Diallel crosses between sources of Black Shank *(Phytophthora parasitica var. nicotianae)* resistance

by

LOUISE VAN DER MERWE

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Promotor: Prof. CZ Roux

Co-promotor: Prof. JMP Geertsen

I the undersigned hereby declare that this dissertation contains my own original work and has not previously in its entirety or in part been submitted at any university for any degree or examination.

Signature: ..... Date: 10-12-02

DEDICATION

I fully dedicate this thesis work to my parents Louis and Anita van der Merwe.

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ABSTRACT

The black shank (*Phytophthora parasitica var. nicotianae*) fungus is a very destructive tobacco disease which is responsible for great losses to farmers worldwide. This disease is also a problem in South Africa, as the most popular South African air-cured tobacco cultivar, CDL28, is very susceptible to black shank. This diallel study focussed on finding the most suitable black shank resistance source to include in a resistance breeding programme with CDL28. Four cultivars were crossed in all possible combinations and planted as an F1 field and greenhouse trial. The F1's were selfed to obtain a segregating F2 population, planted in a greenhouse, to be compared with the mean values of the F1 trials. The field trial was exposed to natural infection while the greenhouse trials were root inoculated. The general combining ability effects of the four parent cultivars differed significantly from each other. The specific combining ability effects of the F1 trials were non significant. These experimental results suggest that additive genetic effects were involved in black shank resistance. The Beinhart 1000-1 source of resistance was significantly better than the Florida 301 source. Burley 37, which possesses the Florida 301 as well as another source of resistance performed better than Domkrag with only the Florida 301 source of resistance. In order to incorporate black shank resistance in CDL28, Beinhart 1000-1 and Burley 37 can be used in a backcross breeding programme with CDL28 which can solve the problems encountered in the cultivation of CDL28 in the presence of black shank.

CHAPTER 1

INTRODUCTION

The semi-aquatic black shank fungus is favoured by high humidity and warm temperatures and therefore it frequently occurs in tropical and subtropical countries, including South Africa. Tobacco is the only natural host reported for the black shank fungus. Losses occur during all stages of plant development and can reach 100% in some fields. Since the first outbreaks of black shank were reported in the early 1900's, this disease has become one of the major tobacco diseases, causing great losses in income to farmers in South Africa and other tobacco-producing countries.

In South Africa, the tobacco industry forms an important part of the agricultural sector, which in turn constitutes part of the country's multifaceted economy. During 1999, 13 962 hectares of tobacco were planted and 30.702 million kilograms of tobacco were produced. The country derived an income of 3 455 million rand in excise duty and 1 250 million rand in VAT, totalling 4 705 million rand. In 1998, the primary tobacco industry employed 26 260 people and 22 450 seasonal workers. Thus, regardless of the government supported anti-smoking campaign, tobacco still has an important role to play as an agricultural crop in South Africa.

Since black shank is a huge problem on tobacco worldwide, a lot of research has been undertaken on the disease. So far, four sources of resistance to black shank have been discovered, but, since the pathogen is able to form new races over a period of time, the research on black shank will have to be an ongoing process. The individual sources of resistance do not provide resistance to all races of the pathogen and combinations of resistance genes, or a new source of resistance, will have to be found in order to satisfy the demand for a factor that is resistant to all the races of black shank.

CDL28 has been the most popular and, in some districts, the only air-cured tobacco

cultivar in South Africa since 1975, but this cultivar has a very low resistance to black shank, which poses a big problem to air-cured tobacco farmers in South Africa. Most producers of air-cured tobacco do not grow flue-cured tobacco and, if they have a big black shank problem on their farms, they receive a much lower income with the result that some have had to stop tobacco farming altogether since no other air-cured tobacco cultivar delivers the same quality as CDL28. No other air-cured tobacco cultivar, which is black shank resistant and equal to or better than CDL28 in quality, has been developed since 1975. It is, therefore, extremely important to find a black shank resistant air-cured cultivar that can fulfill the above-mentioned requirements.

The main aim of this diallel study was to find the most suitable black shank resistant parents for use in combination with CDL28 in a breeding programme for black shank resistance in air cured tobacco.

CHAPTER 2

LITERATURE STUDY

2.1 TAXONOMICAL DESCRIPTION

According to Lucas (1975), Van Breda de Haan named the fungus which causes black shank, *Phytophthora nicotianae* in 1896, but he described a mixed culture and failed to give a Latin description for the organism. In 1913, in India, Dastur described a similar organism pathogenic to castor bean, but not to tobacco, and named the organism *P. parasitica*. Evidence of the possible relationship of these organisms was indicated by Palm, since he was able to infect castor bean with *P. nicotianae*. Similarly, Tisdale and Kelley published in 1927 that an isolate of *P. nicotianae* from Java and one from Florida as well as an isolate of *P. parasitica* all caused similar symptoms when inoculated into castor bean. However, the *P. parasitica* isolate was not able to infect tobacco. Tucker, in 1931, renamed the fungus that attacks tobacco *P. parasitica* Dastur var. *nicotianae* (Breda de Haan) Tucker, and this name is currently used by most tobacco pathologists. In 1963, Waterhouse concluded that the name *P. nicotianae* has priority and stated that the correct name of the black shank pathogen is *P. nicotianae* Breda de Haan var. *nicotianae* Waterhouse. Both these names are now used to describe this organism although they should not be considered synonymous (Shew & Lucas, 1991).

Tucker's trinomial description, *P parasitica* var. *nicotianae*, has been widely accepted since 1974 and is used in most of the later literature. It will, therefore, be the description mostly used in this thesis.

2.2 WHAT CAUSES BLACK SHANK?

According to Furney (1981) and Shew and Lucas (1991), black shank is caused by a fungus *P. parasitica* var. *nicotianae*. This fungus produces mainly two types of spores in its sporangia (Fig 2.1).

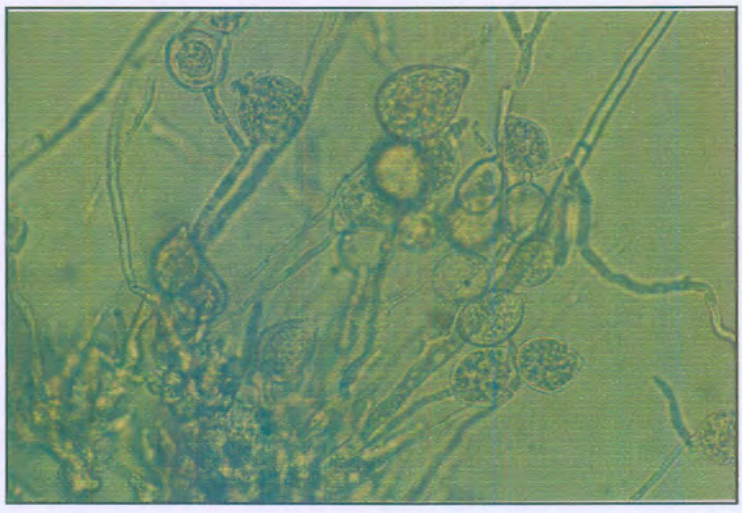

Fig 2.1 Sporangia of *Phytophthora parasitica* var. *nicotianae*.

One spore form, the zoospore, is very delicate and will live for only a short period of time. Sporangia germinate indirectly to produce zoospores or directly by means of a germ tube. Zoospores released during soil saturation are attracted to roots, where they encyst and germinate within an hour. Thus, wounds are not necessary for entrance of the fungus into the root. Infection occurs quickly and the fungus grows rapidly through the epidermis and into the cortex. The rate of growth through roots can reach 2 cm per day in a susceptible cultivar. Lesions continue to enlarge until they reach the stem, resulting in the development of black shank symptoms. The zoospores are produced in large numbers, especially during cloudy, rainy, warm weather and are principally responsible for the rapid build up and spread of the disease during the spring and summer months. The second spore form, the chlamydospore, has a thick wall or outer coat which enables it to withstand heat, cold and drying. Chlamydospores are considered to be the overwintering stage because of their structure and known ability to germinate after months of dormancy. This spore form can remain in the soil in an

active state indefinitely even in the absence of the tobacco plant. That is why black shank is usually considered a threat each crop year. In addition, the fungus may possibly overwinter as oospores or as mycelia in dead tobacco stalks or roots.

Lucas (1975) stated that sporangia and zoospores are important inoculum sources and the severity and incidence of the disease increases with warm temperatures and adequate moisture. During warm, humid weather the fungus sporulates freely and produces plenty of inoculum which is then spread principally by drainage water after heavy rains.

2.3 SYMPTOMS OF BLACK SHANK INFESTED FIELDS AND PLANTS

Black shank causes plants to wilt suddenly and die. The first infections appear in localized areas or low places in the field and the disease may develop at any time during a season.

Lucas (1975) stated that the black shank fungus primarily affects the roots and basal stem region of the tobacco plant, but that all parts of the plant can be infected. Symptoms vary with plant age and weather conditions. Young seedlings are very susceptible and typical damping-off symptoms develop in the seedbed during periods of wet, mild weather. The stem near the soil line becomes dark brown or black (Fig 2.2) and the pathogen rapidly grows up the stem into the leaves. The disease makes its first appearance in low, wet spots or near the edge of the plant bed where surface water was washed in. During cool weather, the disease may develop slowly in plant beds and be overlooked. However, near the end of the planting season, when the weather is warm and the plants are ready to be transplanted, they may wilt during the hot part of the day. All or part of the root system may be infected and turn black, with the black stem lesion extending from the crown of the plant to several centimetres up the stalk. When apparently healthy, but infected, transplants are used to plant a field, a poor stand often results.

The first aboveground symptoms on the field, according to Lucas (1975) and Shew and Lucas (1991), is wilting of the leaves during the middle of the day. Plants usually recover overnight only to wilt more severely the next day. In general, all the leaves wilt (Fig 2.3). This is in contrast to wilting symptoms caused by vascular wilt pathogens

Fig 2.2 Young black shank infected plants become dark brown to black near the soil line.

where wilting of one leaf or of leaves on the one side of the plant occurs. Depending on the level of soil moisture and level of host resistance, leaves begin to turn yellow and hang down the stalk over the next few days to weeks. If a plant is pulled at this early stage and the roots are examined, one or more of the large lateral roots will be

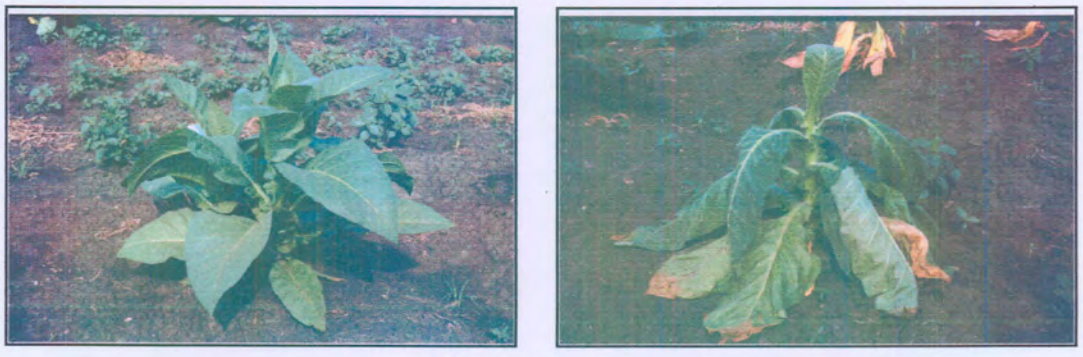

Fig 2.3 Typical wilting symptoms of a black shank infested plant (right) in contrast to a healthy plant (left).

blackened or many of the fine adventitious roots arising from the stem will be decayed. At this early stage, the stalk will be free from decay or discolouration. However, as the disease progresses, infection spreads into the stem of the plant and often into the entire root system. In the final stages of the disease in older, taller plants, the stem turns black 30 cm or more above ground (Fig 2.4), thus the name black shank. At this point leaves turn from yellow to brown, shrivel and in a few days the plant will be dead. The wilted leaves harvested from a black shank affected plant are of little value and usually not marketable.

Fig 2.4 Older plants, in the final stages of the disease, have black stems 30 cm or more above ground and brown, shrivelled leaves.

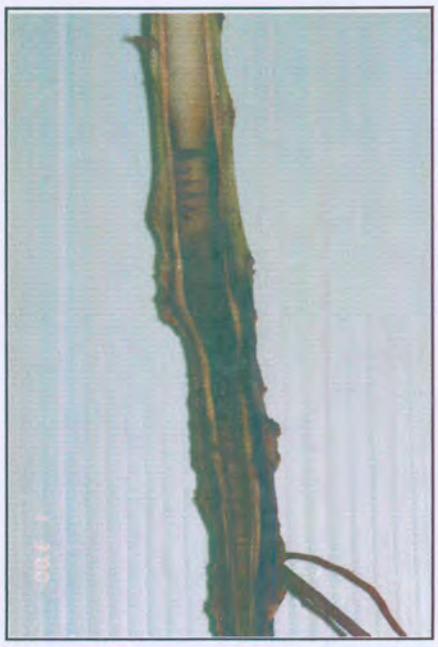

Fig 2.5 A stem of a diseased plant split lengthwise showing the brown pith and plate-like discs.

It is not unusual to see diseased plants late in the season with their stalks black halfway to the top. Such plants may still retain a few small green leaves in the top and bloom prematurely. In resistant cultivars, upper leaves may remain green even after black shank symptoms appear. In addition roots of resistant cultivars may become infected and rot without obvious aboveground symptom development. The affected resistant plants might, therefore, appear healthy. When the stem of a diseased plant is split in half lengthwise through the lesion, the pith appears dry and brown to black

and is usually separated into plate-like discs (Fig 2.5). This is one of the most characteristic diagnostic symptoms of black shank. With the aid of a microscope, the fungus can frequently be seen in the cells of the pith. Anonymous (1958) reported that the pith at the juncture between the dead pith and healthy tissue just above, will appear slightly darkened and watery. Black shank causes a localized infection. There is no discolouration in the stem tissue above the affected part. This also helps to distinguish black shank from the wilt diseases.

According to Lucas (1975), during periods of rainy weather, the lower leaves might be attacked by the fungus and as many as a dozen large, circular lesions, up to 8 cm in diameter, may appear on a single leaf. These leaf spots result from infections initiated by zoospores or sporangia splashed from the soil onto the leaves. Young lesions are paler than the normal green of the leaf. During warm, wet weather the spots enlarge rapidly and the centres turn brown and necrotic with concentric bands of yellow around the margins. The large necrotic lesions cause serious damage to both cigar-wrapper and flue-cured leaves.

2.4 THE FOUR FORMS OF BLACK SHANK

2.4.1 Stalk black shank

Furney (1981) and Prinsloo and Engelbrecht (1989), reported that this form of black shank may occur in both resistant and susceptible varieties and is the form most often observed. Here the fungus enters the roots of the plants, resulting in almost complete destruction of the roots. The disease also advances up the stem for a short distance (up to 30 cm) and results in separation of the pith into discs. Rapid, uniform wilting or drooping of all the leaves follows and the plant dies off. Losses to this form may be extreme.

2.4.2 Stem black shank

Furney (1981) stated that stem black shank is most often observed in resistant varieties after hail or windstorms or when the stalk is bruised. The fungus enters the stalk near the soil line or slightly above. The aboveground portion wilts rapidly but the root system often appears healthy. The pith is separated into discs. The symptoms, especially in resistant cultivars, can be ascribed to the fact that resistance to black shank is located for the most part in the root system and the stalk and leaves are susceptible. Yield loss can be heavy.

2.4.3 Leaf black shank

Furney (1981) and Shew and Lucas (1991) reported that this form may occur in both resistant and susceptible varieties and is usually observed after periods of heavy, splashing rain and overcast days. The spores of the fungus that are present in the soil splash up onto the leaves, resulting in large, rounded, greenish-brown spots on the leaves. The lesions can develop up to 8 cm in diameter. The fungus often grows into the stem from leaf infections and causes typical black shank symptoms. Most damage occurs on the lower leaves and losses to this form are usually low.

2.4.4 Hidden black shank

Furney (1984) stated that hidden black shank is usually observed in resistant varieties. It is caused by the black shank fungus and several other microscopic organisms that may attack the roots of tobacco plants. A portion of the root system is destroyed and is brown to black in colour. The aboveground portions of the plant may wilt slightly during the heat of the day, but usually recover at night. Stunted plant growth is characteristic of this form of black shank and leads to reduced performance. During periods of hot, dry weather the hidden form may become the stalk form and the plants will die. Yield loss may be high, but this form is generally not as destructive as the stalk form.

2.5 FACTORS THAT FAVOUR BLACK SHANK DEVELOPMENT

The literature by Lucas (1975), Furney (1981 and 1984) and Shew and Lucas (1991) can be cited with reference to the factors that favour black shank development.

2.6 SPREADING OF BLACK SHANK

The danger of spreading *P. parasitica* var. *nicotianae* in soil is much greater when the soil is wet. Soil on farm tools, the feet of farm animals, labourers' shoes and the wheels of trucks and automobiles may be responsible for transferring the fungus from field to field. Lucas (1975) reported that the black shank fungus is commonly spread by water. Rainwater transports infested soil and fungus spores along rows, terraces and ditches and from infested fields into plant beds, disease-free fields, drainage ponds, creeks or streams. When water from these infested sources is used for plant bed irrigation, transplanting or field irrigation, it may carry the fungus to new locations and cause outbreaks.

The uniform initial infestation of some fields indicates that windblown dust may be an agency of distribution (Lucas, 1975). An inoculum density of less than one propagule per gram of soil is sufficient to start an epidemic of black shank (Shew & Lucas, 1991).

Lucas (1975) and Shew and Lucas (1991) reported that the fungus is present in stalks of infected plants, so these stalks should not be placed on seedbed sites or non-infested fields. When these black shank infected plants decompose, the chlamydospores formed in the diseased tissue are released into the soil. Under suitable conditions they germinate by germ tubes to form sporangia or other chlamydospores. The sporangia germinate to produce hyphae or zoospores. All these spore forms may be carried long distances by water and all germinate in water. When the spores lodge against a tobacco stem or root or splash onto a leaf, they germinate and produce mycelia which infect the plant.

2.7 CONTROLLING THE BLACK SHANK DISEASE

There are several possible ways to control black shank, namely through biological control (crop rotation), by means of disease-resistant cultivars and by making use of chemical agents.

Biological control implies an effective crop rotation programme. Crop rotation is considered an excellent method of reducing the infestation level of the black shank causal agent and is encouraged where suitable land is available. Rotation does not eliminate the causal agent but rather reduces the infestation level to a point where a resistant variety can be used successfully. A trial was executed by De Villiers (1987) to determine the influence of rotation crops on the occurrence of black shank. The crops evaluated in this study were those which are normally used in rotation with tobacco in South Africa. It was quite clear that blue buffalo grass (*Cenchrus ciliaris)* decreased the black shank population in the soil more effectively than any of the other rotation crops used in the trial. To obtain a significant reduction in the black shank population blue buffalo grass should be planted for at least two years before the tobacco is planted. Although Smiley *et al.* (1966) reported that crop rotation had been used with partial success to control the black shank disease, they also mentioned that many farmers did indeed manage to eliminate the fungus from their farms. These farmers used strict sanitation measures and sowed grass or grass-legume mixtures without applying limestone, which is usually needed to establish a legume. The limestone could cause the fungus to live longer. The grass was grown on the field for at least three years before tobacco was planted on the same field. One year's rotation reduces the amount of inoculum considerably but, because of the rapid build-up of the pathogen, short cropping sequences are of little value when susceptible cultivars are grown (Lucas, 1975).

According to Furney (1981), probably the best way to beat the black shank fungus is to use resistant cultivars. He considers resistant cultivars as the "backbone" of a black shank control programme. Varieties are available with low, moderate and high

resistance. The level of resistance to be used is determined by the infestation level of the fields and the extent to which the disease can be controlled by other control methods. A variety with high resistance is usually the best choice, especially in situations where the infestation level of the causal agent is unknown. Highly resistant cultivars are suggested for all fields where continuous culture is practised and for rotation fields where damage in excess of 5% was caused to the previous crop. Some growers contend that the varieties with low and moderate resistance are usually easier to handle and that they produce leaf of a higher quality. This level of resistance provides adequate protection in rotated fields where the infestation is considered to be low to moderate. It also provides adequate protection when resistance is combined with other control methods. Lucas (1975) mentioned that another consideration to be borne in mind when resistant tobacco cultivars are used is that nematode damage breaks down the tobacco plant's resistance to black shank. Therefore, if black shank is present, even resistant plants will be affected if the soil in which the tobacco is to be planted is not fumigated to reduce the nematode population. When a root-knot resistant crop is grown immediately preceding tobacco, black shank is less severe than when a root-knot susceptible crop is grown. Black shank resistance must be protected as it can be threatened or even destroyed by various factors. Malpractices, such as monoculture with tobacco could allow the fungus population to build up to such an extent that the resistance of the plant can no longer cope with the disease. When breeding, it is important to remember that a tobacco cultivar which is resistant to one race of black shank is not necessarily resistant to the others. Numerous resistant varieties have become useless due to the presence of new highly virulent strains of pathogens.

Few of the chemicals on the market are really effective against black shank and it must be kept in mind that a plant which has already been infected with black shank cannot be healed. Chemicals must be applied as preventive measures within one week after transplanting.

Lucas (1975), Furney (1984) and Nielson (1992 b) all came to the conclusion that satisfactory black shank control can be obtained with a systems control method. This

involves the use of resistant cultivars in conjunction with rotations, soil fumigation with multipurpose chemicals and disease-free transplants. It is, therefore, quite clear that no single preventive measure will be enough to combat black shank but that all the measures must be used to assist each other in order to overcome the disease.

2.8 RESISTANCE IN TOBACCO ROOTS AND STEMS

Disease or pathogen avoidance in plants is a heritable trait and is most likely to be important with pathogens such as *P. parasitica var. nicotianae* that survive in soil at low population levels (Ferrin & Mitchell, 1986).

According to Shew and Shew (1994) and Jones and Shew (1995), the production of fewer roots by black shank resistant cultivars may be important for their survival in soil infested with *P. parasitica var. nicotianae*. Plants that produce fewer roots will be less likely to come in contact with pathogen propagules than plants that have a greater rooting intensity. In other words, cultivars with small root systems would require higher initial inoculum densities to contact the same number of propagules as cultivars with large root systems. Jones and Shew (1995) used the Florida 301(Fla 301) type of resistance in an experiment and determined that tobacco cultivars which produced the smallest root systems had moderate to high levels of partial resistance to *P. parasitica* var. *nicotianae*. They proposed that investigations into the rooting patterns of additional cultivars with the Fla 301 type of resistance might provide insight into whether genes that code for a smaller root system originate from Fla 301. In addition to having fewer infection sites due to the smaller root systems, partially resistant cultivars in the study of Jones and Shew developed fewer lesions per inoculation site than susceptible cultivars. Thus, reduced lesion development appears to be one mechanism of partial resistance to *P. parasitica var. nicotianae* in tobacco. Small root systems may have important effects on interactions other than the plant and pathogen interaction. Water-use efficiency, responses to fertilizer, responses to environmental stress and the effectiveness of pesticides are a few aspects that will be affected by root growth

dynamics. All of these factors may have an impact on the host/ parasite interaction and on the incidence and severity of the disease. The larger root system of susceptible cultivars is primarily the result of increased root branching which greatly increases the number of first order roots present (Fitter, 1982). The rapid proliferation of first order roots may also increase the chances of infection by *P. parasitica var. nicotianae* since first order roots are more susceptible to infection by *P. parasitica* var. *nicotianae* than second or third order roots (English & Mitchell, 1989).

According to Apple (1962), Stokes and Litton (1966), Hendrix and Apple (1967) and Lucas (1975) the latent period for resistant cultivars is long because the fungus grows slowly through the root cortex. Once the fungus penetrates the stem, disease development is similar to that of susceptible cultivars. However, it seems that there is a difference between the different sources of black shank resistance, *Nicotiana plumbaginifolia*, *N. longiflora* and Fla 301, regarding the resistance within their stems. The reaction of lines derived from *N. plumbaginifolia* to stem inoculations with race 0 and 1 of *P. parasitica var. nicotianae* was consistent with their extremely high root resistance to race 0 and high susceptibility to race 1. However, though plants of lines with *N. longiflora* and *N. plumbaginifolia* resistance were rarely lost to black shank in fields heavily infested with race 0, a low percentage of plants having high resistance of the Fla 301 type had died. This can be explained by the fact that plants with Fla 301 type resistance often undergo extensive root damage and when the fungus reaches the base of the stem of a young plant, the entire vascular cylinder may be invaded, resulting in wilting of the plant. Thus, roots of plants with the *N. longiflora* type of resistance have a higher level of resistance than those of the plants with the Fla 301 type of resistance and the plants seldom die if the race 0 fungus reaches the stem. However, stems of all lines and varieties of *N. longiflora*, *N. plumbaginifolia* and Fla 301, tested by Hendrix and Apple (1967), were susceptible to race 1 of *Phytophthora parasitica* var. *nicotianae*.

2.9 BREEDING FOR BLACK SHANK RESISTANCE

Black shank is one of the most destructive soil-borne diseases and it is, therefore, of great economic importance to breed resistant tobacco cultivars.

According to Nielsen (1992 b), inheritance of resistance to black shank may be simple or complex, depending upon the source of resistance. Resistance derived from *N. tabacum* sources is typically oligogenic or polygenic in origin, while single-resistance genes from *N. longiflora* and *N. plumbaginifolia* provide protection against black shank. The *N. longiflora* and *N. plumbaginifolia* gene is effective against only one race of *P. parasitica*. Thus, it will be very important to choose the correct parents in a black shank resistance breeding programme. To a large degree, the key elements which determine the selection of an appropriate breeding method are the inheritance of the resistance and the choice of the parents. Nielsen (1992 b) stated that appropriate breeding methods for developing resistant cultivars include pedigree or modified pedigree methods, although backcross methods may be appropriate in certain circumstances. In general, pedigree breeding methods are the best to use in developing black shank resistant cultivars. Large F2 populations are usually required to obtain recombinants with the desired level of resistance to black shank. Segregating populations or breeding lines may be tested in the field under disease pressure or by inoculating plants grown in controlled environments. Advanced breeding lines need to be evaluated in multiple environments using replicated tests to assess the level of resistance accurately. Backcross methods are generally less appropriate for traits which are quantitatively inherited, but they would be suitable in transferring the *N. longiflora* and *N. plumbaginifolia* genes. In an attempt to use the Beinhart 1000-1 resistance more effectively, Nielsen (1992 b) used phenotypic recurrent selection to increase the level of resistance in a synthetic population. The percentage survival in the population improved considerably following three cycles of selection for resistance. Increasing the number of recombination events by this method may break undesirable linkages present in Beinhart 1000-1 and allow the development of highly resistant cultivars which are commercially acceptable. It is, however, important to remember that change

in pathological races may render a previously resistant cultivar ineffective in reducing disease losses and make the transfer of resistant cultivars from one production area to another a fruitless endeavour. The effect of genes can be altered by factors of both the genetic and external environment. Genetic analysis of black shank resistance and the selection of resistant plants can be influenced by a number of factors, including the severity of the disease, plant age, and the pathogenicity of the *P. parasitica* strain.

2.9.1 Screening Techniques

Identification of useful genotypes as donors of resistance is only the first step in breeding resistant cultivars and the ability to identify resistant plants in segregating populations accurately is just as critical (Nielsen, 1992 b).

With reference to field trials, Campbell and Wernsman (1994) reported that screening for resistance to soil-borne pathogens such as *P. parasitica var. nicotianae* often requires the maintenance of disease nurseries with high pathogen populations. Because inoculum levels and soil conditions are often variable throughout a field, numerous replications must be used to obtain reliable results. In addition, only one field test per year is possible in temperate climates. These factors often make field testing the limiting step in a breeding programme for disease resistance. Nielsen (1992 b) considered field evaluation as the final test of the effectiveness of resistance, but states that selection of resistant plants in infested fields is made difficult by environmental and other factors which may affect disease expression. In addition, the chosen field site must be infested with a form of the pathogen that is representative of that found in the intended area of production. Other limitations of field trials include mixed infestations with other pathogens or unfavourable weather for normal growth and disease development (Nelson, 1973).

Field sites infested with the causal organisms are used in most breeding programmes, but greenhouse and laboratory techniques have also been developed which are desirable because they give quicker results than field tests and are sometimes nondestructive (Carlson *et al.*, 1997).

According to Nielsen (1992 b), screening techniques which are suitable for use in greenhouse environments include the use of small plants which are stem inoculated or which have their roots immersed in inoculum. These techniques allow control of the pathogen isolate used, permit more uniform inoculation and are relatively efficient.

A detached leaf technique for the evaluation of black shank resistance was also developed. Inoculation with a mycelial plug of leaves removed from small plants led to the development of lesions on susceptible genotypes in three to four days. The advantage of this system is that plants possessing a moderate level of resistance reach maturity because the roots and stems are not infected. This method can be used to eliminate susceptible plants from a population, thus reducing populations for subsequent field testing. Plants can, therefore, also be screened for resistance to other diseases, but according to Wills (1971) and Tedford, Miller and Nielsen (1990), the detached leaf technique has had limited accuracy in screens for most root pathogens, other than to identify extreme levels of resistance or susceptibility. In other words, detached leaves and intact plants may differ in resistance to *P. parasitica var. nicotianae*.

Hendrix and Apple (1967) developed an effective stem inoculation technique for detecting monogenic resistance to race 0 of *P. parasitica var. nicotianae*. However, the method was inadequate for differentiating levels of horizontal resistance. Stem inoculation has shown higher correlation to field results than leaf tests but, according to Wills and Moore (1971), it may not reflect root responses to the pathogen. They further stated that resistance detected by stem inoculation techniques has shown good correlation with whole plant resistance but have been too variable for these techniques to be used in tobacco breeding programmes.

Both the detached leaf and the stem inoculation method are often too variable to be used for accurate screening for resistance within segregating tobacco populations (Carlson *et al.*, 1997).

Wills (1971) did some research on inoculations of whole plants and leaf strips. He concluded that resistance as expressed in leaves can sometimes be correlated with the response of whole plants to the pathogen and sometimes not. Thus, leaf strips are not a reliable index of total resistance. They are, however, useful in studying the nature of resistance, since leaves provide a readily accessible and bountiful source of plant material for biochemical and histochemical studies. A study by Wills and Crews (1964) showed that the expression of resistance in leaf tissue depends on the location of the leaf on the stalk. Leaves from the upper and middle stalk positions were the most resistant and tissue from the youngest apical leaf was extremely susceptible.

The root inoculation method of Litton, Collins and Legg (1970 a) is effective for screening the progeny of advanced breeding lines, but the eventual death of even moderately resistant plants limits its use as a selection tool.

2.10 THE RACES OF *Phytophthora parasitica* var. *nicotianae*

Physiological races of a fungus are defined as being similar in morphology but unlike in certain cultural, physiological, biochemical, pathological or other characters (Ainsworth, 1971). Plant pathogens may vary widely as a result of mutation, mitotic recombination, heterocaryosis or genetic recombination arising from interstrain or interspecific hybridization (Apple, 1962).

The black shank pathogen is capable of developing new races. Shew and Lucas (1991) reported that at least four races are known. According to them, race 0 is predominant and other races have been reported from specific geographic locations. These races possibly developed as a result of specific resistance genes used in the cultivars grown in those areas.

Apple (1962) described the races that are nonpathogenic and pathogenic on *N. plumbaginifolia* as races 0 and 1 respectively. Race 1 occurred mainly in a few areas

in Kentucky. The predominant race was race 0, which occurred in many other tobacco-producing states of America and in other countries.

Prinsloo and Pauer (1974) announced their discovery of a new race of *P. nicotianae var. nicotianae*, found in South African soil, in 1974. This race was called race 2 and was unable to infect the tobacco cultivar, DC202, which was susceptible to races 0 and 1 of the pathogen.

McIntyre and Taylor (1978) described race 3 of *P. parasitica* var. *nicotianae*, found in fields in Connecticut, in 1978. Their results showed that their new race caused typical black shank symptoms and had a similar morphology to races 0 and 1, but that it differed significantly from these races in pathological, physiological and biochemical traits. They showed that the South African cultivar, A23, which is resistant to race 2, was susceptible to their Connecticut isolates. Thus, according to the accepted definition of physiological races of Ainsworth (1971), these Connecticut isolates qualified as a new race, race 3, of *P. parasitica* var. *nicotianae*.

According to Nielsen (1995 b), numerous studies suggest that population dynamics are often more stable for soil-borne pathogens than for pathogens that may be disseminated by other means. Significant changes in *P. parasitica* populations would therefore not be expected in a short period. However, cooperators in the Cooperation Centre for Scientific Research Relative to Tobacco (CORESTA) Black Shank Collaborative Study do suggest a few changes in the pathogen's population structure over a four-year period.

The ultimate goal is to control the black shank disease through immunity or high resistance to all of the above-named *P. parasitica* var. *nicotianae* races and those that will still develop in future.

2.11 THE FOUR SOURCES OF BLACK SHANK RESISTANCE

2.11.1 *Nicotiana longiflora* and *Nicotiana plumbaginifolia*

Stokes and Valleau (1957) reported that, in the section *Alatae* of *Nicotiana,* four species have been found which show high resistance to several field strains of black shank. Two of these species, *N. longiflora* and *N. plumbaginifolia*, were closely related and could be hybridised, apparently with complete fertility in the progeny. According to Collins *et al.* (1971 b) the absence of segregation in F2 populations from crosses among accessions of *N. longiflora* and *N. plumbaginifolia* indicated that the resistance locus is identical in the two species. Therefore, there is little reason to believe that breeding with these two species will give different results so far as reaction to virulent strains of *P. parasitica* var. *nicotianae* is concerned.

According to Hendrix and Apple (1967) the stem resistance derived from *N. longiflora* behaved in the same way as that derived from *N. plumbaginifolia* and Goodspeed (1954) considered the two species to be quite similar by saying of *N. plumbaginifolia,* it "might be considered a variety of *N. longiflora*". However, in a study of Wernsman, Matzinger and Powell (1974) to determine if the chromosome that bears the inserted factors for resistance from *N. longiflora* and *N. plumbaginifolia* is the same chromosome that carries genes for Fla 301 resistance, they were able to identify the *N. longiflora* chromosome associated with black shank resistance, but found that the genetics of resistance of *N. plumbaginifolia* was so complex that chromosomes carrying major genes for disease resistance could not be positively identified. On the contrary, according to Collins *et al.* (1971 a) and Goins and Apple (1971) resistance from both sources is controlled by a single dominant gene which is recovered in the homozygous condition from segregating populations less frequently than expected.

None of the crosses made with, and breeding lines developed from *N. longiflora* and *N. plumbaginifolia,* has as high a level of resistance as the original *Nicotiana* species from which the resistance was obtained (Chaplin, 1966).

In 1948, Smith and Clayton (1948) reported that race 1 of *P. parasitica var. nicotianae* is highly pathogenic to *N. longiflora and N. plumbaginifolia*. Later work by Valleau, Stokes and Johnson (1960) and Hendrix and Apple (1967) showed that both *N. longiflora* and *N. plumbaginifolia*, as well as breeding lines developed from them, have resistance to the original race, race 0. They were, however, susceptible to race 1 of black shank. However, Litton, Collins and Legg (1970 b) and Wills (1971) stated that, although the species *N. longiflora* and *N. plumbaginifolia* have been considered susceptible to race 1 and highly resistant to race 0, it has been shown by their work that *N. longiflora* has resistance to race 1 as well.

Valleau, Stokes and Johnson (1960) and Silber and Heggestad (1963) raised a question in 1960 as to whether the *N. longiflora* and *N. plumbaginifolia* resistances, which are probably identical, and the resistance found in other wild species would be of any permanent value in the control of black shank. They suggested that it would probably prove more profitable, in the long run, to attempt to raise the level of resistance of the Fla 301 type rather than to resort to species hybridization, unless a better source could be found than was then known. But on the contrary, black shank resistance from *N. plumbaginifolia* in the variety NC2326 and from *N. longiflora* in McNair 20 and McNair 30 has since exemplified successful utilization of interspecific gene transfer in commercial flue-cured tobacco production in the United States. Wernsman, Matzinger and Powell (1974) did, however, state that other interspecific gene transfers have been plagued by reduced leaf quality and have generally been unacceptable to the trade.

As a summary of the utilization of the two species of *Nicotiana* as sources of black shank resistance, it will probably be best to quote Legg, Litton and Collins (1982) saying that, "Interspecific transfers of genes for disease resistance have been used extensively in breeding tobacco, but some transferred segments have changed agronomic and chemical traits". According to them a vertical source of resistance to race 1 has not yet been identified and if such a source of resistance to race 1 could be found, the breeders could combine that resistance with race 0 resistance from *N.*

longiflora and *N. plumbaginifolia*.

Nicotiana longiflora

Silber and Heggestad (1963) reported that black shank resistance from *N. longiflora* was transferred to Burley tobacco by Valleau et al., but the best of their Burley lines containing the *N. longiflora* factor had a survival rate of only 60 to 80% in field trials. Attempts to secure homozygous black shank resistant Burley tobacco from these breeding lines were not successful. Valleau later found a homozygous black shank resistant breeding line designated as L8. L8 transmitted black shank resistance in a Mendelian fashion to the F1 of crosses made with black shank susceptible varieties. However, resistance was not transmitted in a Mendelian fashion beyond the F1 generation. Nielsen (1992 b) stated that L8 possesses a single dominant gene, derived from *N. longiflora*, which causes very high resistance to race 0, but it is believed to be completely susceptible to other races. According to Smiley et al. (1966), Stokes and Litton (1966) and Nielsen (1992 b), L8 is not satisfactory for commercial use because it develops a physiological leaf spot. However, this trait is recessively inherited and poses no problem to F1 hybrids of this line with standard varieties which are highly resistant to black shank race 0. F1 hybrids with L8 as one of the parents are not resistant to race 1 of *P. parasitica var. nicotianae*. L8 hybrids represented 35% of the US Burley acreage in 1992. However, the frequent occurrence of *P. parasitica* races to which L8 is susceptible has diminished the effectiveness of this *N. longiflora* derived resistance.

Hendrix and Apple (1967) suggested that the expression of resistance from *N. longiflora* was not entirely root-specific and that plants with black shank resistance from *N. longiflora* would survive stem inoculations, whereas those with the Fla 301 source of resistance would die.

There is evidence that *N. longiflora* resistance is dominant and simply inherited, but apparently modifying genes are also involved, for expected genetic ratios are not obtained from crosses with susceptible genotypes (Lucas, 1975). In a study of Legg,

Litton and Collins (1982), which they conducted to study the effect of *N. longiflora* resistance on race 0 of *P. parasitica* var. *nicotianae* and the effect of this resistant gene on agronomic and chemical traits, they concluded that although *N. longiflora* provides complete field resistance to race 0, the effects of this resistance varied in different genetic backgrounds. Furthermore, the resistant selections had agronomical and chemical traits less desirable than those of the susceptible cultivars. These results indicated that each of the resistance genes had been transferred to Burley lines with a little additional alien chromosomal material. Valleau, Stokes and Johnson (1960) suggested that the abnormal transmission of *N. longiflora* resistance into *N. tabacum* lines was due to the substitution of a pair of *N. longiflora* chromosomes that do not pair normally with *N. tabacum*.

However, Legg, Litton and Collins (1982) felt that the observed effects of race 0 resistance on agronomic and chemical traits should not prevent the development of acceptable cultivars with the *N. longiflora* resistant allele, if germplasm sources and selection procedures are carefully chosen. The breeder should firstly start with a group of cultivars or lines. Secondly, during the selection phases, major emphasis should be placed on the resistant lines that show the smallest changes in agronomic and chemical traits when compared with standard checks. These procedures should give the breeder a good chance of identifying and using germplasm in which the association of disease resistance and undesirable changes in other traits is absent or minimal.

Nicotiana plumbaginifolia

N. plumbaginifolia was originally used by Apple (1962) to describe the races nonpathogenic and pathogenic to it as races 0 and 1 respectively. Apple also reported that two types of resistance were recovered from an *N. tabacum* x *N. plumbaginifolia* hybrid breeding programme. One type was highly resistant to race 0, but susceptible to race 1, as was *N. plumbaginifolia*, the second was resistant to both race 0 and race 1, but resistance to race 0 was of a lower magnitude. This species-fungal race reaction was further complicated when five accessions of *N. longiflora* were found to be highly resistant to both races 0 and 1, while *N. plumbaginifolia* behaved as previously

reported, i.e. resistant to race 0, susceptible to race 1 (Litton, Collins & Legg, 1970 b). It was suggested by Wernsman, Matzinger and Powell (1974), that the inconsistencies between previously published results and the findings above may have been due to the use of different accessions of *N. longiflora*. However, *N. plumbaginifolia* and most varieties of *N. longiflora* are very similar morphologically and it is possible that identification errors have been made in some investigations.

Chaplin (1962) reported that *N. plumbaginifolia,* which is believed to be closely related to *N. longiflora*, was used by him as a source of black shank resistance in 1951. He transferred the black shank resistance factor in *N. plumbaginifolia* to *N. tabacum* and concluded that this type of resistance was controlled by a partially dominant single factor and that genetic modifiers might have been present in susceptible parent material with which *N. plumbaginifolia* was crossed. However, later efforts by others characterised this resistance as a single dominant gene (Nielsen, 1992 b).

Like Valleau, Stokes and Johnson (1960), who struggled to produce homozygous lines using black shank resistance from *N. longiflora*, Cameron (1958) was unable to obtain stable black shank resistant lines carrying the resistance factor from *N. plumbaginifolia*. Goins and Apple (1971) also observed a trend towards a lower recovery of homozygous resistant genotypes in families segregating for the resistance factor from *N. plumbaginifolia*.

Unfortunately, in flue-cured varieties, the association of lowered quality with black shank resistance from *N. plumbaginifolia* has been a concern to breeders (Nelson, 1973) and because of pathogenic specialization within *P. parasitica* the *N. plumbaginifolia* resistance has not proved to be completely effective in controlling black shank induced losses (Nielsen, 1992 b).

2.11.2 Florida 301

According to Lucas (1975) and Nielsen (1992 b), Florida 301 was the original line used in hybridizations in order to generate black shank resistant commercial tobacco

cultivars. This *N. tabacum* resistance to black shank has since been called Fla 301 resistance. From the 1940's till about 1964, resistance from this line was used in virtually all of the developed cultivars which were described as possessing black shank resistance. Currently, the improved cultivars from this type of resistance are preferred to the original source cultivar in breeding programmes. One of the reasons why Fla 301 became much more popular as a source of black shank resistance than *N. longiflora* and *N. plumbaginifolia* was that tests with L8 (*N. longiflora* type of resistance), its F1 hybrids and seedlings of *N. plumbaginifolia* revealed that certain strains of *P. parasitica var. nicotianae* killed this type of material, whereas Burley 11A with its Fla 301 type of resistance survived (Silber & Heggestad, 1963).

In 1992, all cultivars which had resistance to both race 0 and race 1 had been derived from Florida 301 as the source of resistance (Legg and Nielsen, 1992). Although Lucas (1975) said that Fla 301 resistance was sufficiently effective against all races of the black shank pathogen known in 1975, this source has since provided only moderate levels of resistance and breeders have continued to search for other sources.

Fla 301 type of resistance is genetically complex. The inheritance of the Fla 301 type of resistance was reported to be polygenic (Smith & Clayton,1948; Stevenson & Jones, 1953). Later Clayton (1958), referring to a major gene that provides resistance, described the inheritance as simple and recessive. Moore and Powell (1959) and Tedford and Nielsen (1990) hypothesised that Fla 301 resistance is not simply inherited, although it may be inherited as a single factor which may be partially dominant and which is expressed to varying degrees because of the presence of modifier genes in susceptible parents. As a result, cultivars with low to high resistance have been bred. Apple (1962) stated that this source of resistance was apparently controlled by a major recessive gene that must be enhanced by several modifiers to obtain a usable level of resistance. Chaplin (1966) mentioned that varieties of flue-cured tobacco with the Fla 301 type of resistance had been developed and that these varieties had varying degrees of resistance, classified as low, moderate and high. These degrees of resistance suggest that resistance is inherited quantitatively. For

example, the basic resistance in Coker-187 was derived from Florida 301. However, Coker-187 exhibited more resistance than Florida 301. The increased resistance is probably due to modifying factors being added from the susceptible varieties used in the development of Coker-187. According to Wills (1971) and Lucas (1975), this type of resistance was generally thought to be polygenic. Nielsen, (1992 b) stated that Fla 301 resistance was reportedly polygenic, that it might be recessive, and that it might be modified by genes from a susceptible parent.
From the above it is quite clear that inheritance of resistance from Florida 301 has been variously interpreted, but most investigators agree that more than a single dominant gene is involved.

Although inheritance of the Fla 301 type resistance has provided economic control of the disease, root damage could lead to great losses of plants. This loss of plants due to root damage can be explained by a study of Hendrix and Apple (1967). When stems of plants with Fla 301 type resistance were inoculated with races 0 and 1 of *P. parasitica* var. *nicotianae*, they wilted and died, showing susceptibility to races 0 and 1. Thus, when root damage occur the fungus may reach the base of the stem of a young plant, the entire vascular cylinder may be invaded which results in wilting and death of the plant (Hendrix & Apple 1967). It is a known fact that the aboveground organs of resistant cultivars are more susceptible than the roots (Lucas, 1975), hence the death of the Fla 301 type of resistant plants when their stems were inoculated.

According to Valleau, Stokes and Johnson (1960), the Fla 301 type of resistance gives satisfactory resistance in relatively short rotations and in moist seasons, but in dry periods the disease may cause appreciable damage. Heavy losses can, therefore, sometimes occur with the Fla 301 type of resistance.

2.11.3 Beinhart 1000-1

Nielsen (1995 b) considered Beinhart to be consistently one of the most resistant genotypes tested at all the locations worldwide in the CORESTA Black Shank Collaborative Study. Heggestad and Lautz (1957) concluded that Beinhart 1000-1

(B1000-1) had a high level of resistance to both race 0 and 1 of black shank and Abdul Wajid, Shenoi and Moses (1986) stated that B 1000-1 was resistant to races 0, 1 and 2 of *P. parasitica* var. *nicotianae*.

According to Nielsen (1995 b), previous genetic studies of the black shank resistance in B1000-1 have been somewhat inconclusive. Some researchers have reported that this type of resistance may be controlled by only a few genes, but others have reported a more complex inheritance of resistance. Silber and Heggestad (1963) reported that black shank resistance from B1000-1 appears to be a quantitatively inherited character, which is partially dominant and in this characteristic is similar to that obtained from Fla 301. B1000-1 resistance may be modified by genes from some susceptible parental sources. Nielsen (1992 b) stated that B1000-1 resistance appears to be partially dominant and that it may be oligogenic.

According to Tedford and Nielsen (1990) and Legg and Nielsen (1992), the difficulty in using the B1000-1 source of resistance is caused by a close association between undesirable agronomic traits and resistance. This association seems to stem from tight chromosomal linkages or pleiotropic effects between cigar tobacco characteristics and black shank resistance. However, Telford and Nielsen (1990) found increased black shank resistance through three cycles of selection and further experiments by Legg and Nielsen (1992) showed that the increased resistance occurred without changes in means or genetic variation for the six agronomic traits they studied. Thus, any associations between black shank resistance and these agronomic traits can be broken by recombination during the development and advancement of the synthetic lines.

2.12 VALUABLE DATA USED IN THIS STUDY FROM THE CORESTA BLACK SHANK COLLABORATIVE STUDY

Nielsen (1992 a) reported on the CORESTA Black Shank Collaborative Study which was initiated as a result of discussions within the Phytopathology Group at the CORESTA Congress held at Kallithea, Greece in 1990. At that time the main goals established for the Black Shank Collaborative Study were the characterization of the extent of *P. parasitica* var. *nicotianae* variability, using genotypes with known levels of resistance, and the identification of useful sources of resistance to black shank. It was decided that the best means to accomplish these goals would be to solicit the cooperation of those CORESTA members and nonmembers who were stationed in production areas in which black shank was a recognised problem. The group decided to include some cultivars from other participating countries in their own trials. Cooperators were asked to plant a minimum of 20 plants per plot with three replications at each test site. The number of plants in each plot, as determined two weeks after transplanting, was used as the initial stand count. Counts of living, non-diseased plants in each plot were recorded at intervals throughout the growing season. Results were reported as the number or percentage of living plants at the end of the growing season.

The CORESTA study group collected valuable data which are referred to in numerous sections of this study. The sources of resistance to black shank, which were dealt with by the CORESTA group and which were used or are referred to in this study, are *N. longiflora*, Burley 37, B1000-1 and Fla 301. These sources are summarised in Table 2.1.

Cultivars which were evaluated in the CORESTA Black Shank Collaborative Study and which are mentioned in this study are L8, Burley 37, Beinhart 1000-1, Speight G28, TL33, CDL28, A23 and Domkrag. A summary of these cultivars is given in Table 2.2.

Table 2.1 Sources of resistance to black shank used by the CORESTA Black Shank Collaborative Study Group and referred to in this study

Source/Cultivar	Tobacco Type	Type of resistance	Level of resistance *
L8	Burley	*N.longiflora*	Resistant to race 0 Susceptible to race 1
Burley 37	Burley	---	Medium resistance
B1000-1	Cigar	*N.tabacum*	Very high resistance
Fla 301	Cigar	*N.tabacum*	---

* Level of resistance designated by previous field studies

Note: This table was compiled using data from Nielsen (1992 a)

Table 2.2 Cultivars used in the CORESTA Black Shank Collaborative Study and their survival percentage

Cultivar	Survival percentage (%) *	
KY X L8	87.7%	(1991-average of all CORESTA countries)
Burley 37	80.0%	(1991-average of all CORESTA countries)
Beinhart 1000-1	96.0%	(1991-average of all CORESTA countries)
Speight G28	99.0%	(1992 - Zimbabwe)
Speight G28	100.0%	(1995 - Greece)
TL33	98.7%	(1993 - Zimbabwe)
TL33	84.0%	(1994 - South Africa)
CDL28	0.0%	(1994 - South Africa)
A23	0.0%	(1994 - South Africa)
Domkrag	91.0%	(1994 and 1995 - South Africa)

* The year and country in which the data were collected are given in brackets

Note: This table was compiled using data from Nielsen (1992 a), Nielsen (1993), Nielsen (1995 a) and Nielsen (1995 b)

2.13 BACKGROUND OF THE CULTIVARS USED IN THIS DIALLEL STUDY

2.13.1 CDL28

During 1975, CDL28 was released as a dark air-cured tobacco cultivar by the Department of Agriculture - Institute for Tobacco and Cotton near Rustenburg. Currently this institute is known as the Agricultural Research Council - Institute for Industrial Crops (ARC-IIC).

According to Boshoff *et al.* (1992) CDL28 has resistance to powdery mildew (*Erysiphe chichoracearum*), tobacco mosaic virus (TMV) and shows tolerance against brown spot (*Alternaria alternata*). CDL28 was the first cultivar with such a wide spectrum of disease resistance to be commercially grown in South Africa. If CDL28 is planted on fertile soil with the correct amounts of fertilization and with correct spacing, it produces dark air-cured tobacco of high quality. Although it has been released as a dark air-cured tobacco cultivar light air-cured tobacco is unfortunately sometimes obtained when specific cultivation practices are used.

Table 2.3 Black shank resistance counts of CORESTA cultivars at the ARC-IIC for the seasons 1998/1999, 1999/2000 and 2000/2001

	1998/1999			1999/2000			2000/2001		
Cultivar	**a**	**b**	**c**	**a**	**b**	**c**	**a**	**b**	**c**
CDL28	-	-	-	38	0	0	-	-	-
A23	-	-	-	54	1	2	-	-	0
Domkrag	80	75	94	69	61	88	-	-	80
Burley 37	78	72	92	79	70	89	-	-	90
Beinhart 1000-1	78	78	100	76	70	92	-	-	90

a Stand Count
b Number of Healthy Plants
c % Healthy

Note: This table was compiled using data from Prinsloo *et al.* (1999), Prinsloo *et al.* (2000) and Prinsloo et al. (2001)

The main disadvantage of CDL28 is its total susceptibility to black shank. During the ARC-IIC's CORESTA trials of 1999/2000 (Table 2.3), stand counts were taken twelve weeks after the trial was planted on a black shank infested field. None of the original 38 CDL28 plants survived. The CORESTA data in Table 2.2 show that CDL28 had a 0% survival rate in South Africa in 1994. According to the ARC-IIC's resistance expression in Table 2.4, these nil survival percentages show the total susceptibility of CDL28.

Table 2.4 The scales for black shank resistance expression used by Todd and the ARC-IIC

	Ratio[a]	% Healthy Plants	Resistance Expression
Todd	0	100	high
	0.01 - 0.96	51 - 99	medium
	1 - 6	14.3 - 50	low
	> 6	0 - 14.3	susceptible
ARC-IIC	0 - 0.25	80 - 100	high
	0.27 - 0.49	67 - 79	medium to high
	0.52 - 1.94	34 - 66	medium
	2.03 - 4.00	20 - 33	low
	> 4.3	0 - 19	susceptible

a Ratio = Number of diseased plants / Number of healthy plants

Note: This table was compiled using data from Todd (1981) and Prinsloo *et al.* (1996)

2.13.2 Domkrag

According to Breet (Personal communication, 2001) this narrow-leaved air-cured cultivar, which was registered during the late 1980's, was developed by the ARC-IIC. The cultivar never became very popular and was removed from the registration list in 1999. Domkrag (DK) was developed from a cross between the narrow-leaved breeding line, C11, and the black shank resistant breeding line, WOSB. WOSB inherited its black shank resistance from one of its parents, TL33. As recorded by Boshoff et al. (1992), TL33 was released in 1975 by the ARC-IIC as a flue-cured tobacco cultivar for

commercial cultivation, especially in areas which were badly stricken by black shank. TL33 was derived from the cross between black shank resistant Speight G-28 and susceptible A23. According to the CORESTA data in Table 2.2, A23 had a survival rate of 0.0% in South Africa in 1994 and in the CORESTA trials of the ARC-IIC (Table 2.3), it had a survival rate of 2% in the 1999/2000 season. According to Lamprecht and Prinsloo (1977), A23 is resistant to race 2 but susceptible to race 1 of the black shank fungus. However, both races of the fungus were present in the ARC-IIC's fields where their CORESTA trials were conducted and A23 was, therefore, highly affected by race 1 and turned out to have a very low survival percentage as can be seen in Tables 2.2 and 2.3. Numerous opinions exist about the black shank resistance of Speight G-28. Abdul Wajid *et al.* (1986) typified Speight G-28 as resistant to race 2 only and Jones and Shew (1995) referred to Speight G-28 as being moderately black shank resistant. However, the data in Table 2.3 show that DK had a survival rate of 94, 88 and 80% in the consecutive seasons of 1998/1999, 1999/2000 and 2000/2001. This clearly indicated that DK, with its Speight G28 type of resistance, showed a high level of resistance at the ARC-IIC's black shank infested trial site. According to the data of the CORESTA trials in Table 2.2, Speight G -28 had a survival rate of 99.0 % in Zimbabwe in 1992 and 100.00 % in Greece in 1995 while DK had a survival rate of 91.0 % in South Africa in 1994 and 1995. These high survival percentages, reflected in Tables 2.2 and 2.3, will put Speight G-28 and DK in the high resistance category according to the ARC-IIC's resistance expression in Table 2.4. According to an unknown source, the resistance of Speight G-28 has been described as the Fla 301 type of resistance and has been recorded as dominant and oligogenic.

TL33 is resistant to races 1 and 2 of *P. parasitica* var. *nicotianae* (Lamprecht *et al.*, 1975; Boshoff *et al.*, 1992) and is also resistant to TMV and powdery mildew.

2.13.3 Burley 37

According to Skoog, Neas and Heggestad (1961), the University of Tennessee Agricultural Experiment Station and the United States Department of Agriculture jointly released this cultivar in 1960 as a Burley tobacco. It has moderate resistance to black

root rot (*Thielaviopsis basicola*) and fusarium wilt (*Fusarium oxysporum* var. *nicotiana*) and was the first commercially available cultivar with combined resistance to wildfire (*Pseudomonas syringae* pv. *tabaci*) and black shank. It had improved characteristics over the then only other commercially available black shank resistant Burley cultivars, Burley 11-A and Burley 11-B. Burley 37 (B37) is more resistant to black shank than either Burley 11-A or Burley 11-B.

According to Hendrix and Apple (1967) and Tedford and Nielsen (1990), the original source of black shank resistance used in the development of Burley 11A and Burley 11B was the cultivar, Florida 301. Skoog, Neas and Heggestad (1961) reported that B37 was developed from a cross between progenitors of the two well-known cultivars of the fifties and sixties, black shank susceptible Burley 21 and black shank resistant Burley 11-A. Although the wild species, *N. longiflora*, which is highly resistant to black shank, was used in Burley 21's parentage, this cultivar is susceptible to black shank. Continuous selection for black shank resistance in Burley 11-A was performed in a trial that was conducted annually for several years. Seed was harvested only from those plants with high resistance to black shank. Even with this selection, B37 still had higher resistance than the selected material. B37, the end result of a cross between black shank susceptible Greeneville 25 and black shank resistant Greeneville 42, is even more resistant than Greeneville 42, Burley 11-A and the selected lines from Burley 11-A. Skoog, Neas and Heggestad (1961) suggested that this phenomenon could be attributed to the slight possibility that the original parent plant of Greeneville 42 that was used for the cross was more highly resistant than other plants in the breeding line. But, according to them, a more probable theory was that the susceptible parent, Greeneville 25, contributed inheritable factors to the cross which made Burley more resistant than its resistant parent, Greeneville 42.

Although the CORESTA Black Shank Collaborative Study Group classified B37 as having medium resistance (Table 2.1), the data from the CORESTA trials in Table 2.2 and the data from the ARC-IIC's CORESTA trials in Table 2.3 show B37 to be highly resistant according to the ARC-IIC's resistance expression in Table 2.4.

Skoog, Neas and Heggestad (1961) reported that, because B37 has resistance to and not immunity to black shank, it is recommended that it be grown in rotation with other crops. Crop rotation will aid in preventing the build-up of disease organisms and new strains of the pathogens should be less likely to occur.

2.13.4 Beinhart 1000-1

According to Silber and Heggestad (1963), E. G. Beinhart sent seed of a selection of the tobacco variety Quin Diaz, which was grown locally in the Dominican Republic, to Tobacco Investigations, Maryland, USA in 1955. This strain was then called Beinhart 1000 and was shown to be highly resistant to black shank. The black shank resistance of Beinhart 1000 was much better than that of other tobacco cultivars.

Nielsen (1992 a) referred to Beinhart 1000-1 (B1000-1) as being one of the most, if not the most, black shank resistant varieties/variety known. Unfortunately, efforts to transfer black shank resistance from Beinhart, which is a cigar tobacco, to other tobacco types have been complicated by apparent linkages or pleiotropy between genes conferring black shank resistance and those affecting cigar tobacco properties.

According to the CORESTA data in Table 2.1, B1000-1 is considered to have very high resistance to black shank. B1000-1 showed an average survival rate of 96.0 % in all the CORESTA countries in 1991 (Table 2.2). The ARC-IIC's CORESTA trial data in Table 2.3 show that B1000-1 has a higher resistance than the other two resistant tobacco cultivars, B37 and DK, used in these trials. It is considered to be highly black shank resistant according to the ARC-IIC's resistance expression in Table 2.4.

This literature study revealed that the information, with regards to the different sources of black shank resistance, is not always coherent and that different authors had different views on, i.e. the genetic basis of the available sources of resistance. A relevant question for the South African situation is whether the different sources of black shank resistance will deliver the same results in crosses with CDL28, the popular black shank susceptible cultivar, and if not, which of the different sources of black

shank will be the best to use in a breeding programme to incorporate black shank resistance in CDL28. This dialel study was done to clarify which cultivar/source of black shank resistance should be used in future to incorporate black shank resistance in CDL28.

CHAPTER 3

MATERIAL AND METHODS

3.1 GENETIC MATERIAL

Since the aim of this diallel study was to find the most suitable black shank resistant source to be incorporated into susceptible CDL28, three resistant tobacco cultivars were selected from the available tobacco germplasm at the ARC-IIC, to be combined with CDL28. All four cultivars were pure breeding lines and the resistant cultivars differed from each other regarding their source of black shank resistance. As susceptible parent, the most popular South African dark air-cured cultivar, CDL28, was chosen.

Domkrag (DK), Beinhart 1000-1 (B1000-1) and Burley 37 (B37) were chosen as black shank resistant cultivars. DK and B1000-1 have respectively the Florida 301 (Fla 301) and B1000-1 sources of black shank resistance and B37 possess the Fla 301 source of resistance with the possibility of *N. longiflora* as an additional source of resistance. These cultivars and the different sources of resistance were discussed in detail in Chapter 2.

3.2 EXPERIMENTAL PROCEDURE

Diallel trials according to Griffing method 1 crossing system

According to the method 1 diallel crossing system of Griffing (1956) these four cultivars were crossed in all possible combinations (all p^2 combinations) resulting in 16 entries. These 16 entries included all four parents, one set of F1's and the reciprocal F1's (Table 1 of Appendix1). To monitor the F1 diallel's susceptibility or resistance to/against black shank, the trial was planted in a greenhouse and repeated on a heavily black shank infested field.

Although the Griffing method 1 diallel procedure does not include F2 generations, it was decided to self the twelve F1 crosses, to obtain a segregating F2 generation which could be compared with the mean values of the sixteen entries of the F1 trials. The entry names and numbers were the same as those used in the F1 field and F1 greenhouse trials and are given in Table 1 of Appendix 1. The F2 trial was planted in a greenhouse and was not repeated on the black shank infested field.

3.2.1. F1 field trial

Trial site

The trial site at the ARC-IIC (Agricultural Research Council - Institute for Industrial Crops) where the F1 field trial was planted, is an ideal, highly infested, natural environment in which to screen for black shank resistance or susceptibility.

The predominant race on this field is race 1 though other races of *P. parasitica* might also be present (Prinsloo, 1998. Personal communication). The 1996 and 1997 data of the CORESTA Black Shank Collaborative Study (Nielsen, 1996; Nielsen, 1997), correlates with this statement of Prinsloo, revealing that a mixed population of races are present at the ARC-IIC's black shank infested field.

Trial design

The trial was planted as a randomised complete block design with three replications. The trial layout is given in Table 2 of Appendix 1. Each plot consisted of 20 plants, spaced in two rows. The rows were spaced 1.3 m apart. Each row contained 10 plants spaced 0.5 m from each other.

The cultivars were sown in separate small containers and were later transplanted into seed trays (Fig. 1 of Appendix 2). When these seedlings were strong enough they were transplanted to the black shank infested field described above. Normal cultivation practices (Blignaut, 1993) were further followed throughout the duration of the trial.

Data collection

Initial stand counts of the healthy plants were taken 7 days after transplanting,

thereafter stand counts was taken at 44, 66, 72 and 99 days after transplanting. At day 99, CDL28, which served as the negative control cultivar had died away totally and no further stand counts were, therefore, taken. The stand count of the healthy plants on the individual plots are given in Table 3 of Appendix 1.

Plants were regarded as diseased when they were dead, severely wilted (Fig 2 of Appendix 2) or developed black lesions at the base of the stem. Such lesions were usually also associated with yellowing of the leaves (Fig. 3 of Appendix 2).

3.2.2 F1 and F2 greenhouse trials

Trial design

The same sixteen F1 entries (Table 1 of Appendix 1), which were planted on the field, were planted as a randomised complete block design with three replications (Table 4 of Appendix 1) in a greenhouse. In the following season, the F2 crosses were planted together with the four parents (Table 5 of Appendix 1) in the same way as the F1 greenhouse trial, as a randomised complete block design with three replications, in the same greenhouse than the F1 greenhouse trial. Each plot consisted of 20 plants, spaced in two rows of 10 plants each.

The seed was sown in separate containers. When the seedlings became strong enough to be transplanted they were directly planted, one plant per pot, into small 10 cm pots. These pots were placed directly next to each other. The plants were, therefore, spaced approximately 10 cm from each other. The lay out of one of the replications of these greenhouse trials can be seen in Fig. 4 of Appendix 2.

Greenhouse temperature ranged from 24-30°C which is the optimum temperature for *Phytophthora parasitica* (*P. parasitica*) growth.

Inoculation of tobacco plants

Preparation of P. parasitica var. nicotianae inoculum: The preparation of inoculum was carried out under sterile conditions. A mixed population of the pathogen was isolated on cornmeal agar from a diseased plant which grew on the highly infested F1 trial site. Virulent colonies of the isolated organism were grown for 2 weeks at 26°C on V8-juice

(Campbell Soup Company, Camden, NJ, USA) agar in petri dishes with nystatin at a pH of 5.9 (Fig.5 of Appendix 2). The nystatin is used in the preparation of these pure cultures because a yeast normally isolated together with *Phytophthora* causes it not to form sporangia. Nystatin makes this yeast latent and therefore sporangia can form freely.

Since the number of sporangia is directly proportional to the severity of infection, care must be taken that the cultures have developed a large amount of sporangia before using them as inoculum. When the amount of sporangia was not sufficient, incubation continued at 26°C until enough sporangia developed.
Sterilised tap water was added to the agar medium containing the fungal mycelium in a 1:1 proportion, and mixed in a Waring blender to form a homogenous inoculum. (Prinsloo, 2001. Personal communication)

Inoculation technique: Ten weeks after transplanting, the tobacco plants were root inoculated with the pathogen. Root damage was inflicted by cleaving the soil with a scalpel, wounding the roots approximately 1 cm from the stem (Fig. 6 of Appendix 2), after which 2 ml of the inoculum was injected into the opening (Fig. 7 of Appendix 2). The opening was then closed up afterwards by pressing the soil together.

Data collection

Initial stand counts of the number of healthy plants were taken on the days when the plants of the two trials were inoculated. The first black shank symptoms became visible about 7 days after inoculation. Stand counts of the number of healthy plants in the F1 greenhouse trial were taken at 7, 10 and 14 days (Table 6 of Appendix 1) and in the F2 greenhouse trial at 9 and 21 days (Table 7 of Appendix 1) after inoculation. No further stand counts were taken after the date when CDL28, the very susceptible negative control cultivar, had died away totally.

Plants were regarded as diseased when they were dead, severely wilted, or developed black lesions at the bottom of the stem which was usually also associated with

yellowing of the leaves (Fig. 8 of Appendix 2). After these symptoms were experienced, the plants died away shortly thereafter.

3.3 STATISTICAL ANALYSIS

The raw data of the stand counts of healthy plants per plot, obtained in the three trials as described in sections 3.2.1.1 and 3.2.1.2, were converted to percentage plants surviving at the specific days of data collection. The data of the percentage plants surviving was used for the further analyses, since the initial stand counts differed from plot to plot in certain instances and this difference in stand counts are taken into account with raw data counts being expressed as percentages. The percentage data of the stand counts per plot of the F1 field, F1 greenhouse and F2 greenhouse trials can respectively be viewed in Tables 8, 9 and 10 of Appendix 1.

The mean percentages of surviving plants in the two F1 trials and the F2 trial were calculated from Tables 8, 9 and 10 of Appendix 1 and were plotted as percentage plants survived in Figures 9, 10 and 11 of Appendix 2.

The data obtained at the last day of data collection, i.e., when CDL28 had 0% healthy plants, were used for the statistical diallel analysis in an attempt to use data obtained when the disease incidence was basically the same for all three trials.

3.3.1 ANOVA

By using the statistical programme, ANALYSIS OF DIALLEL CROSSING EXPERIMENTS, written in 1996 by Dr H van Ark for the ARC-Biometry unit, an ANOVA for a randomized block design was done on the mean percentage data on the last day of data collection for all three trials as calculated from Tables 8, 9 and 10 of Appendix 1. These ANOVA data are respectively given in Tables 11, 12 and 13 of Appendix 1.

To test for the homogeneity of the genotype variances the Bartlett chi-squared test was done on a 5% significance level.

The Anderson-Darling, Cramer-von Mises and Watson tests were done at a 5% test level in order to test for the normality of the plot residuals.

3.3.2 Genotype mean values

The genotype means of the three replications of the percentage surviving plants for the three trials on the last day of data collection were calculated from the data in Tables 8, 9 and 10 of Appendix 1 and are respectively given in Tables 4.1, 4.2 and 4.3, the p x p diallel tables.

To identify significant differences among the 16 entries of each trial, the "Bonferroni multiple comparison test" in the statistical programme, COMPMEAN, was used. COMPMEAN was developed for the use of the ARC-Biometry unit by Dr Van Ark. The test was done at a 5% test level. Significant differences between the entries are indicated with small letters in the tables of genotype mean values of the percentage surviving plants (Tables 4.1, 4.2 and 4.3). Mean values differing significantly from other mean values do not share the same small letters.

3.3.3 Diallel analysis

The diallel analysis in this study was done by using the Griffing Model 1, Method 1 (Griffing, 1956) procedure, of the statistical programme, ANALYSIS OF DIALLEL CROSSING EXPERIMENTS. The diallel was done to test for parents, crosses and reciprocal effects.

Combining abilities

In order to see if significant general combining ability (GCA), specific combining ability (SCA) and reciprocal differences were obtained, an ANOVA for combining ability was done for each of the three trials. These ANOVA results can be seen in Tables 4.4, 4.5 and 4.6. If these ANOVA results showed non significant differences for either the GCA, SCA or reciprocal effects further study of these abilities or effects were unnecessary.

Significancy of the GCA effects

The Student-Newman-Keuls multiple range test was used to test for significant differences between the GCA effects of the three trials. These data are given in Table 4.7.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 ANOVA

The ANOVA results of the three trials in Tables 11, 12 and 13 of Appendix 1 show F-probability values for genotypes < 0.001 which indicate that there were highly significant differences between the entries (genotypes) regarding the mean number of plants that survived in all three trials. However, in all three these trials the Bartlett chi-squared test revealed that the error variances were heterogeneous and the Anderson Darling, Cramer von Mises and Watson tests revealed non normality of the plot residuals. These could be ascribed to the concentration of very high values of B1000 (up to 100%) and the concentration of very low values (0%) of the negative control cultivar, CDL28, with the majority of genotypes having intermediate values (Tables 8, 9 and 10 of Appendix 1). Thus, this non normality of plot residuals and heterogeneous error variances were taken into account and balanced by studying the coherence of the results of the three trials and by being more careful in drawing conclusions.

4.2 GENOTYPE MEAN VALUES

In the F1 field trial (Table 4.1) no significant differences were obtained between the resistant parents and the crosses between the resistant parents. All of these genotypes showed a reasonably high degree of black shank resistance. With the single exception of the Burley 37 (B37) X CDL28 cross, no significant differences were found in the crosses between the resistant parents and the negative control cultivar, CDL28. However, this exception is not repeated in the reciprocal and could, thus, probably be a chance effect. When studying the row and column means it seems as if Domkrag

(DK) tends to have a lower mean value than the other two resistant parents, Beinhart 1000-1 (B1000-1) and B37.

Table 4.1 The diallel table of the genotype mean values of percentage survival at the last day of data collection of the F1 field trial.

		Female Lines								Row Means
	Genotype	B37		DK		B1000-1		CDL28		
Male Lines	B37	95.00	e	90.00	de	95.00	e	72.16	cde	88.04
	DK	93.33	e	73.33	cde	88.16	de	35.00	ab	72.46
	B1000-1	96.67	e	94.82	e	93.33	e	63.33	bcde	87.04
	CDL28	43.95	bc	28.33	ab	55.00	bcd	0.00	a	31.82
Column Means		82.24		71.62		82.87		42.62		

Bonferroni LSD (p = 0.05) = 36.02 CV % = 18.4

Values followed by the same letters do not differ according to the Bonferroni multiple comparison test.

Table 4.2 The diallel table of the genotype mean values of percentage survival at the last day of data collection of the F1 greenhouse trial

		Female Lines								Row Means
	Genotype	B37		DK		B1000-1		CDL28		
Male Lines	B37	93.33	e	71.67	cde	98.33	e	40.00	abcd	75.83
	DK	71.67	cde	55.00	bcde	76.67	de	26.67	ab	57.50
	B1000-1	78.33	de	91.67	e	96.67	e	75.00	de	85.42
	CDL28	28.33	abc	28.33	abc	66.67	bcde	0.00	a	30.83
Column Means		67.92		61.67		84.59		35.42		

Bonferroni LSD (p = 0.05) = 43.90 CV % = 25.1

Values followed by the same letters do not differ according to the Bonferroni multiple comparison test.

The F1 greenhouse trial (Table 4.2) basically shows the same pattern as the F1 field trial with the exception that both DK and B37 have lower average values in the row and column means than the remaining resistant parent, B1000-1. This can possibly be explained by the fact that both DK and B37 possess the Florida 301 (Fla 301) type of

resistance which is known to have no stem resistance (Hendrix & Apple, 1967) and when cuts were made into the bigger roots during the inoculation procedure the pathogen possibly moved into the stem, resulting in the death of plants which would have had root resistance if the roots were not manually damaged. Thus, the severity of the specific inoculation method used in the greenhouse trial probably circumvented the Fla 301 type of root resistance resulting in the lower than expected survival values of DK and B37.

The general lack of significant differences between the crosses of the two F1 trials (Tables 4.1 and 4.2) is due to a lack of sensitivity of the current analysis and indicates the necessity of a Griffing diallel analysis which tests for differences between averages, i.e. general combining ability (GCA) effects.

Table 4.3 The diallel table of the genotype mean values of percentage survival at the last day of data collection of the F2 greenhouse trial

		Female Lines								Row Means
	Genotype	**B37**		**DK**		**B1000-1**		**CDL28**		
Male Lines	**B37**	21.70	abc	45.00	cd	46.70	cd	5.00	ab	29.60
	DK	25.00	abc	5.00	ab	20.20	abc	5.20	ab	13.85
	B1000-1	65.00	d	36.70	bcd	68.00	d	15.00	abc	46.18
	CDL28	1.70	a	25.00	abc	25.00	abc	0.00	a	12.93
Column Means		28.35		27.93		39.98		6.30		

Bonferroni LSD (p = 0.05) = 34.78 CV % = 48.4

Values followed by the same letters do not differ according to the Bonferroni multiple comparison test

When studying the F2 greenhouse trial (Table 4.3), three sets of patterns are observed namely, DK and B37 and the crosses between them, secondly B1000-1 and its crosses and thirdly the crosses between the resistant parents and CDL28. There are almost no significant differences within these three sets of crosses with the few exceptions not repeated in the reciprocals and which could, therefore, be explained as chance effects. Thus, as was observed in the F1 trials, a general lack of significant differences is also present in the F2 greenhouse trial accentuating the insufficiency of the current analysis

and the need for a Griffing diallel analysis. Generally it seems as if resistance has largely been lost in most crosses. Only B1000-1 and some of its crosses show resistance, although the parental resistance is much lower than in the F1 tests. Part of the general drop in resistance of the crosses could be ascribed to segregation of the F2 generation, but since the homogenous resistant parent cultivars, DK and B37, did not differ significantly from the negative control cultivar, CDL28, segregation can not be the only cause of the lower than expected resistance. The most appropriate explanation for the very low resistance seems that the infection in the F2 greenhouse trial was so severe that the resistance of B37, DK and their crosses was largely destroyed.

4.3 COMBINING ABILITIES

A significant general combining ability (GCA) indicates real differences between the additive effects of the parents. The probabilities of F-ratios for GCA of all three trials (Tables 4.4, 4.5 and 4.6) were < 0.0001 which indicate that highly significant GCA differences existed. The null hypothesis could, therefore, be rejected and we could assume that there were indeed highly significant differences between the additive effects of the four parent cultivars used in this study.

A significant specific combining ability (SCA) would indicate real differences between the SCA effects and, therefore, non additive genetic effects of the parents involved in the crosses. Although the SCA effects of the two F1 trials (Tables 4.4 and 4.5) were not significant, the F2 greenhouse trial (Table 4.6) showed significant SCA effects. These significant SCA effects in the F2 trial were quite unexpected since the F1 trials did not pick up any significancy and this can only be explained in two ways. Firstly, the F1 tests could have been not sensitive enough to pick up SCA effects which manifested themselves in the test of the F2 trial. However, the F2 trial had a very high coefficient of variation of 48.4% and should therefore be even less sensitive to pick up SCA effects than the F1 field and F1 greenhouse trials with coefficients of variation of 18.4%

and 25.1% respectively. The second explanation, which seems more likely, is that the severity of the specific inoculation method, in the F2 trial, did not allow some additive genetic differences to manifest themselves sufficiently, thus, indicating significant SCA effects. Some values of the F2 crosses did not differ significantly from the negative control. This type of effect mimics dominance and would manifest itself as SCA.

Table 4.4 ANOVA for combining ability of the F1 field trial

Source of variation	df	ss	ms	F Value	F pr
GCA	3	12250.734	4083.5781	74.352	<0.0001
SCA	6	485.859	80.9766	1.474	0.2205
Reciprocal Effects	6	483.999	80.6665	1.469	0.2225
Error	30	1647.674	54.9225		

Table 4.5 ANOVA for combining ability of the F1 greenhouse trial

Source of variation	df	ss	ms	F Value	F pr
GCA	3	11723.953	3907.9844	47.900	<0.0001
SCA	6	992.539	165.4232	2.028	0.0928
Reciprocal Effects	6	416.667	69.4444	0.851	0.5413
Error	30	2447.571	81.5857		

Table 4.6 ANOVA for combining ability of the F2 greenhouse trial

Source of variation	df	ss	ms	F Value	F pr
GCA	3	4665.629	1555.2096	31.227	<0.0001
SCA	6	1414.756	235.7926	4.734	0.0017
Reciprocal Effects	6	762.500	127.0833	2.552	0.0407
Error	30	1494.099	49.8033		

Since it was already mentioned in section 4.2 that segregation in the F2 trial seems less important than the effects of the severe inoculation method, one can conclude that the severity of the inoculation method played a major role in the apparent significant

SCA effects. Hence it is fair to conclude that, although the F2 trial showed significant SCA effects, no real heterotic effects were present in any of the three trials. Furthermore in the F2 trial (Table 4.6) the F value of 4.734 for SCA in comparison with the 31.227 for GCA indicates that GCA effects were by far the most important effect measured.

Significant reciprocal effects would be an indication of maternal effects. If significant reciprocal effects exist, it would be important to make future crosses in the correct direction, i.e. to use the correct parent as the female. The analysis of variance data (Tables 4.4, 4.5 and 4.6) very clearly indicate that the reciprocal effects were not of real significance in any of the three trials. Thus, it seems that maternal effects are not important regarding these three sources of black shank resistance and it will not matter in which direction crosses are made.

4.4 GCA EFFECTS

When studying the GCA effects (Table 4.7), it is clear that the values of B1000-1 differed significantly from the rest of the parent cultivars in the F1 and F2 greenhouse trials and that B1000-1, therefore, seems to be the most resistant parent cultivar.

Table 4.7 The general combining ability effects for the F1 field, F1 greenhouse and F2 greenhouse trial

Genotypes	General combining ability effects		
	F1 field trial	F1 greenhouse trial	F2 greenhouse trial
Beinhart 1000-1	15.1174 a	22.6042 a	17.1875 a
Burley 37	15.2993 a	9.4792 b	3.4375 bc
Domkrag	2.2007 b	-2.8125 c	-4.6875 cd
CDL28	-32.6174 c	-29.2708 d	-15.9375 d

LSD = 2.16

GCA effects within the same column followed by the same letter are not significantly different at P = 0.05.

The Student-Newman-Keuls multiple range test was used to test for significant differences.

However, in the F1 field trial B37 had a slightly higher GCA effect value than B1000-1 but these values did not differ significantly from each other. Thus, in the F1 field trial, the GCA effect values of B1000-1 and B37 were equal. In all three trials the GCA effect values of DK were the lowest of the three resistant parents. It is, thus, again clear that the Fla 301 type of resistance of B37 and DK is much lower in the greenhouse trials than in the F1 field trial. As already explained in section 4.2 this can be the result of the severe inoculation method used which circumvented the Fla 301 type of resistance. In all three trials (Table 4.7) the GCA effects of B1000-1 differed significantly from those of DK. B37's GCA effects differed significantly from DK in both F1 trials but not in the F2 trial. However, it must be kept in mind that the F2 generation possesses more variability and due to the severe infection method the significance test in the F2 trial could possibly be less sensitive. With the latter in mind and since B37's GCA effects are better than those of DK in the other two trials, it can be accepted that both B37 and B1000-1 GCA's are better than DK. DK had the lowest resistance of the three resistant cultivars in terms of GCA effects. Thus, when taking all trials into consideration, it can be concluded that B1000-1 and B37 are likely to be the most resistant cultivars, followed by DK.

The generally significant differences between the GCA effects of the parent cultivars B1000-1, B37 and DK can be ascribed to the fact that each of the resistant parents had different sources of resistance to black shank (Table 1 of Appendix 1). According to the literature, B37 contains the Fla 301 type of black shank resistance (Hendrix & Apple, 1967) with the added possibility of *N. longiflora* resistance (Skoog, Neas & Heggestad, 1961). B1000-1 contains the Beinhart 1000-1 source of resistance and DK got its black shank resistance from Speight G-28 which contains the Fla 301 type of resistance. From this diallel study it can now be concluded that the Beinhart 1000-1 type of resistance is generally the most black shank resistant source currently available. Since B37 had significantly better GCA effects than DK, which had only the Fla 301 type of resistance, this study confirms that B37 does indeed contain a black shank resistant source other than Fla 301. This source can possibly be *N. longiflora* as proposed by Skoog, Neas and Heggestad (1961).

CHAPTER 5

CONCLUSIONS

Heterogeneous error variances and non-normality of plot residuals were obtained in the data of this study. This non-normality of plot residuals and heterogeneous error variances were taken into account and balanced by studying the coherence of the results of the three trials and by being more careful in drawing conclusions. These heterogeneous error variances could be ascribed to the concentration of high survival percentages of some of the resistant types and the low survival percentages of CDL28. In more than one of the three replications of the last day of data collection of the two F1 trials B1000-1 yielded survival percentages of 100% and CDL28 had survival percentages of 0% in all three replications of all three trials. Such concentrations of very high and very low values contribute to heterogeneous error variances and non normality of plot residuals. It must, therefore, be kept in mind for future studies that stand counts should be taken at shorter intervals than what was done during this study, i.e. when CDL28 still has some surviving plants and not a survival rate of 0% in all repetitions. This adjustment to the method of data collection can result in more homogeneous error variances.

The significant GCA's found in all trials confirmed that additive effects were indeed present between the parent cultivars. Although the SCA effects of both F1 trials were non significant, the SCA effects of the F2 trial appeared to be significant. The significant SCA effects of the F2 trial could have been caused by the specific inoculation method used in the F2 trial. It was, therefore, concluded that SCA effects were not of real importance in any of the trials. The three trials revealed no reciprocal effects. Thus, maternal and heterotic effects can be ignored.

Although B1000-1 seemed to be the more resistant parent cultivar regarding GCA effects in the F1 greenhouse trial, it did not differ from B37 in the F1 field trial. B37 and

DK possess the Fla 301 type of resistance which can easily be broken down by root inoculation methods such as cutting into the larger roots. Thus, it was concluded that B37's resistance was circumvented in the greenhouse trials and that its resistance is equal to that of B1000-1. DK proved to have the lowest resistance to black shank of the three resistant parent cultivars.

It can be concluded that the B1000-1 source of resistance is better than that of DK with its Fla 301 source of resistance. B37 did better than DK in both F1 trials. B37 contains the Fla 301 source of resistance together with another source, which can possibly be *Nicotiana longiflora*. Thus, although B1000-1 can cause quality problems when used in air-cured tobacco crosses it, and B37, must be considered for inclusion in a backcross programme with CDL28. In such a programme, the B1000-1 and B37 resistance can be built into CDL28 while focussing on maintaining the CDL28 characteristics. It is hoped that, by using this breeding method, black shank resistance will be incorporated into CDL28. This can solve the problem of air-cured tobacco farmers who have been struggling to cultivate CDL28 in the presence of black shank since 1975.

CHAPTER 6

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APPENDIX 1

TABLES

Table 1 The entry numbers, parent cultivars and their crosses used in the F1 field, F1 greenhouse and the F2 greenhouse trials

Entry	Parent Cultivars/Crosses	Source of resistance to black shank
1	B37 (Burley 37)	Fla 301 and possibly *N. longiflora*
2	B37 X CDL28	
3	B37 X B1000-1	
4	B37 X DK	
5	DK (Domkrag)	Fla 301
6	DK X CDL28	
7	DK X B1000-1	
8	DK X B37	
9	B1000-1 (Beinhart 1000-1)	B1000-1
10	B1000-1 X CDL28	
11	B1000-1 X B37	
12	B1000-1 X DK	
13	CDL28	no resistance
14	CDL28 X B1000-1	
15	CDL28 X B37	
16	CDL28 X DK	

Table 2 The trial layout of the F1 field trial

Replication 1			
3	5	9	11
13	15	6	8
2	1	7	14
10	12	16	4

Replication 2			
10	4	16	12
5	11	3	9
7	15	6	13
14	1	8	2

Replication 3			
10	1	4	7
11	14	5	12
13	9	2	8
6	15	16	3

Table 3 Stand counts on the individual plots of the F1 field trial 44, 66, 72 and 99 days after transplanting

	Entry	Initial stand counts 7 days after transplanting			Stand counts 44 days after transplanting			Stand counts 66 days after transplanting			Stand counts 72 days after transplanting			Stand counts 99 days after transplanting		
		Rep1	Rep 2	Rep 3	Rep1	Rep 2	Rep 3	Rep1	Rep 2	Rep 3	Rep1	Rep 2	Rep 3	Rep1	Rep 2	Rep 3
1	B37	20	20	20	20	20	20	20	20	20	20	20	20	20	19	18
2	B37 X CDL28	20	20	19	20	20	19	18	18	13	18	18	13	9	10	7
3	B37 X B1000-1	20	20	20	20	20	20	20	20	20	20	20	19	20	20	18
4	B37 X DK	20	20	20	20	20	19	20	20	19	20	20	19	18	20	18
5	DK	20	20	20	20	20	19	20	20	18	20	19	17	19	13	12
6	DK X CDL28	20	20	20	19	20	19	14	16	16	11	16	14	5	8	4
7	DK X B1000-1	19	20	20	19	20	20	18	19	20	18	19	20	17	19	20
8	DK X B37	20	20	20	20	20	20	18	18	20	18	18	20	17	18	19
9	B1000-1	19	20	20	19	20	19	19	20	16	19	20	16	19	20	16
10	B1000-1 X CDL28	20	20	20	20	20	20	17	18	18	15	15	18	8	9	16
11	B1000-1 X B37	20	20	20	20	20	20	20	20	19	20	20	18	20	19	18
12	B1000-1 X DK	20	20	19	20	19	18	19	19	18	19	19	17	16	19	17
13	CDL28	20	20	20	17	17	19	4	2	10	4	2	6	0	0	0
14	CDL28 X B1000-1	20	20	20	20	20	20	15	20	19	15	19	18	6	16	16
15	CDL28 X B37	17	20	20	17	20	20	16	18	18	16	15	18	13	11	17
16	CDL28 X DK	20	20	20	20	20	19	12	15	16	12	13	16	8	5	8

Table 4 The trial layout of the F1 greenhouse trial

Replication 1			
3	5	9	11
13	15	6	8
2	1	7	14
10	12	16	4

Replication 2			
10	4	16	12
5	11	3	9
7	15	6	13
14	1	8	2

Replication 3			
10	1	4	7
11	14	5	12
13	9	2	8
6	15	16	3

Table 5 The trial layout of the F2 greenhouse trial

Replication 1			
2	3	9	6
8	13	10	14
15	7	4	12
11	16	5	1

Replication 2			
3	6	11	2
10	5	4	13
1	7	14	15
12	8	9	16

Replication 3			
6	13	1	4
12	7	8	16
3	11	10	14
9	15	5	2

Table 6 Stand counts on the idividual plots of the F1 greenhouse trial 7, 10 and 14 days after inoculation

Entry	Stand counts at inoculation			Stand counts 7 days after inoculation			Stand counts 10 days after inoculation			Stand counts 14 days after inoculation		
	Rep1	Rep 2	Rep 3	Rep1	Rep 2	Rep 3	Rep1	Rep 2	Rep 3	Rep1	Rep 2	Rep 3
1 B37	20	20	20	18	20	20	18	20	19	18	20	18
2 B37 X CDL28	20	20	20	9	12	10	8	6	8	8	4	5
3 B37 X B1000-1	20	20	20	19	19	16	19	17	12	19	17	11
4 B37 X DK	20	20	20	14	18	20	10	17	16	10	17	16
5 DK	20	20	20	17	15	13	12	15	11	12	14	7
6 DK X CDL28	20	20	20	9	14	5	8	10	3	6	10	1
7 DK X B1000-1	20	20	20	19	20	16	19	20	16	19	20	16
8 DK X B37	20	20	20	17	19	11	17	18	9	17	18	8
9 B1000-1	20	20	20	20	20	20	20	19	20	20	18	20
10 B1000-1 X CDL28	20	20	20	14	17	18	11	13	18	11	11	18
11 B1000-1 X B37	20	20	20	20	20	20	19	20	20	19	20	20
12 B1000-1 X DK	20	20	20	19	18	11	19	18	9	19	18	9
13 CDL28	20	20	20	2	1	0	1	1	0	0	0	0
14 CDL28 X B1000-1	20	20	20	15	19	19	12	16	19	12	16	17
15 CDL28 X B37	20	20	20	11	18	12	7	18	6	6	13	5
16 CDL28 X DK	20	20	20	7	11	6	6	11	3	5	8	3

Table 7 Stand counts on the idividual plots of the F2 greenhouse trial 9 and 21 days after inoculation

Entry	Stand counts at inoculation			Stand counts 9 days after inoculation			Stand counts 21 days after inoculation		
	Rep1	Rep 2	Rep 3	Rep1	Rep 2	Rep 3	Rep1	Rep 2	Rep 3
1 B37	20	20	20	4	8	8	2	5	6
2 B37 X CDL28	20	20	20	0	0	4	0	0	1
3 B37 X B1000-1	20	20	20	18	10	17	14	10	15
4 B37 X DK	20	20	20	0	5	10	0	5	10
5 DK	20	20	20	2	5	2	0	3	0
6 DK X CDL28	20	20	20	6	4	7	6	3	6
7 DK X B1000-1	20	20	20	5	11	13	4	10	8
8 DK X B37	20	20	20	8	14	16	4	10	13
9 B1000-1	19	20	20	16	14	17	15	13	12
10 B1000-1 X CDL28	20	20	20	5	3	10	3	3	9
11 B1000-1 X B37	20	20	20	14	9	10	12	9	7
12 B1000-1 X DK	19	20	20	3	2	8	2	2	8
13 CDL28	20	20	20	0	0	1	0	0	0
14 CDL28 X B1000-1	18	20	20	1	7	6	0	4	5
15 CDL28 X B37	20	20	20	1	3	3	0	2	1
16 CDL28 X DK	19	19	20	1	3	4	0	2	1

Table 8 Percentage surviving plants on the individual plots of the F1 field trial 44, 66, 72 and 99 days after transplanting

Entry	Percentage surviving plants 44 days after transplanting			Percentage surviving plants 66 days after transplanting			Percentage surviving plants 72 days after transplanting			Percentage surviving plants 99 days after transplanting		
	Rep1	Rep 2	Rep 3	Rep1	Rep 2	Rep 3	Rep1	Rep 2	Rep 3	Rep1	Rep 2	Rep 3
1 B37	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	95.0	90.0
2 B37 X CDL28	100.0	100.0	100.0	90.0	90.0	68.4	90.0	90.0	68.4	45.0	50.0	36.8
3 B37 X B1000-1	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	95.0	100.0	100.0	90.0
4 B37 X DK	100.0	100.0	95.0	100.0	100.0	95.0	100.0	100.0	95.0	90.0	100.0	90.0
5 DK	100.0	100.0	95.0	100.0	100.0	90.0	100.0	95.0	85.0	95.0	65.0	60.0
6 DK X CDL28	95.0	100.0	95.0	70.0	80.0	80.0	55.0	80.0	70.0	25.0	40.0	20.0
7 DK X B1000-1	100.0	100.0	100.0	94.7	95.0	100.0	94.7	95.0	100.0	89.5	95.0	100.0
8 DK X B37	100.0	100.0	100.0	90.0	90.0	100.0	90.0	90.0	100.0	85.0	90.0	95.0
9 B1000-1	100.0	100.0	95.0	100.0	100.0	80.0	100.0	100.0	80.0	100.0	100.0	80.0
10 B1000-1 X CDL28	100.0	100.0	100.0	85.0	90.0	90.0	75.0	75.0	90.0	40.0	45.0	80.0
11 B1000-1 X B37	100.0	100.0	100.0	100.0	100.0	95.0	100.0	100.0	90.0	100.0	95.0	90.0
12 B1000-1 X DK	100.0	95.0	94.7	95.0	95.0	94.7	95.0	95.0	89.5	80.0	95.0	89.5
13 CDL28	85.0	85.0	95.0	20.0	10.0	50.0	20.0	10.0	30.0	0.0	0.0	0.0
14 CDL28 X B1000-1	100.0	100.0	100.0	75.0	100.0	95.0	75.0	95.0	90.0	30.0	80.0	80.0
15 CDL28 X B37	100.0	100.0	100.0	94.1	90.0	90.0	94.1	75.0	90.0	76.5	55.0	85.0
16 CDL28 X DK	100.0	100.0	95.0	60.0	75.0	80.0	60.0	65.0	80.0	40.0	25.0	40.0

Table 9 Percentage surviving plants on individual plots of the F1 greenhouse trial 7, 10 and 14 days after inoculation

	Entry	Percentage surviving plants 7 days after inoculation			Percentage surviving plants 10 days after inoculation			Percentage surviving plants 14 days after inoculation		
		Rep1	Rep 2	Rep 3	Rep1	Rep 2	Rep 3	Rep1	Rep 2	Rep 3
1	B37	90	100	100	90	100	95	90	100	90
2	B37 X CDL28	45	60	50	40	30	40	40	20	25
3	B37 X B1000-1	95	95	80	95	85	60	95	85	55
4	B37 X DK	70	90	100	50	85	80	50	85	80
5	DK	85	75	65	60	75	55	60	70	35
6	DK X CDL28	45	70	25	40	50	15	30	50	5
7	DK X B1000-1	95	100	80	95	100	80	95	100	80
8	DK X B37	85	95	55	85	90	45	85	90	40
9	B1000-1	100	100	100	100	95	100	100	90	100
10	B1000-1 X CDL28	70	85	90	55	65	90	55	55	90
11	B1000-1 X B37	100	100	100	95	100	100	95	100	100
12	B1000-1 X DK	95	90	55	95	90	45	95	90	45
13	CDL28	10	5	0	5	5	0	0	0	0
14	CDL28 X B1000-1	75	95	95	60	80	95	60	80	85
15	CDL28 X B37	55	90	60	35	90	30	30	65	25
16	CDL28 X DK	35	55	30	30	55	15	25	40	15

Table 10 Percentage surviving plants on the individual plots of the F2 greenhouse trial 9 and 21 days after inoculation

Entry		Percentage surviving plants 9 days after inoculation			Percentage surviving plants 21 days after inoculation		
		Rep1	Rep 2	Rep 3	Rep1	Rep 2	Rep 3
1	B37	20.0	40.0	40.0	10.0	25.0	30.0
2	B37 X CDL28	0.0	0.0	20.0	0.0	0.0	5.0
3	B37 X B1000-1	90.0	50.0	85.0	70.0	50.0	75.0
4	B37 X DK	0.0	25.0	50.0	0.0	25.0	50.0
5	DK	10.0	25.0	10.0	0.0	15.0	0.0
6	DK X CDL28	30.0	20.0	35.0	30.0	15.0	30.0
7	DK X B1000-1	25.0	55.0	65.0	20.0	50.0	40.0
8	DK X B37	40.0	70.0	80.0	20.0	50.0	65.0
9	B1000-1	84.2	70.0	85.0	78.9	65.0	60.0
10	B1000-1 X CDL28	25.0	15.0	50.0	15.0	15.0	45.0
11	B1000-1 X B37	70.0	45.0	50.0	60.0	45.0	35.0
12	B1000-1 X DK	15.8	10.0	40.0	10.5	10.0	40.0
13	CDL28	0.0	0.0	5.0	0.0	0.0	0.0
14	CDL28 X B1000-1	5.6	35.0	30.0	0.0	20.0	25.0
15	CDL28 X B37	5.0	15.0	15.0	0.0	10.0	5.0
16	CDL28 X DK	5.3	15.8	20.0	0.0	10.5	5.0

Table 11 ANOVA for a randomised block design of the F1 field trial

Source of variation	d.f	s.s	m.s	F Value	F pr
Blocks	2	43.662	21.8311	0.132	0.8764
Genotypes	15	39661.832	2644.1221	16.048	<0.0001
Error	30	4943.022	164.767		
Total	47	44648.516			

Table 12 ANOVA for a randomised block design of the F1 greenhouse trial

Source of variation	d.f	s.s	m.s	F Value	F pr
Blocks	2	1957.29	978.6458	3.998	0.0289
Genotypes	15	39399.48	2626.6321	10.732	<0.0001
Error	30	7342.71	244.7571		
Total	47	48699.48			

Table 13 ANOVA for a randomised block design of the F2 greenhouse trial

Source of variation	d.f	s.s	m.s	F Value	F pr
Blocks	2	1251.04	625.5208	4.187	0.0249
Genotypes	15	20528.65	1368.5764	9.160	<0.0001
Error	30	4482.30	149.4099		
Total	47	26261.98			

APPENDIX 2

FIGURES

Fig. 1 Seedlings were transplanted from small containers to this type of seed trays from where they were, when strong enough, transplanted to the black shank infested field

Fig. 2 When the stand counts of the F1 field trial where taken, plants were regarded as diseased when they showed severe wilting

Fig. 3 After severe wilting occurred, black lesions at the bottom of the stem developed together with yellowing of the leaves

Fig. 4 The first replication of the 1998 F1 greenhouse trial which was planted in small 10 cm pots

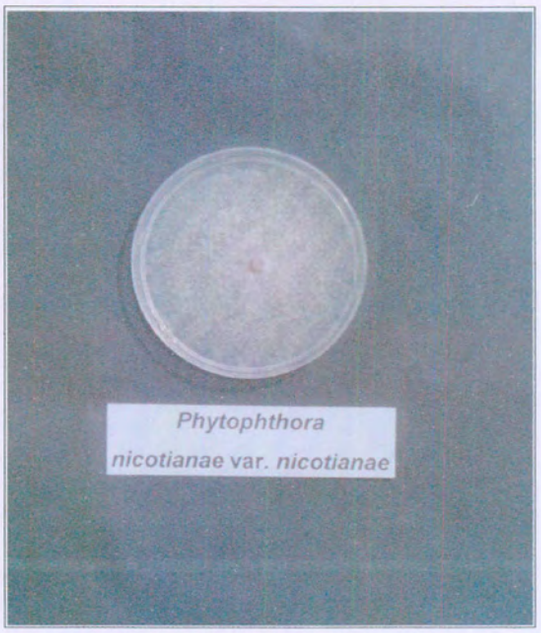

Fig. 5 Virulent colonies of *Phytophthora parasitica* var. *nicotianae* (*Phytophthora nicotianae* var. *nicotianae*) were grown for 2 weeks at 26°C on V8-juice agar in petri dishes with nystatin at a pH 0f 5.9

Fig. 6 In order to inoculate the greenhouse trials root damage was inflicted by making a single incision into the roots approximately 1 cm from the stem

Fig. 7 Two ml of the inoculum were injected into the incisions before it was closed up

Fig. 8 During the greenhouse trials plants were regarded as diseased when they were dead, severely wilted, or developed black lesions at the bottom of the stem which was usually also associated with yellowing of the leaves

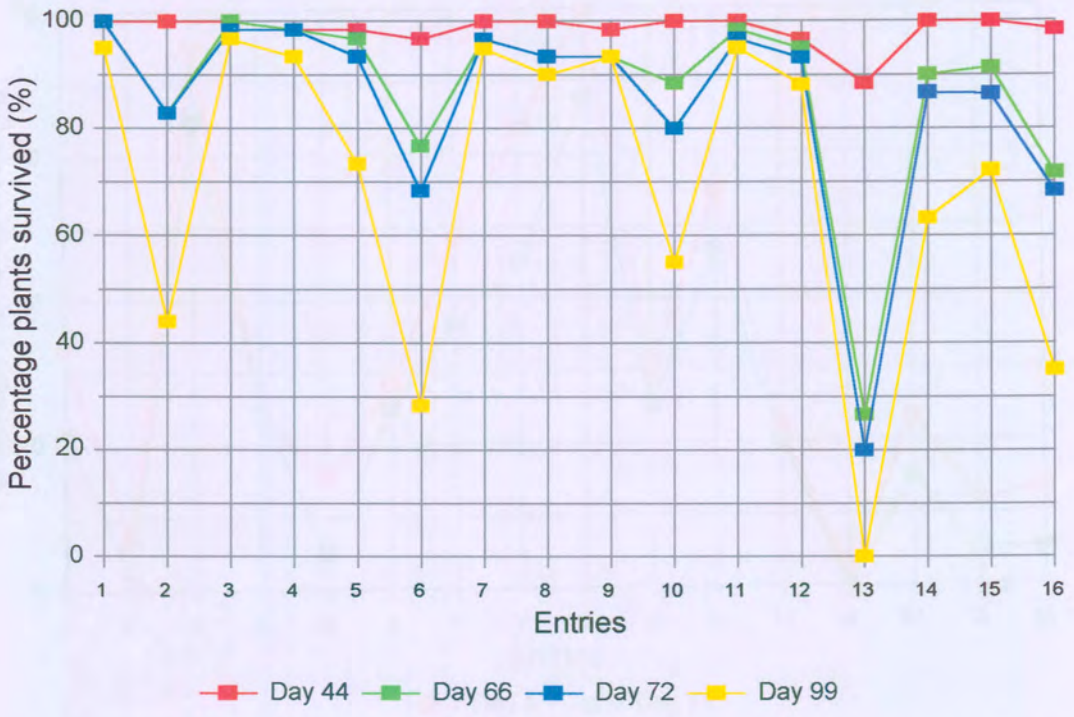

Fig. 9 Mean percentages of surviving plants of the 1998/1999 F1 field trial 44, 66, 72 and 99 days after transplanting

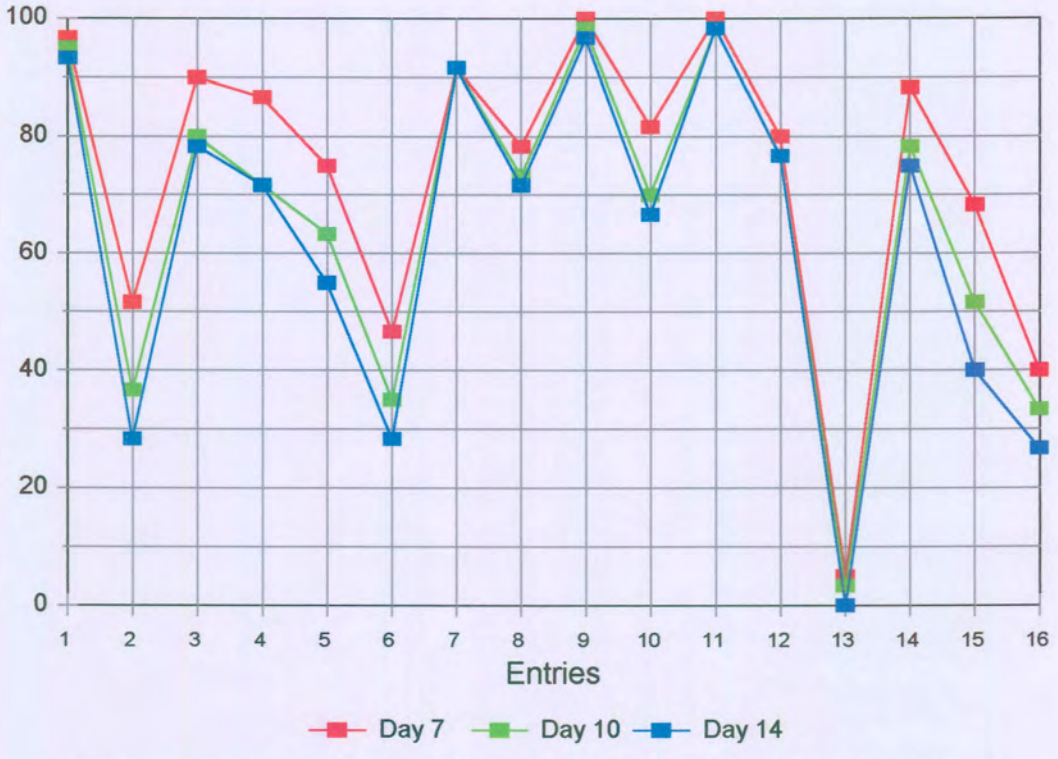

Fig10 MearpercentagesofsurvivingplantsoftheF1greenhousetrial7,10 and 14 days after inoculation

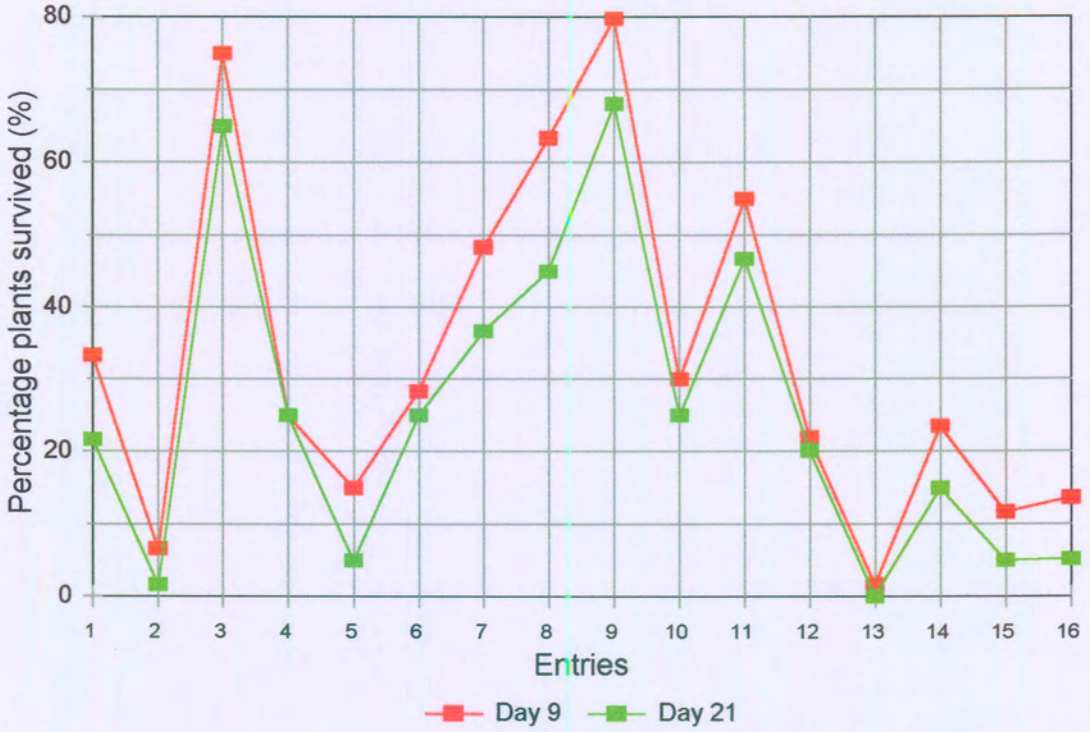

Fig. 11 Mean percentages of surviving plants of the F2 greenhouse trial 9 and 21 days after inoculation