoculum initially played the role of initially infecting a crucifer crop, thereby establishing infected debris in one or more production fields. Given the lack of rotation in the affected lands, this inoculum built up slowly in each crop, and was spread over the field by land preparation practices. The level of blackleg incidence in initial crops was below the farmer's level of disease perception, which Vanderplank (1963) has estimated to fall between the 5-10% infection level. Finally, a land with a large quantity of *L. maculans*-infected debris on it was used for a seedbed. With appropriate environmental conditions, a serious epidemic of blackleg then developed.

For example, consider a case where a cabbage seedlot had 1 in 10 000 seeds infected with *L. maculans*. Planted into a seedbed, each infected seed initiated infection of 10 seedlings. These infected 1000 other seedlings plucked in the same batch of transplants. These seedlings each infected their two nearest neighbouring plants in the rows (a 0.5 m spread distance, well within the 1.5 m suggested for *Phoma exigua* Desm. pycnidiospores (Logan, 1976)). Thus, 3 000 plants would have been infected with blackleg.

Consider then that the next season a seedbed was placed over the infected debris, and 100 seedlings became infected with *L. maculans*. Each of these then infected 1 000 other seedlings at transplanting, a total of 100 000. If each one then infected only one neighbour in a parallel row (work on a 50% chance that the other neighbour is already infected, the equivalent of Vanderplank's correction factor, (1-X)) (Vanderplank, 1963), then a total of 200 000 plants would be infected, affecting an area of about 4 ha with a current value of about R126 000 [5].

A possible anomaly arises in this scenario because ascospores play little or no role. However, the patterns of disease spread in all the field surveys indicate that this is indeed the case. The results of various inoculum trials reported in Chapter 7 also indicated that ascospores play no detectable role in the KwaZulu-Natal blackleg pathosystem. A similar situation occurred with cauliflower production in Australia. Carter (1982) observed that the sexual stage (*L. maculans*), whilst widely distributed on residues of rapeseed crops, was seldom found on cauliflower or other *Brassica* vegetables in market garden areas remote from rapeseed fields, despite a high incidence of blackleg disease. He considered that stem lesions only developed on cauliflowers if early infection pycnidiospores occurred, usually via the cotyledons.

[5] 200 000 cabbages x 90% harvest @ R0.70 ea. = R126 000

In contrast, it is clear that ascospores are the dominant infection structure in the epidemiology of *L. maculans* on canola (e.g., Alabouvette, 1970; Barbetti, 1975a; 1975b; 1976; Gugel and Petrie, 1992; McGee and Petrie, 1979; Petrie, 1994).

In the case of SBT, it is understandable that pycnidiospores should play the dominant role: ascospores are not generated on live hosts, and pycnidiospores are effectively disseminated by the wet transplanting process. However, in the five cases arising directly from field infection as a result of infected debris left over from prior crops, ascospores should have been present, and could have played a role. However, the relative contributions of ascospores and pycnidiospores could not be determined in this survey.

A further anomaly arises: Gabrielson *et al.* (1978) considered blackleg to be polycyclic in the USA. Yet in KwaZulu-Natal, this disease is clearly mono- or at most, bi-cyclic, the disease not spreading more than 1.5 m from the initial infection, because spread of the pycnidiospores is so limited and ascospores appear to play no role. Hammond and Lewis (1986) and Hall (1992) also found *L. maculans* to be predominantly monocyclic on canola in the UK and Canada.

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CHAPTER 9. THE Rôle OF CRUCIFEROUS WEEDS IN THE EPIDEMIOLOGY OF *LEPTOSPHAERIA MACULANS* IN KwaZulu-NATAL

The history of weeds is the history of man.

Anderson, 1954

A weed is a plant in the wrong place at the wrong time. Any plant can be a weed: pretty ones, ugly ones, rare ones, even crop plants such as maize and wheat. A weed must be a nuisance, just sometimes.

Bromilow, 1995

Abstract

Over 6 yr, cabbage fields of 26 farms were each examined once for cruciferous weeds infected with *L. maculans*. All the fields surveyed were sites of blackleg epidemics. All stem or foliar lesions were collected and laboratory isolation of pathogens undertaken. None of the lesions discovered on cruciferous weeds were caused by *L. maculans*, suggesting that weeds play no role in the local crucifer blackleg pathosystem.

. . . . . .

9.1 Introduction

In Section 1.2, numerous crop and weed crucifers which can be infected by *L. maculans* are identified. Barbetti (1978) found no evidence of host specificity in isolates of *L. maculans* from *R. raphinistrum* (wild radish). The latter is a common weed in the Australian canola production area and is therefore a likely alternate host. Petrie (1969; 1975; 1979) and Petrie and Vanterpool (1965; 1974), reported numerous weed hosts of *L. maculans* in Canada as discussed previously in Section 1.2.7. The

epidemiologically important point of their findings is that the *Sisymbrium* strain isolated from *S. altissimum*, *S. loeslii* and *Descurainia* spp., and the *Brassica* strain found on *B. kaber* (wild mustard) and *B. hirta* (white mustard) are both pathogenic on canola. It is therefore probable that in Canada these weeds play some role in the *L. maculans* canola pathosystem. In that the *Thlaspi*, *Lepidium* and *Richardsonii* strains of *L. maculans* are weakly pathogenic on *Brassica* species, they probably play little direct role in the epidemiology of *L. maculans* on canola. However, it remains unclear from the work of Petrie (1969; 1975; 1979), and Petrie and Vanterpool's (1965; 1974) whether the various strains of *L. maculans* studied were sexually compatible; i.e., would pseudothecia be produced if the different mycelia were cultured together (Venn, 1979) and if so, what is the pathogenic nature of their ascosporial offspring ? It would be a powerful ESS for the various biotypes to retain compatibility and for their offspring to be pathogenic on either or both hosts (Taylor and Borgmann, 1994). In this one step, there would be a broad increase in the pathogen's genetic pool and, therefore, its diversity. This possibility was recently raised by Petrie (1995) as extremely likely.

As in Australia and Canada, wild radish and wild mustard are common weeds in South Africa (Bromilow, 1995), and furthermore, are selected for by the widely-used acetanilide group of herbicides, as discussed in Section 1.2.7.2. It was feasible, therefore, that some cruciferous weeds could act as secondary hosts to *L. maculans* biotypes pathogenic on cultivated crucifers, within the epidemiological framework discussed in Section 1.2.7.3.

A study was therefore initiated with the aim of establishing the role of local cruciferous weeds in the blackleg pathosystem in KwaZulu-Natal.

9.2 A Survey of Weeds on Cabbage Farms

9.2.1 Introduction

Over a 6 yr period, fields of 26 cabbage farmers in various regions of KwaZulu-Natal were surveyed for the presence of cruciferous weeds infected with *L. maculans*. All

All the fields surveyed were planted to cabbage and were sites of blackleg epidemics. There was a high level of inoculum present in the fields, and the potential for weeds to become infected with *L. maculans* was high.

The districts covered in the survey were Dargle, Greytown, Harding, Highflats, Howick, Ixopo, Kokstad, Lion's River, Richmond, Tala Valley, Thornville Junction, Tugela Ferry, Umzimkulu and Weenen (Fig. 9.2.A).

9.2.2 Materials and Methods

The target plants of the surveys were: wild radish (*Raphanus raphanistrum* L.), wild mustard (*Rapistrum rugosum* (L.) All., *Sisymbrium capense* Thunb., *S. thellungii* L.) and birdsweed (*Lepidium africanum* (Burm.f.) DC. and *L. bonariense* L.). Plants of these species were examined for the presence of any foliar or stem lesions similar to the lesions caused by *L. maculans*. Samples were taken of any plant displaying a stem, silique or foliar lesion. The lesion material was excised, surface sterilized for three minutes with a 1% NaOCl solution, washed in sterile distilled water, and transferred in the laboratory onto water agar and V8 agar. Fungal colonies were reisolated onto V8 agar and incubated under black light to stimulate sporulation. Once fruiting bodies formed, the colonies were examined under the dissecting microscope for the presence of pycnidia of *P. lingam*, which exude a characteristic ruby-red cirrus when wet, with characteristic pycnidiospores. *Alternaria* species were identified according to the key of Ellis (1971).

9.2.3 Results

Most of the foliar lesions investigated were the result of *Alternaria* infections. *A. raphani* Groves & Skolko, *A. brassicae* (Berk.) Sacc. and *A. brassicicola* (Schw.) Wilts. were all isolated. The pathogen most commonly isolated from *R. raphinistrum* and *S. thellungii* was *A. raphani*. From the stem lesions examined, phycomycetous fungi and *Rhizoctonia solani* Kuhn were also isolated. *L. maculans* was not isolated from any of the lesions examined.

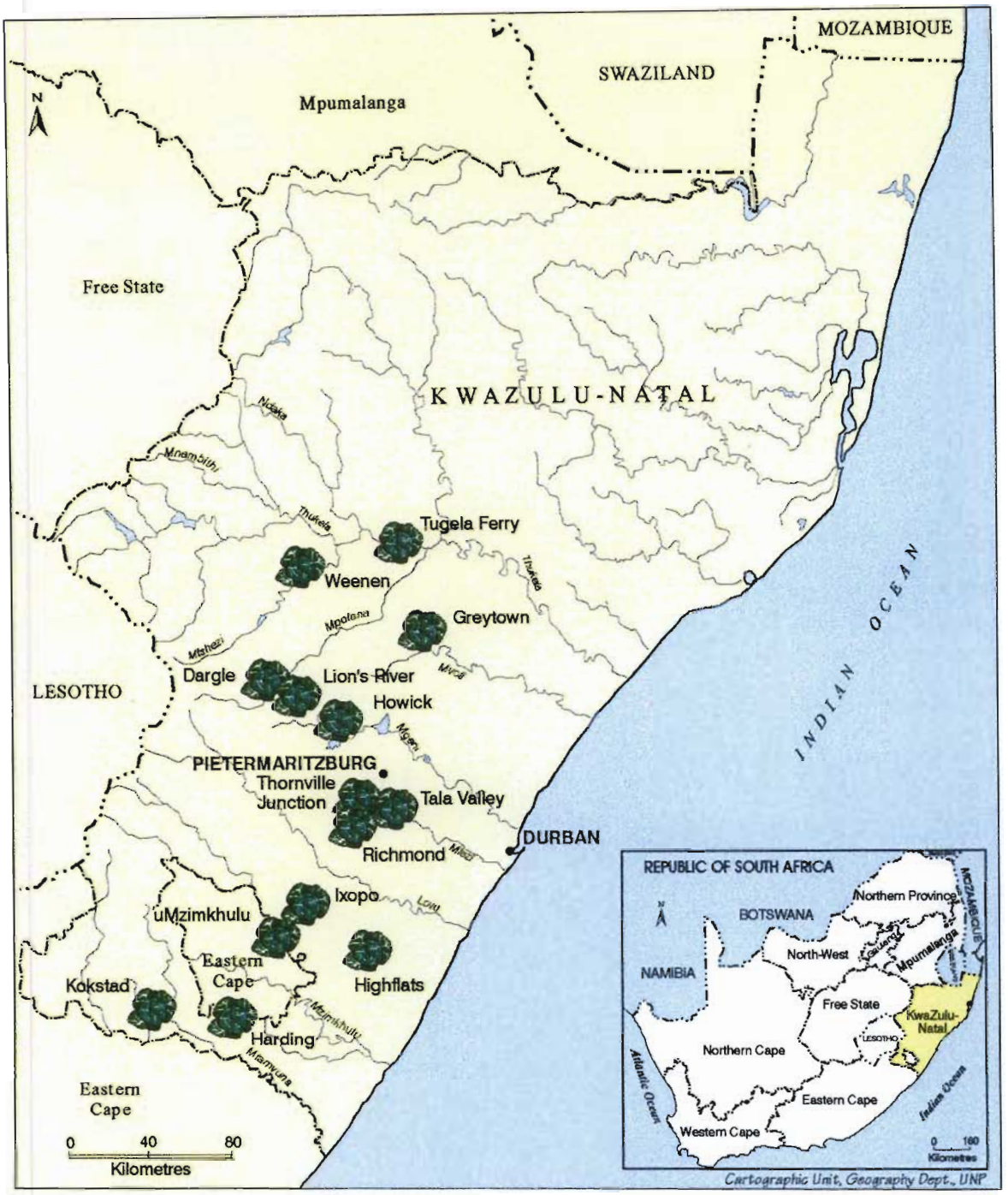

Fig. 9.2.A : A Map of Natal with the Weeds Survey Areas Marked

9.2.4 Discussion

No evidence was found to implicate local cruciferous weeds in the epidemiology of crucifer blackleg in KwaZulu-Natal. This result contrasts with the findings of Petrie (1979) in Canada and Barbetti (1978) in Australia, who found that weeds were regularly infected with *L. maculans*. One difference is that in KwaZulu-Natal, cabbage was the cruciferous crop concerned, whereas in Canada and Australia canola was the susceptible crop. One can speculate that there was greater cross-susceptibility between canola and cruciferous weeds such as *R. raphanistrum*.

Another explanation is that perhaps ascospores are needed to infect cruciferous weeds, and since these do not play a role in the KwaZulu-Natal pathosystem, despite being present, weed species are not infected. However, this theory does not explain the presence of uninfected cruciferous weeds in ex-cabbage fields which had experienced severe blackleg outbreaks, and in which there were high levels of pycnidial and pseudothecial inoculum.

A third possible explanation is that these survey results were false negative; i.e., that infection of cruciferous weeds does occur in KwaZulu-Natal, but that this survey failed to detect infected plants. However, this is unlikely because the survey was conducted over a 6 yr period at many farms, in several regions of KwaZulu-Natal, in all seasons, and under a wide range of environmental conditions.

Overall, the potential role of weed strains of *L. maculans* in the local blackleg pathosystem may be less significant than was initially theorized. Control options should therefore focus on other options, and resources should not be expended on control of cruciferous weeds.

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CHAPTER 10. THE INTERACTION OF THE HOST PHENOLOGY AND PHYSIOLOGY WITH THE PATHOGEN'S LIFE CYCLE

Hybrids resistant to the disease had a higher sugar content than susceptible hybrids when grown under recommended cultural practices.

Mortimore and Ward, 1964
quoted by Vanderplank, 1984

Abstract

A theory is proposed that phenological windows of disease susceptibility open and shut during the different stages of a plant's development, and that these phenological windows of susceptibility are specific and different for the various primary organs of plants. It is also postulated that blackleg is a "low sugar disease". A trial was conducted to test this theory, applying different fertilizer levels to cabbages grown semi-hydroponically. Disease incidence was significantly lower in well fertilized cabbage plants than in minimally fertilized plants. Given that a large head sink should provide for a high sugar content, but a high susceptibility to low sugar pathogens in the stem, roots and outer leaves, a further trial was conducted to test for a relationship between horticultural characteristics of several cabbage cultivars, particularly organoleptic tests of taste and texture, and blackleg susceptibility. As theorized, superior taste evaluations in cabbage were generally correlated with high susceptibility to blackleg.

10.1 Host Phenology

The cabbage plant effectively undergoes five primary stages of growth during its life cycle. Chronologically, the stages are seed germination, seedling growth, young plant growth, adult plant growth and seed formation. The first four stages are critical to the cabbage farmer, but when using CGS, only the middle three stages occur on-farm. The nutrient status of the crop during these different stages is different in the various plant organs; i.e., the roots, stem, leaves, and heads or flowers.

The theory of low sugar diseases was first mooted by Holbert *et al.* (1935) and De Turk *et al.* (1937) and promoted further by Horsefall & Dimond (1957) who classified a range of diseases into low and high sugar diseases. Their theory was that each plant pathogen may be classified according to the nutrient status of the plant (and the specific organ) it successfully attacks. The two extreme poles are low-sugar and high sugar pathogens, where the pathogens attack a host organ which has a distinctly low or high nutrient status, respectively. The topic was studied by Dodd (1980a; 1980b), who showed conclusively that the theory applied to low sugar stem and root rots of maize. Another paper in this sphere is that by Davet and Serieys (1987) who show that infection of sunflower by *Macrophomina phaseolina* (Tassi.) Goid. is inversely related to the levels of reducing sugars present in host plants. The subject has been reviewed by Vanderplank (1984).

I suggest that the phenotypic stages of cabbage growth are associated with fluctuating levels of photosynthates in the different organs during the different growth stages, and that as these fluctuate, so does the susceptibility or resistance of the particular organs. In other words, the theory is that there are phenological windows of disease susceptibility which open and shut during the different phenological stages of a crucifer's life, affecting the various plant organs differently. I also suggest that like black rot, blackleg is a "low sugar disease" (*sensu* Horsefall and Dimond, 1957). In contrast, I would describe white mould of crucifers (caused by *Sclerotinia sclerotiorum*) as a "high sugar disease". I would therefore predict that blackleg and black rot would cause the greatest levels of disease at the same periods of plant growth, although they

attack different organs (stems and outer leaves, respectively). In particular, I predict that these two pathogens would cause the most serious disease during two phenological stages: seedling growth, and heading or flowering. In the case of seedling growth, no reserves accumulate because all photosynthates are utilized immediately to supply the rapidly expanding foliage and root systems. At heading, the rapidly expanding head functions as a major sink, stripping photosynthates and nutrient reserves from the leaves and stem of each plant, leaving these organs with a low nutrient status. I further predict that white mould will be limited to attacking cabbage only after headfill, when there is an organ with a sufficiently high nutrient status, the filled head. The theory also extends further, because it can partially explain the differential susceptibility of different cultivars to blackleg: those cultivars which most efficiently strip the stem, roots and leaves of nutrients to supply the head sink should provide the sweetest heads, but should leave the stems and leaves highly susceptible to *L. maculans* and *X. campestris* pv. *campestris* and the head most susceptible to *S. sclerotiorum*.

This theory is presented in Table 10.1.A.

Table 10.1.A: A Model of Phenotypically Conditioned Susceptibility of Crucifers to *L. maculans*, *X. campestris* and *S. sclerotiorum*

Phenotypic Stage	Organ	Physiological Status	*L. maculans* Susceptible	*X.campestris* Susceptible	*S.sclerotiorum* Susceptible
Seedling	Stem	Low nutrient: rapid growth	High	N/A	Very Low
	Foliage	Low nutrient: rapid growth	High	High	Very Low
Early Growth: Pre-heading	Stem	High nutrient: sink	Low	N/A	Very Low
	Foliage	Moderate nutrient: partial source	Low	Low	Very Low
Mature Growth: Heading and Flowering	stem	low nutrient: source	high	n/a	high
	foliage	low nutrient: source	high	high: outer leaves	bottom leaves
	Head	High nutrient: primary sink	Very Low	Very Low	Very High

10.2 Host Physiology

The above theory is supported by the strong evidence that whenever the host's physiological status is sub-optimal, its susceptibility to blackleg is increased. In particular, wounding increases the rates of successful infection of host plants by pycnidiospores of *L. maculans* (Alabouvette *et al.*, 1974) and ascospores (Brunin and Lacoste, 1970). Other common causes of stress to crucifer crops in the field are herbicide damage (Petrie, 1973; Gladders and Musa, 1982; Sudarmadi and Wallace, 1984), frost (Van der Spek, 1981), pigeon damage (Rawlinson and Muthyalu, 1979; Gladders and Musa, 1980), insect damage (Van Keulen, 1926; Cottier, 1930; 1932; Buryhuna, 1950; Van der Spek, 1981; Newman and Plumridge, 1983; Newman, 1984; Schulz and Daebeler, 1984; Broschewitz *et al.*, 1993), heat stress (Petrie, 1986; Brun and Jacques, 1991) or crucifer light leaf spot (caused by

Pyrenopeziza brassicae Sutton et Rawlinson) (Daebeler *et al.*, 1992). In all these cases, an increased level of blackleg infection occurred as a result of the various stress factors.

Research conducted by Smith (1986) showed that black rot susceptibility in cabbages (caused by *Xanthomonas campestris* pv. *campestris*) was inversely related to nitrogenous top-dressing (Fig. 10.2.A), which supports the theory of Low Sugar/High Sugar Crop Susceptibility as it applies to black rot, on the basis that the nutrient status of cabbages is related to fertilization levels, within an asymptote of fertilizer uptake.

Extrapolation from Smith's (1986) observations may have an important bearing on cabbage management, especially fertilization and irrigation practices, and possibly in the selection of cabbage cultivars. The use of fertilization to manage "low-sugar" diseases has a wider applicability to other crops and vegetables, and even to the understanding of the epidemiology of fynbos root diseases (Bond, pers. comm.). Lowe and Laing (1996) have also showed that bacterial speck of tomato (*Pseudomonas syringae* pv. *tomato* (Okabe) Young *et al.*) is also a low sugar pathogen.

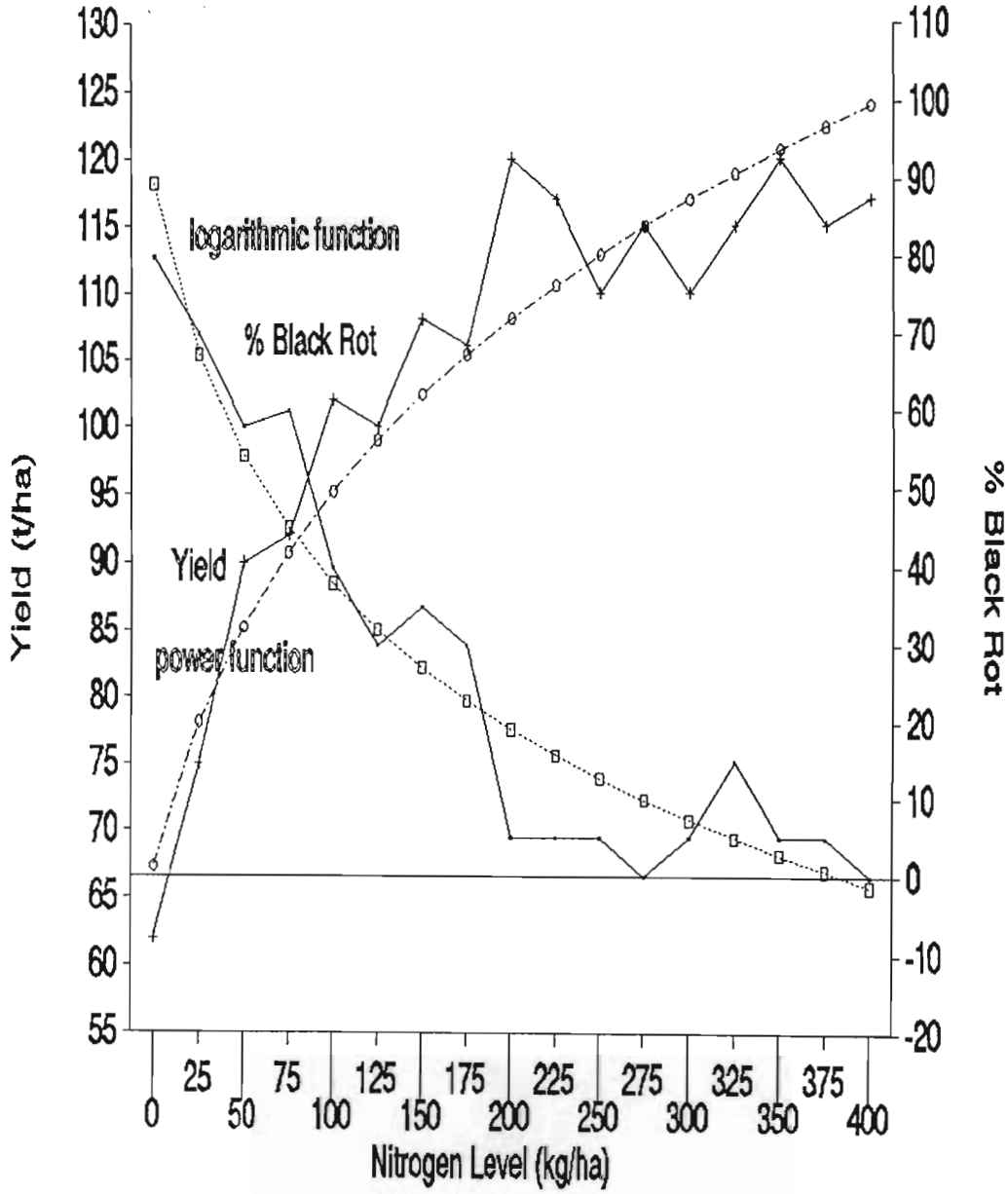

Fig. 10.2.A Cabbage Yield and Black Rot Severity as a Function of Nitrogen Application Rates

10.3 The Effect of Host Physiology on Incidence of Blackleg

10.3.1 Introduction

A trial was designed to test the theory that *L. maculans* is a "Low Sugar" pathogen when attacking cabbage, and that the nutritional status of cabbages will affect their susceptibility to *L. maculans*.

10.3.2 Material and Methods

Cabbage container grown seedlings (CGS) were produced at a commercial nursery (Sunshine Seedlings) and transplanted into composted pine bark (Gromed Organics, Crammond) in large plastic bags (300 mm diameter), with one plant per bag. A single cabbage cultivar, Hercules, was used. Inoculum was applied to the base of each seedling in the form of chopped, infected cabbage stems, at a rate of approximately 2.5 g per bag (based on the rate of inoculum found to be most effective in Section 7.4). Each bag was individually drip-irrigated three times daily with different levels of a balanced fertilizer (Ocean Agriculture 3.1.3 (38), plus Microplex (micronutrient mixture), hydroponic fertilizers). The design used was a randomized complete blocks layout, with 5 replicates, and 10 bags per replicate. Five different levels of fertilizer were used, to provide the following levels of N and K with each irrigation (P at ⅓ of this level):

300, 200, 100, 50, 25 ppm (mg ℓ^{-1}, w/v).

Trial evaluation was for the presence of blackleg lesions on the stem of each cabbage at plant maturity (i.e., % incidence of stem infection). Analysis was by ANOVA, using Fisher's LSD test for means separation. The statistics package used was Statsgraphics 5.0.

10.3.3 Results

The results are tabulated in Table 10.3.A and displayed in Fig. 10.3.A. The F-Test was highly significantly, and all but the lowest two fertilization treatments were significantly different. A linear regression of blackleg incidence on fertilization levels was also highly significant.

Table 10.3.A: Incidence of Cabbage Stem Infection by *Leptosphaeria maculans* as a Function of Fertilization

Treatment: Nitrogen Level	Blackleg Incidence *
25	83.1 a
50	74.4 a
100	52.0 b
200	14.6 c
300	5.2 d

* Figures with different alphabetic characters differ at the level of P = 0.05, using Fisher's LSD Test.

ANOVA Details:

F Test = 34.6 **

LSD Means Separation = 9.2 CV% = 12.7%

Linear Regression: $r = 0.98$ $r^2 = 0.95$

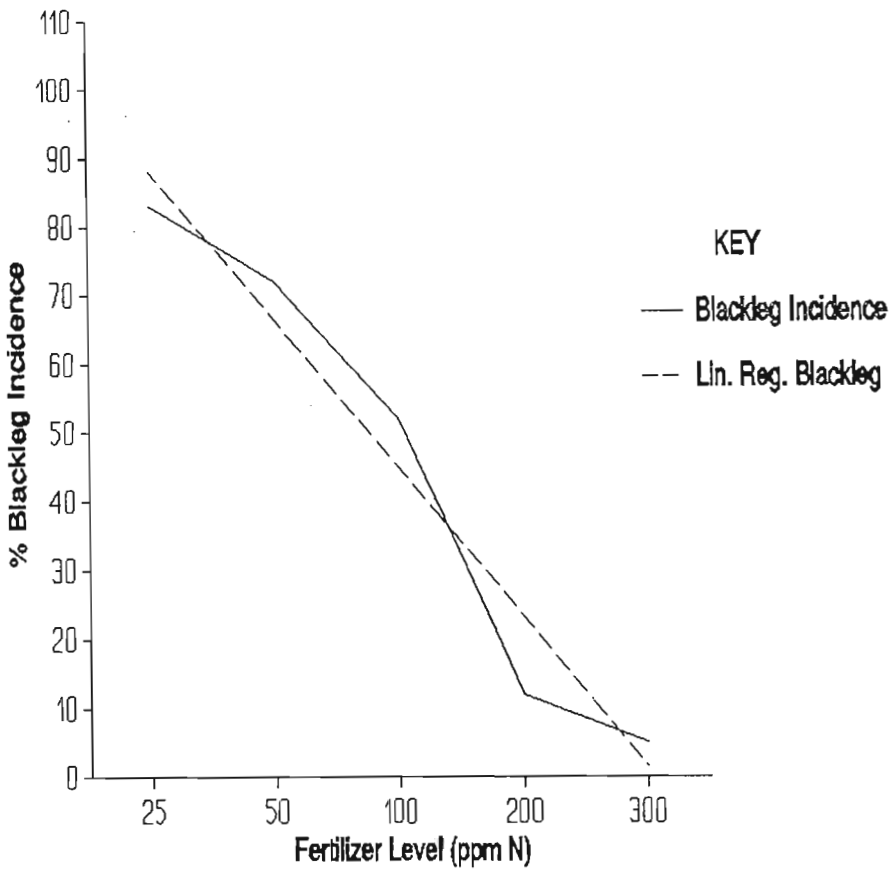

Fig. 10.3.A Cabbage Blackleg Incidence as a Function of Fertilizer Level

The fertilizer used was 3.1.3 (38). Levels of N = levels of K = 3 x levels of P, in ppm.

10.3.4 Discussion

The results suggest that the theory that crucifer blackleg is a Low Sugar Disease is correct. In this, they reflect the results of Smith (1986) with respect to crucifer black rot.

Constraints on interpretation of the result lie in the artificial system used to provide a *ceteris paribus* experimental system, the limited numbers of plants per plot, and the fact that even at the highest level of fertilization, blackleg still occurred, albeit at a low level. Further, this trial was based on the assumption that fertilization levels directly affect nutrient levels in the cabbages. This trial was also conducted once only.

This trial protocol could be used to test the theory on other diseases such as downy mildew, black rot and Sclerotinia white mould; to test the effect of stress on disease susceptibility; and to test the theory of phenological windows of susceptibility. It has been used successfully to test the effect of fertilization on bacterial speck severity on tomato seedlings (Lowe and Laing, 1996) and crucifer chocolate spot, a recently discovered disease of crucifers (considered to be a new pathovar *Xanthomonas campestris*) (Laing, unpublished). An added level of sophistication would be to monitor levels of sugars and free amino acid in the cabbages as a function of time or fertilizer applications or stress levels.

Two practical propositions can be extrapolated from this result. Firstly, injury and other stress factors should be avoided in order to minimize blackleg incidence. This reflects the results of other studies on the effect of stress on blackleg incidence, including herbicide or insect damage, mentioned above. Secondly, high levels of fertilization may help to suppress, but not control, the disease at sites with high inoculum or with cultivars of known high susceptibility.

10.4 The Relationship Between Cabbage Taste and Texture, and Blackleg Susceptibility

10.4.1 Introduction

A trial was conducted to test for relationships between horticultural characteristics and blackleg susceptibility. In particular, the taste and texture characteristics of a range of cultivars were correlated with blackleg susceptibility. The concept tested was that a cabbage with a very strong nutrient sink in the head should develop a very sweet, turgid head but a highly susceptible stem. In contrast, a cabbage with a weak nutrient sink in the head should have a poor taste and texture, but a reduced stem susceptibility.

10.4.2 Materials and Methods

The cabbages used in this trial came from another trial, Cultivar Trial 2 (Section 5.2).

From the blackleg resistance trial conducted there, 5 cabbages per plot were harvested and evaluated in a blind test by staff and students of the Dept of Dietetics and Home Economics, Univ. of Natal. Each score was the mean of six values, given by the evaluators, with three replications. The following 5-point scale was used:

1. very poor
3. poor
5. adequate
7. good
9. excellent

The cabbage cultivars were each evaluated using the above five point scale for the following parameters:

1. Crispness
2. Head Tightness

3. Texture of Cabbage in the Form of:
    a. Coleslaw
    b. Steamed
    c. Boiled
4. Taste of Cabbage in the Form of:
    a. Coleslaw
    b. Steamed
    c. Boiled
5. Blackleg: % Stem Infection

The mean of all scores for horticultural characteristics were calculated in a Horticultural mean (Hort Mean).

For each cultivar linear regressions were run, regressing the means of each horticultural character against blackleg incidence of each cultivar.

10.4.3 Results

Table 10.4.A presents a summary of the evaluation of 10 cabbage cultivars. Fig. 10.4.B-C present linear regressions of blackleg susceptibility regressed on horticultural evaluations.

Table 10.4.A: Evaluation of Ten Cabbage Cultivars for Blackleg Incidence and Horticultural Characteristics

	C	U	L	T	I	V	A	R		
Parameters	1	2	3	4	5	6	7	8	9	10
% Blackleg Incidence	95.3	80.0	79.8	91.1	84.9	50.6	72.1	89.0	77.7	53.0
Blackleg Susceptibility Rank	1	5	6	2	4	10	8	3	7	9
Hort. Rank Mean	4.2	4.5	4.8	5.0	6.8	6.9	7.0	8.4	8.6	13.7
Overall Rank	1	2	3	4	5	6	7	8	9	10
Taste Mean: 1-9	6.1	5.4	5.4	6.0	5.1	5.5	5.0	5.0	5.3	2.2
Taste Mean: Rank	1	4	5	2	7	3	8	9	6	10
Texture Mean: 1-9	5.7	6.1	5.8	6.0	5.7	5.1	5.8	5.2	4.9	2.9
Texture Mean: Rank	6	1	4	2	5	8	3	7	9	10
Taste Slaw Score: 1-9	6.8	6.3	5.4	7.2	5.2	6.4	4.8	5.2	7.4	2.4
Taste Steam: 1-9	6.3	5.4	5.4	5.3	4.0	6.8	6.7	5.4	5.2	2.7
Taste Boiled: 1-9	5.8	4.7	5.3	5.5	5.2	3.2	3.5	4.3	3.3	1.6
Texture Slaw: 1-9	5.0	6.6	5.7	5.6	6.0	6.6	5.8	4.8	4.8	4
Texture Steam: 1-9	6.3	6.0	5.8	5.8	4.8	6.0	6.2	3.0	4.7	2.8
Texture Boil: 1-9	5	5.6	5.9	6.5	6.2	2.7	5.4	5.6	4.9	1.8
Crispness: 1-9	9.0	7.0	7.0	7.0	7.5	5.0	7.0	8.3	3.0	3.0
Head Tight: 1-9	5	9	7	1	7	5	3	4	1	3

Key to Cultivars

1. Dynasty
2. Bonanza
3. Green Coronet
4. Grand Slam
5. Gloria
6. Rotan
7. Green Star
8. Hercules
9. Sunlander
10. Glory of Enkhuisen

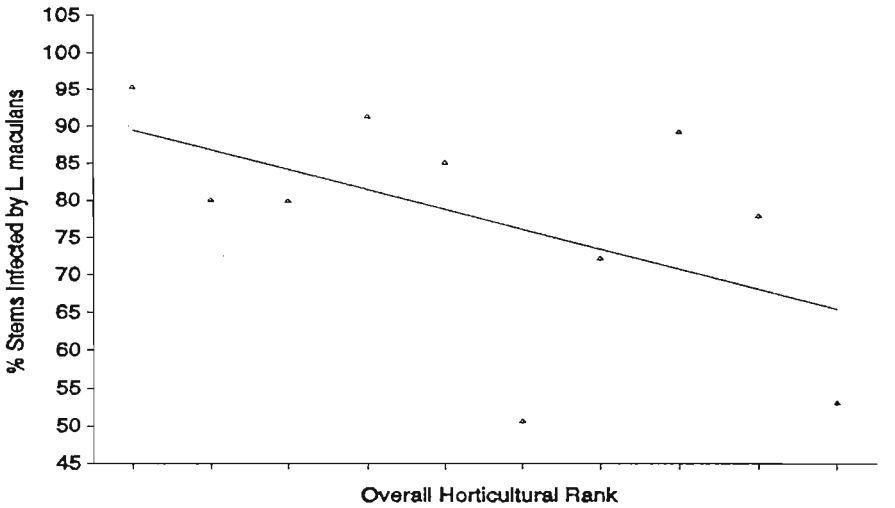

Fig. 10.3.B: Regression of Blackleg Incidence On Overall Horticultural Rank

$r = -0.54$ $r^2 = 0.29$

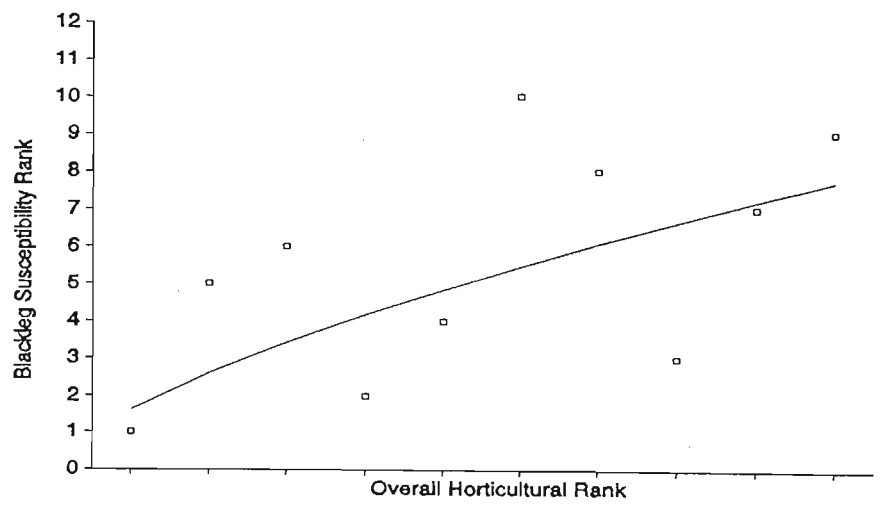

Fig 10.3.C Regression of Blackleg Rank On Overall Horticultural Ranking

$r = 0.67$ $r^2 = 0.45$

10.4.4 Discussion

A correlation appeared to exist between poor cabbage texture and taste, and good disease resistance, and good texture and taste with high susceptibility to blackleg. Cabbage breeders are constrained by two conflicting goals: breeding for good taste or for good blackleg resistance. It may be difficult to combine both characteristics in one cultivar. In particular, the cultivar Dynasty displays a negative correlation of the best overall score for taste, but the highest susceptibility to blackleg (ranked 1st for susceptibility and for taste). In contrast, Glory of Enkhuisen combines poor taste with good resistance to blackleg (ranked 9th for susceptibility and 10th for taste). Hercules is perhaps an exception, combining high susceptibility (ranked 3rd) with poor taste (ranked 8th). Rotan offers the converse picture of low susceptibility (ranked 10th) and moderate taste (ranked 6th). Overall the rank correlations were high at 45%.

This result generally fits the theory advanced in Sections 10.1 and 10.2, with regards cabbage disease resistance relating to the scale of the sugar sink of each cabbage head.

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CHAPTER 11. INTEGRATED DISEASE MANAGEMENT

Experts at curing diseases are inferior to specialists who warn against diseases.

an eleventh-century Chinese physician
quoted by Root, 1980

The groundwork for decision is a symphony of parts and processes brought together by people who share a variety of roles and responsibilities. The recommendation system for plant disease control is structured from scientific concepts and knowledge of crop production to form a disease control strategy. It represents a partnership between the scientific community and producers.

Horne, 1989

Abstract

An integrated management strategy is proposed, based on seed treatment with fungicides, the use of container-grown seedlings rather than seedbed transplants, 3 yr rotation of crucifer lands, implementation of either deep-ploughing or accelerated biodegradation to eliminate debris, the development of horizontal resistance to *L. maculans* in cruciferous vegetables, application of field fungicides in high risk areas (benzimidazoles or triazoles, or combinations), and the minimization of stress and optimization of host nutrition.

11.1 Introduction

The objective of this study was to develop a set of management practices which KwaZulu-Natal farmers could practically and economically implement to control crucifer blackleg. The trials described above were conceived to do just that. However, the final task was to integrate the various options into a single management package and communicate this to the farming community in KwaZulu-Natal, and the scientific community.

The literature reports various programmes designed to provide integrated control of blackleg in cabbage and canola. Table 11.1.A summarizes the measures proposed in some of these programmes.

Table 11.1.A: Integrated Management of Crucifer Blackleg [6]

	T R E A T M E N T S									
Author	Seed	Debris, Rotate Tillage	Sep. Field	Alt. Hosts	Resis. Cvs	Plant Space	Field Fcs	Fert.	Avoid stress	Bio-ctrl
1					X		X			
2	X	D,R,T								
3		D,R	X	X			X	X	X	X
4	X	D			X	X				
5		D,T					X			
6	X	D,R		X	X					
7		D								
8	X	D,R,T		X					X	
9										X
10	X		X							
11	X		X	X						
12		D,R	X							
13	X ?	R,T				X	X ?		X	
14					X		X			
15	X								X	X
16	X		X							
17		D,R,T		X	X				X	
	Seed	Debris Rotate Tillage	Sep. Field	Alt. Hosts	Resis. Cvs	Plant Space	Field Fcs	Fert.	Avoid Stress	Bio ctrl
	T R E A T M E N T S									

Key to Recommended Control Measures

Seed:	Use either certified clean seed or treat seed with an effective measure.
Debris:	Eliminate debris or *L. maculans* on debris
Rotate:	Follow a long rotation to ensure debris has disappeared
Tillage:	Use deep-ploughing to bury debris
Separate Field:	Ensure that current lands are far from previous lands, and inoculum on debris
Alternate Hosts:	Eliminate crucifer weeds and volunteer crops
Res. Cvs:	Use resistant cultivars
Plant Space:	Use an appropriate plant spacing to reduce disease
Field Fcs:	Use field fungicides to control *L. maculans*
Fert.:	Use of fertilization to reduce effects of blackleg
No Stress:	Eliminate insect damage, herbicide drift
Biocontrol:	Apply a biocontrol agent to control *L. maculans*

[6] *Continued on next page*

Table 11.1.A (continued): Numbered Authors

1. Barbetti (1975)
2. Cook and Evans (1979)
3. Daebeler *et al.* (1981)
4. Grayston and Germida (1991b; 1991b)
5. Greathead (1984)
6. Gugel and Petrie (1992)
7. Humpherson-Jones and Ainsworth (1982); Humpherson-Jones and Burchill (1982); Petrie, 1995
8. Ndimande (1976)
9. Novotna (1990)
10. Pauvert (1971)
11. Petrie (1979)
12. Rempel and Hall (1993)
13. Seidel *et al.* (1984)
14. Steinbach *et al.* (1991)
15. Sudarmadi and Wallace (1984)
16. Williams (1974)
17. Winter *et al.* (1993a; 1993b)

. . . . . .

11.1.1 The Rôle of Epidemiology in Formulating a Management Strategy

Epidemiology allows, indeed demands, a holistic view of a pathosystem at all systems levels (Robinson, 1979). It therefore provides the structural tools with which to construct a overall management strategy. As a subset within epidemiology, ethographs provide a visual picture of the conceptual models, and are, therefore, a powerful tool for simplifying the issues. The ethograph presented in Fig. 3.1.A portrays the key steps of the blackleg pathosystem in KwaZulu-Natal, leading to the synthesis of an overall management strategy.

11.1.2 The Role of Economics in Modifying a Disease Management Strategy

The key to any management strategy is that it has to be cost-effective. Farmers are both risk and cost-averse, and will not implement practices which are difficult to perform or expensive to implement, unless they provide rapid, clearly visible, and substantial savings. Thus, steps such as fungicide applications will not be accepted if they are only occasionally needed. The Decision Theory model shows that

application of disease control measures such as fungicide applications are an integrated decision process by the farmer, balancing perceived risks and predicted benefits against known costs of application of the control measure (Carlson, 1969; Horne, 1989). Each step in a management plan therefore has to be evaluated in terms of its potential impact, cost and likelihood of adoption.

11.2 A Management Strategy for the Control of Crucifer Blackleg in KwaZulu-Natal, South Africa

11.2.1 Seed-Borne Inoculum

The first infection step identified in the blackleg ethograph is seed-borne inoculum (Fig. 3.1.A). Virulent strains of *L. maculans* probably enter South Africa regularly but at fairly low levels as seed-borne inoculum in crucifer seed produced overseas (mostly by Japanese seed companies). This inoculum probably does not generate epidemics directly. However, it does play two critical epidemiological roles:

1. It introduces *L. maculans* into virgin lands;
2. It introduces new strains of *L. maculans* into South Africa.

The control of seed-borne inoculum would therefore be extremely valuable for crucifer production in South Africa in the long term.

The obvious option would be to introduce routine testing of all crucifer seedlots for the presence of virulent strains of *L. maculans*, as is done in the USA (Gabrielson, 1974; Williams, 1974), and thereby to enforce the international phytosanitary regulations of zero blackleg-infected seeds in 30 000 seeds. However, the costs of establishing and running a sophisticated seed pathology laboratory would be substantial, and almost certainly greater than South Africa's current economic position would allow (Holtzhausen, pers. comm.).

An alternative would be to assume that all seedlots of all crucifers are contaminated with *L. maculans* and to treat them with an effective systemic fungicide. Such a practice would have to be implemented by either seedsmen or CGS nurserymen because cabbage farmers today do not deal with seeds directly.

The current fungicide of choice is iprodione as it has been shown to be effective and was subsequently registered elsewhere at 5 g a.i. kg^{-1} seed (Humpherson-Jones et *al.*, 1980). Iprodione is available locally under the trade name of Rovral Flo (250 g a.i. ℓ^{-1}), and sells for R65.91 ℓ^{-1} (ICI-Farmers Organization). Thus, the cost of treating 1 kg of seed is:

(R65.91 x 4 (a.i.) x 5 ml) ÷ 1000 ml = R1.31

This equals R4.37 per million seeds (approximately 300 cabbage seed weigh 1 g) or about 0.4 **c** per thousand seedlings.

Application of iprodione to all crucifer seed would also eliminate other seed-borne fungal pathogens. *Alternaria* spp. are commonly carried on crucifer seed, and sclerotia of *S. sclerotiorum* are occasionally distributed in seed. Seed-borne dissemination of these pathogens would also be prevented by routine treatment of crucifer seed with iprodione. However, it is unlikely that the nurserymen could be persuaded to adopt this approach for two reasons:

1. The treatment provides the nurseries with little or no direct and visible advantage; it would benefit their clients, the cabbage farmers, in the long run. Furthermore, the link between seed-borne infection and blackleg epidemics is long and tenuous, and it is unlikely that farmers would recognize it unless they experienced regular, serious blackleg epidemics.
2. None of the nurseries have the equipment with which to treat seed efficiently with accurate doses of fungicides. The few larger CGS nurseries which have tried to treat crucifer seed regularly with a recommended fungicide have run into problems with inaccurate fungicide application rates and times, resulting in loss of entire seed lots (Laing, unpublished).

Of all the links in the crucifer production chain, it is the seedsmen who should understand the significance of seed-borne pathogens best. They are also the community best equipped technically to apply a systemic fungicide to the seed before it is planted, and could easily substitute or add an effective systemic to the standard treatment of seed with captan or thiram. If the seedsmen conducted the treatment, and passed the cost onto the customers, the addition of R0.65 to the cost of each 500 g tin of cabbage seed would be acceptable, given that it would only add about 0.07% to the total cost (based on a price of R965 for a 500 g tin of the F1 hybrid cabbage, Green Coronet, at McDonald's Seeds, 10/3/96).

Another problem would be to persuade Maybaker to register their fungicide, iprodione, for this use. Specifically, the problem is that the return on registration of iprodione for vegetable crucifer seed would be so small, perhaps R3 200 p.a. for the entire country. The development of canola as an alternative field crop for wheat farmers of the Cape Province may alter the situation, however, because this crop is direct seeded over large areas, with much larger quantities of seed being planted. If a joint registration for seed treatment of all crucifer seed is allowed, then this might make the registration of iprodione more economically interesting to Maybaker.

11.2.2 Seedling Infection

As suggested in the ethograph (Fig. 3.1.A), and confirmed by the various trials undertaken in this study, seedbeds form the single most important link in the epidemiology of cabbage blackleg in the KwaZulu-Natal pathosystem:

1. The presence of infected crucifer debris in seedbeds allow primary inoculum in to infect seedbed transplants (SBT) (Ethograph Step 2). Given the efficiency of this form of inoculum (see Section 7.3 and 7.4), seedbeds planted over infected debris will almost certainly produce infected seedlings.
2. When SBT are pulled from the seedbed, packed and carried to the production land, many uninfected seedlings become infected (Ethograph Step 3).

3. SBT are highly susceptible to infection during and after transplanting, probably because of the stress they suffer during the transplanting process (see Section 7.3). In contrast, the use of container-grown seedlings (CGS) eliminates all these problems, and provides a much hardier seedling, and better yield prospects. It is therefore essential that all crucifer farmers adopt the CGS system if crucifer blackleg is to be controlled.

If farmers cannot afford CGS (and there are many subsistence farmers who cannot), then they must produce SBT with extreme circumspection, with a focus on rotation and sanitation of the sites chosen for seedbeds:

1. Seedbed sites should not have been used for crucifer production for at least 3 yr.
2. They should be carefully picked clean of any crucifer debris of any kind on the soil surface.
3. All cruciferous weeds, and volunteer vegetable or fodder plants should be eliminated from the seedbed sites and from the immediate surrounds.
4. Before transplanting, application of benomyl fungicide sprays to the seedlings would be desirable on any farm where blackleg has occurred previously.
5. When transplanting SBT, the containers used to carry them from the seedbed to production lands should be designed to carry fewer seedlings than is the current practice.
6. The containers should be washed down, then sterilized with a fresh 1% NaOCl solution (or equivalent sterilant) before re-use, to ensure that no viable pycnidiospores are left on the containers.
7. Horticultural conditions should be optimized to avoid any stress factor, including herbicide, insect and bird damage.

11.2.3 Field Infection

As seen in Sections 7.3 and 7.4 and Chapter 8, blackleg epidemics can occur even on farms where CGS are used, if sanitation is not practised, and a build-up of infected debris occurs (Ethograph Steps 4 and 5). The first and most important form of sanitation is therefore crop rotation. The process allows for debris from previous crops (Ethograph Steps 6 and 7) to be degraded by saprophytic organisms as discussed in Section 7.2. A period of greater than 3 yr is recommended for KwaZulu-Natal.

Based on the results from Section 7.2, it is suggested that commercial cabbage farmers should practice field sanitation, followed by rotation periods of at least 3 yr. Alternatively, very intensive cabbage production with no rotation would be possible if all crucifer stems and leaves from production lands were efficiently collected and eliminated by composting. This practice would also reduce inoculum of other debris-borne crucifer diseases such as black rot and *Sclerotinia* white mould. It would not help to control diseases such as clubroot and wirestem, which are truly soil-borne diseases. However, active sanitation requires considerable resources be devoted to what is essentially an unproductive activity and which might be unnecessary. It is, therefore, doubtful whether it would be widely adopted. Over the period 1982-1987, it was suggested to a number of leading cabbage farmers that they adopt this practice. However, just one responded positively, and then only after he had suffered a severe blackleg epidemic in a field planted to cabbages for 12 successive crops of cabbage. Other farmers declined to adopt the practice of debris removal, submitting that labour costs and the less-than-obviously-productive nature of the practice as their reasons.

The alternative option of deep-ploughing of debris is probably more acceptable to cabbage farmers, and two intensive crucifer farmers in KwaZulu-Natal have sporadically followed this practice. However, it does carry the penalties of increased fuel costs, soil erosion, loss of soil moisture and damage to the soil structure, the reasons why minimum tillage has been developed and adopted world-

wide (Berry, *et al.*, 1985; 1987; Berry and Mallett, 1988; 1989; Mallett *et al.*, 1981; Mallett and Johnston, 1983; Mallett *et al*, 1985).

Another approach is to eliminate the inoculum in debris. This approaches substantially reduces the rotation period required before a field is safe to plant to crucifers again. Humpherson-Jones and Burchill (1982) found a number of chemicals that inhibited the production of ascospores in infected canola debris, or accelerated biodegradation to the extent that *L. maculans* mycelium in the debris was destroyed. The chemicals tested included the fungicides triarimol, fenarimol and ethylmercury phosphate (all at 0.025% a.i.), the herbicides dinoseb, dinoquat and paraquat (all at 0.1% a.i.), the surfactants Manoxol N, Bradasol, Cetrimide and Deciquam (5% a.i.), and the nitrogenous fertilizer urea (5% a.i.). All were 97-100% effective in preventing the formation of pseudothecia on debris. The cheapness, simplicity and great efficacy of the urea treatment suggest that this treatment should be adopted widely by canola and possibly cabbage farmers. I suggest that urea was effective because it cause an accelerated biodegradation of the canola debris, by reducing the high C:N ratio of the debris to a ratio closer to the ideal of 30:1. It is a technique with potential application on any infected crop debris with a high C:N ratio. Petrie (1995) tested a similar range of fungicides, wetters, herbicides and urea, and found similar reductions in the discharge of ascospores occurred as infected debris had been treated.

There are reports of biological control of *L. maculans*. A French paper (Anon., 1954) reports the reduction, but not elimination, of *L. maculans* by a combination of soil treatment with *Penicillium claviforme* and seed treatment with *Trichothecium roseum*. A Russian article (Upitis, 1956) reports that *Trichoderma viride* cultures sprayed onto cabbages reduced the incidence of cabbage blackleg by a factor of 2-3. *In vitro* studies by Grayston and Germida (1991a; 199b) suggest that sulphur-oxidizing microorganisms may have a role to play in control of *L. maculans*. However, until biocontrol technologies are developed and commercialized, no such option is available to cabbage farmers.

11.2.4 Resistant Cultivars

Step 8 of the blackleg ethograph (Fig. 3.1.A) highlights seed production. This has two aspects, the genetic qualities of the cultivars made available to KwaZulu-Natal farmers, and the phytosanitary cleanliness of the seed provided.

Whilst crucifer seed is not produced in KwaZulu-Natal, local farmers could influence, nevertheless, the overseas seed companies by demanding particular qualities in their cabbage cultivars, such as blackleg resistance, and the provision of disease-free seed.

Results presented in Chapter 5 (Cultivar Trials) highlighted the substantial differences in blackleg resistance in existing cabbage and cauliflower cultivars. It is therefore feasible for the international seed companies to breed for, and incorporate, blackleg resistance into their crucifer cultivars. However, it is doubtful whether blackleg resistance will ever be recognized as a major breeding object in cruciferous vegetables, given the sporadic occurrence of this disease. Compared to perennial and ubiquitous disease problems such as black rot and clubroot, blackleg of cabbages is a minor problem. Where blackleg has been a crop-limiting factor, in canola production, committed plant breeding programmes immediately were introduced, with blackleg resistance as the primary goal, and these programmes have produced effective resistance relatively quickly (Roy, 1978).

An alternative would be to breed cabbage cultivars locally, aiming for parent lines with high levels of blackleg and other disease resistances, which can then be licensed to the international seed companies to use for the production of hybrids. This process has already been followed in South Africa by Mayfords Seeds, who developed a number of parent lines from the local open pollinated cultivar, Spitskop, a sugarloaf cabbage. The net result of their programme was a high-yielding F1 hybrid called Spitzo, produced by Sakata and sold world-wide, but especially in South Africa (Zingel, Mayfords Seeds, pers. comm.).

A concerted breeding programme, based on recurrent selection, would develop blackleg resistance in cabbages and other vegetable crucifers. Such a programme would be relatively inexpensive to establish, would select for multiple characters, both pathological and horticultural and should arrive at useful, stable resistance relatively rapidly (Robinson, 1987).

11.2.5 Field Fungicides

As discussed in Chapter 6, application of field fungicides for the control of crucifer blackleg can be cost effective. However, mitigating against the use of field fungicides is the sporadic distribution and occurrence of blackleg; if it occurred as regularly in unsprayed field as early blight occurs on tomatoes in KwaZulu-Natal (Putter, 1980), then farmers would be more willing to follow this practice. However, routine spraying of all crucifer seedlings and field plantings with benomyl would be uneconomic if one considers the relatively small proportion of the crop affected in KwaZulu-Natal. Thus, it is unlikely that the routine application of benomyl as a field fungicide would be adopted widely. An exception might occur if a large-scale crucifer farmer who has experienced repeated blackleg outbreaks because of poor sanitation combined with the continued use of SBT. If canola production comes to KwaZulu-Natal, and if, as might be expected, blackleg becomes a problem in this crop, then spraying with mixed triazole/benzimidazole fungicides would probably become routine, as has occurred with maize for the control of maize grey leaf spot (*Cercospora zeae-maydis* Tehon) (Ward *et al.*, 1996).

11.2.6 Management of Host Physiology

The theory proposed in Sections 10.1 and 10.2 and the results presented in Section 10.3.3 suggest that blackleg can indeed be managed by maintaining a well-fertilized cabbage crop without stress. The key elements involved in KwaZulu-Natal are optimizing soil pH, minimizing soil acid saturation, avoiding manganese toxicity (associated with the previous two parameters), optimizing macro- and micro-nutrient application and avoiding water stress (Askew, 1995). Other factors such as

avoiding bird, insect and herbicide damage also play a part, as discussed above in Section 10.2.

11.2.7 Cultural Practices

Attempts to reduce the rate of blackleg spread by roguing has been reported both as successful (Whitehead and Jones, 1929; Millard, 1945) and unsuccessful (Neill, 1929). In light of the fungus' long latent period, there is little chance that roguing would be successful, the practice would be a case of "shutting the stable door after the horse had bolted". Varying fertilizer levels has been researched as a possible control method. Levy (1919; 1920) found that, "the better the crops in an infected area the more subject to disease they were. Doubtless this is accounted for by the fact that a rapidly developing (swede) bulb is thin in the skin and loose in the tissues, and thus more liable to afford ingress to the spores of the disease" (Levy, 1919). In contrast, Kupryanova (1957) reported a reduction in cabbage blackleg following application of mixed chemical and organic fertilizers, compared with manure only. Cockayne (1918) recommended the liming of soil to delay blackleg progress in swedes. Antonov (1978) recommended the addition of colloidal sulphur (5 g per m^2) as a measure to control blackleg. However, the results presented in Chapter 10 showed conclusively that blackleg can be managed by optimizing fertilizer application levels.

11.2.8 Conclusions

Blackleg is a disease which is can be managed if certain basic steps are followed, based on the disease cycle and requirements of the pathogen, *L. maculans,* and the production cycles of susceptible host crops.

11.3 Suggested Protocol for Cabbage Production in KwaZulu-Natal, Designed to Minimize Blackleg Incidence

The following measures are suggested as practical measures which will significantly reduce the risks of blackleg occurrence, within the known production pattern of the KwaZulu-Natal Midlands.

1. **Seed:**

Treat seed with iprodione (Rovral EC) at 5 g a.i. kg^{-1} seed as a seed slurry.

2. **Seedlings:**

   a. Use CGS if reliable nurseries are available; check critically that strict phytosanitary practices are applied to all components of CGS production.

   b. If CGS are unavailable, then produce SBT seedlings in seedbeds; ensure that the seedbed is not situated in old production lands of any cruciferous crops, It must also be isolated from crucifer production lands and from any vegetable gardens with crucifers.

   c. After removal of SBT for transplanting into production fields, remove all excess SBT and compost them.

   d. At transplanting, spray or dip the seedling in a benomyl suspension.

   e. Use smaller rather than larger containers to move the SBT transplants from seedbeds to the production fields.

3. **Lands:**

A minimum of a 3 yr rotation, with at least a 100 m distance from old fields, and more,, if these are situated upwind.

4. Irrigation:

Irrigate with high volume, short duration, overhead sprays in the middle of the day, or at night if dew is present already. This approach will minimize leaf wetness periods, during which infection can occur.

5. Insects:

Good insect control is important, to avoid plant stress.

6. Harvesting of All Crucifers:

Harvest heads by removing the entire plant from the ground and then cutting the head off. Alternatively, ensure that Step 7 is carried out meticulously.

7. Sanitation:

At the end of the season, uproot all unharvested plants, windrow, then collect all debris and bury or ensilage it.

8. Weeds:

a. Kill wild crucifers throughout the year.

b. Do not use herbicides that do not kill cruciferous weeds.

c. Spray all old production lands with 50 kg per ha. urea in solution, to accelerate saprophytic degradation of crucifer debris. The addition of a wetting agent to the urea solution may assist microbial degradation of debris.

9. Alternate Hosts:

Avoid production of cruciferous fodder crops in proximity to crucifer vegetable production lands.

10. Fertilization

Ensure high levels of fertilization (especially nitrogen) if the crop is considered to be at risk. However, this may increase the risk of Erwinia soft rot and Sclerotinia white mould.

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