THE PATHOGENESIS OF *FUSARIUM OXYSPORUM* F. SP. *NARCISSI* AND THE ROLE OF ANTAGONISTIC BULB-BORNE FUNGI IN THE CHEMICAL CONTROL OF BASAL ROT

AAN MIJN OUDERS

NORANNE EN NELLEKE

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STELLINGEN

I

Bij "vroeg planten" van narcissen kan onderzoek naar het vóórkomen van latente bolrot infecties en inoculum van *Fusarium oxysporum* f. sp. *narcissi* in de grond veel schade voorkómen.

Dit proefschrift.
Gregory, P.H., 1932. Annals of Applied Biology 19: 475-514.

II

De effecten van pimaricine op *Fusarium oxysporum* f. sp. *narcissi* illustreren mede dat een andere inrichting van het traditionele fungicidenonderzoek en het daarop afgestemde toelatingsbeleid gewenst is.

Dit proefschrift.

III

In de land- en tuinbouw danken thiram en organische kwikverbindingen hun populariteit voornamelijk aan hun vermogen een biologisch bestrijdingsmechanisme te induceren en te stimuleren.

Dit proefschrift.

IV

Isolatie van antagonisten van plantpathogenen, èn toepassing ervan tegen die pathogenen, dienen bij voorkeur onder sub-optimale omstandigheden plaats te vinden.

V

Een biologische of geïntegreerde bestrijding van *Rhizoctonia solani* met behulp van *Verticillium biguttatum* biedt, de gewekte verwachtingen ten spijt, geen perspectieven voor een verantwoorde vervanging van kwikhoudende fungiciden in de pootaardappelteelt.

Boogerts, P.H.J.F. en G. Jager, 1984. Netherlands Journal of Plant Pathology 90: 117-126.
Jager, G., 1984. Pootaardappelwereld, augustus: 11-16.

VI

Kwantificering van zaadzwakte, middels meting van de ethyleenproduktie aan verouderd zaad van *Phaseolus vulgaris*, vraagt om een betere definitie van de term "vigour".

Samimy, C. en A.G. Taylor, 1983. Journal of the American Society for Horticultural Science 108: 767-769.
Perry, D.A., 1976. Advances in Research and Technology of Seeds, part 2: 62-85.

VII

De genetisch bepaalde vatbaarheid voor *Drechslera sorokiniana* bij gramineeën dient niet veronachtzaamd te worden in onderzoek dat ten doel heeft effecten van auxine-achtige herbiciden op de vlekkenziekte te verklaren.

Hodges, C.F., 1984. Plant Disease 68: 213-215.
Langerak, C.J., 1984. Annual Report of the Government Seed Testing Station (1983/1984), pag. 54.

VIII

Het achterwege laten van gezondheidsonderzoek op zaden, bestemd voor bewaring in een genenbank, impliceert voor plantenziektenkundigen voldoende werkgelegenheid in de toekomst.

Neergaard, P., 1980. National Academy of Sciences, India. Golden Jubilee Commemoration Volume, pag. 495-530.

IX

Milieu-organisaties realiseren zich onvoldoende dat een volledig verbod op toepassing van kwikhoudende fungiciden schadelijk is voor milieu, economie en volksgezondheid.

X

Biologisch-dynamische voedselproduktie is een luxe, die men zich kon gaan veroorloven nadat onderzoek naar en toepassing van chemische ziektenbestrijding effectief bleek.

XI

Zolang het "Westen" en het "Oosten" de kop in 't zand blijven steken is voor een Sahelbewoner, *Luctor et Emergo*, niet meer dan een utopie.

XII

Het is economisch verantwoord afschaffing van de maximum snelheid op autosnelwegen te koppelen aan het creëren van meer verkeersdrempels in de bebouwde kom.

XIII

Hoe meer nullen achter een project, hoe duurder een project.

Proefschrift van C.J. Langerak
'The pathogenesis of *Fusarium oxysporum* f. sp. *narcissi* and the role of antagonistic bulb-borne fungi in the chemical control of basal rot'.

Wageningen, 18 januari 1985.

C.J. Langerak

THE PATHOGENESIS OF *FUSARIUM OXYSPORUM* F. SP. *NARCISSI* AND THE ROLE OF ANTAGONISTIC BULB-BORNE *FUNGI* IN THE CHEMICAL CONTROL OF BASAL ROT

Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. C.C. Oosterlee
in het openbaar te verdedigen
op vrijdag 18 januari 1985
des namiddags te vier uur in de aula
van de Landbouwhogeschool te Wageningen

ISW = 217 850-03

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CONTENTS

1 GENERAL INTRODUCTION

Various organomercury compounds were approved for use in agriculture and horticulture since the early nineteen thirthies. Because of their high activity against fungal plant pathogens, their low costs and chemical stability, new and good possibilities arose for control of especial those fungal pathogens which were transmittable via seeds, bulbs or tubers from one generation to the next. Treatment of such plant material through dressing with a mercurial or soaking in solutions of the fungicide became the best standard procedure in the production of sowing seed and reproduction of flower bulbs and seed potatoes. However, in view of environmental hazards and toxic side effects, the need arose to replace these fungicides by less noxious compounds. The development and introduction of new types of fungicides in the nineteen sixties, e.g. the systemic benzimidazoles and oxathiins with a much lower toxicity to man and animals, contributed then partly to a reduced use of organomercury fungicides. Application of these mercurials on plant material intended for production of consumption goods became forbidden in The Netherlands, just as in most of the other industrialized countries. Further, special precautions were prescribed for treatment of basic seed, bulb and tuber material grown for further reproduction as far treatment with mercurials remained allowed. This, far from ideal, solution has led in the early nineteen seventies to the establishment of a working group for the replacement of mercurial fungicides by the Ministry of 'Volksgezondheid en Milieuhygiëne' in The Netherlands. Representatives of several agricultural institutes and the chemical industry participated in a steering committee, and the author was appointed to conduct research on this topic.

As still a substantial quantity of organomercury compounds was used at that time for control of basal rot in narcissus bulbs, caused by *Fusarium oxysporum* f. sp. *narcissi*, priority was given to a study of the possibility to control this disease by non-mercurials. After a preliminary screening two compounds appeared to be especially promising, namely thiram and the antibiotic pimaricin. The latter compound, produced by Gist-Brocades N.V. at Delft, The Netherlands had earlier found to be active for treatment of seeds against deep seated fungal infection (Dekker, 1957).

Soon after the start of the investigations, it appeared necessary to pay detailed attention to the pathogenesis of the causal organism, as

several parts of this process had been understood only poorly or even wrongly in the past (chapter 2). Insight in the relation between infection of bulbs in the autumn and the development of the disease in the following season appeared to be important for the grower to devise appropriate control measures in addition to a treatment with fungicides (chapter 3). As fungal antagonists, present on the bulbs and in soil, appeared to play a role in disease development and in control with fungicides, it was decided to pay attention also to this aspect (chapter 4). Finally, the influence of environmental conditions had to be investigated both on the development of basal rot in bulbs in the field and in store and on the effectiveness of chemical control measures (chapter 5). General conclusions have been drawn and are discussed in chapter 6.

2 THE PATHOGENESIS OF *FUSARIUM OXYSPORUM* F. SP. *NARCISSI*

2.1. Introduction

Basal rot of narcissus is caused by *Fusarium oxysporum* Schlecht. f. sp. *narcissi* (Cooke & Massee) Snyder & Hansen. Since the occurrence and cause of the disease were described by Gregory (1932), the aetiology of the disease has been elucidated only partly.

Hot-water treatment of the plant material is usually carried out for control of parasitic insects, mites and nematodes (Van Slogteren, 1931). Such a treatment, however, may cause spread of inoculum of the pathogen from diseased to healthy bulbs, and thus favours new infections. Hawker (1935, 1943b) and McClellan (1952) have reported that the pathogen invades the central disc of the bulb, the 'basal plate', from senescent roots, which have been infected in early summer when soil temperature exceeds 13 °C. The inoculum infecting the roots is considered to be bulb-borne (Gregory, 1932; Hawker, 1940) and/or soil-borne (Hawker, 1943b; Price, 1975, 1977a).

This generally accepted view on the aetiology of the disease is questionable for at least two reasons. Firstly, Hawker (1935, 1943b) ascertained, that more than 4 weeks old roots were no longer susceptible to infection, which is not in agreement with the assumption that senescent roots may become infected 30 weeks later in early summer. Secondly, field experiments have revealed that the date of lifting in early summer generally has less influence on the basal rot incidence during storage than the date of planting in the previous autumn (B.H.H. Bergman, pers. comm.).

Field observations on the occurrence of basal rot are sometimes difficult to explain. Low soil temperatures before harvest do not correlate with low levels of basal rot during storage. On the other hand, growers often assume that a two year growing period does not appear to cause a considerable increase in the percentage of rotted bulbs after lifting. Such a conclusion at harvest time is often wrong as many infected bulbs originally planted will have been decayed completely during the long stay in the soil (Price, 1977a).

Addition of fungicides may prevent spread of inoculum during the hot-water treatment. Effective chemicals in use for many years are organic mercury compounds (Hawker, 1940; McClellan, 1948; Miller & Gould, 1967)

and formalin (Gregory, 1932; Hawker, 1935, 1940, 1943a, b). Control of basal rot with these compounds, however, is not always satisfactory in practice and results from field experiments are often inconsistent. Therefore a more efficient treatment is being sought.

In preliminary laboratory and field experiments the polyene antibiotic pimaricin gave promising results in control of root rot and basal rot (Langerak, 1975, 1977) and therefore it was decided to investigate the potential of this antibiotic to replace the mercurials in the hot-water treatment (Langerak, 1977).

To evaluate the effect of pimaricin formulations in practice, supplementary knowledge about the pathogenesis of *Fusarium oxysporum* f. sp. *narcissi* appeared necessary. Knowledge of the time of penetration of roots and bulbs will allow to determine whether fungitoxic action will be needed after application and planting. Therefore experiments were carried out to study the progress of infection, the development of symptoms, and changes in the morphology and the physiology of natural growing healthy narcissus bulbs which were contaminated with the pathogen prior to planting. Histological techniques were applied to aid observation.

2.2. Materials and methods

Plant material. 'Round bulbs' were obtained from commercial stocks of narcissus bulbs (*Narcissus pseudonarcissus*, cv. Carlton) and were stored and used in all experiments as described by Langerak (1977).

Fungi. *Fusarium oxysporum* f. sp. *narcissi* was isolated from roots and diseased scales of narcissus bulbs. Single-conidium cultures were made from microconidia of each of the isolates. The most pathogenic culture of *F. oxysporum* f. sp. *narcissi* (code F_9) was used for inoculation experiments. *Penicillium corymbiferum* was isolated from healthy roots grown in natural soil. One culture (code P_1) with antagonistic properties against F_9 was used in the pre-rooting experiment. All cultures were maintained at 5 °C in a mixture of oatmeal and peat soil to preserve either pathogenicity or antagonistic activity.

Soil. Sandy soil in which no flower bulbs had grown for two years was obtained from the experimental garden of the Bulb Research Centre at Lisse, The Netherlands. This soil was sterilized in an autoclave (30 min at 115 °C) on two subsequent days.

Chemicals. Clove-oil, Orange G and Safranin (Chroma Ges., Stuttgart, F.R.G.); formalin (37% a.i.) and Methylviolet KB (E. Merck AG, Darmstadt, F.R.G.); Fast Green FCF and Sudan IV (British Drug Houses Ltd, Pool, U.K.); Cotton Blue (G.T. Gurr, London, U.K.) were used.

Isolation and culturing of fungi. Fungi were isolated from diseased bulb scales and healthy roots. Isolations were made by plating the colonized plant material on an oxgall-PCNB medium (Papavizas, 1967) and by sub-culturing on potato dextrose agar with 50 mg/l vendarcin (PDA+V) as described by Langerak (1977).

Testing of pathogenicity. Single-conidium cultures of *F. oxysporum* isolates on PDA+V were tested for their pathogenicity on narcissus bulbs. Twenty-five agar pieces per culture (5 mm diam.) with mycelium were transferred into an equal number of fresh wounds of the outer white scales of five bulbs; wounds were sealed up with cellotape, and the bulbs were incubated for 14 days at 25 °C. The cultures causing more than 20 positive infections with symptoms typical for *F. oxysporum* f. sp. *narcissi* were maintained in mixtures of oatmeal and peat soil.

Assessment of antagonistic activity. Single-conidium cultures of *Penicillium corymbiferum* were tested *in vitro* using the double-layer technique as described by Langerak (1977) for antagonistic activity against *F. oxysporum* f. sp. *narcissi*.

Culturing and harvesting of conidia. Conidia used in the inoculation experiments were cultured on autoclaved narcissus bulb scales. The bulb scales were contaminated with inoculum from the oatmeal and peat soil stock-cultures, incubated and finally liberated from conidia as described by Langerak (1977).

Survival of hot-water treated inoculum. Conidia and mycelial fragments were seeded on PDA+V. Roots were cut to pieces of 5 mm and plated on Papavizas medium. Both seedings and platings were incubated at 25 °C. Germination of seeded inoculum was determined after three days and mycelial growth from root pieces after six days. Survival was expressed in percentage of controls, in which the inoculum was cold-water treated (2 h at 15 °C) and incubated on agar directly after treatment.

Achievement of constant soil temperatures in the field. Performance of the experiments B and C required soil temperatures of 10 °C and 15 °C respectively, which should vary at a depth of 10 cm as little as possible

for a period of two weeks from the day of planting. This aim was met by planting bulbs at 16 °C and covering the soil immediately after planting with a 20 cm thick layer of coarse-chopped reed. Temperatures varied under these circumstances between 13-15 °C for two weeks. When this layer was removed, the temperature decreased gradually to a level of 10 °C, due to a strong nightly radiation. Recordings showed that temperatures varied between 8-12 °C for another two weeks and decreased to lower levels thereafter.

Pre-rooting of bulbs. Bulbs of 5 cm diameter were carefully cleaned by removing old roots and dead cork tissue from the bases of the bulbs and brown papery scales from the fleshy white bulb scales. Residual fungal and bacterial contamination was eliminated by treatment in a 0.1% formaldehyde solution (2 h at 43.5 °C). After washing in sterile tap water, bulbs were transferred to sterile bottomless multipot sets which had been placed upside down in PVC trays. Trays were filled with tap water so that the bulbs just touched the surface, closed and incubated at 10 °C in darkness. The water was changed at three day intervals. Air humidity was near 100% in the closed trays. Bulbs with abnormal and incomplete rooting were discarded and not used in the inoculation experiments.

Histological procedures for localization of mycelium and suberin-like substances in root and basal plate tissue. Roots and blocks of basal plate tissue (5x5x10 mm) were fixed in 50% ethanol for 30 min and 2 h, respectively, immediately prior to sectioning. Material was sectioned with a Hooker microtome (Hooker, 1967) and collected in 50% ethanol.

The presence of fungal mycelium was demonstrated by staining the tissue with Cotton Blue in lactophenol (CB) for 2 min at boiling point, and subsequent differentiation in lactophenol at room temperature.

Lignin in cell walls was stained with crystalline violet by using the four-fold staining procedure of Johansen (Gerlach, 1969).

Suberin in cell walls of basal plate periderm and exodermis of roots was stained in a saturated and filtered solution of Sudan IV in 70% ethanol (S). For a clear contrast between suberin-containing and suberin-free cell walls, the S-staining was combined with CB-staining (CB-S). S-stained sections were placed in a CB-solution, gently heated for 1 min to boiling point, transferred to 96% ethanol for 30 sec and then to lactophenol for 1 min to remove the excess of dye. Thereafter, CB-stained sections were boiled for 2 min in a S-solution, transferred to glycerin to wash off the

excess dye.

All stained and differentiated sections were mounted in glycerin.

2.3. Anatomy and development of the basal plate of a narcissus bulb

The anatomy of a segment of the bulb *discus*, during storage in August has been sketched in Fig. 2.1. The bulb *discus*, mostly called basal plate, is the squat stem, which consists of two regions, the cortex and the central cylinder (De Mason, 1979). Internal differentiation based on histological studies has been included in Fig. 2.1.

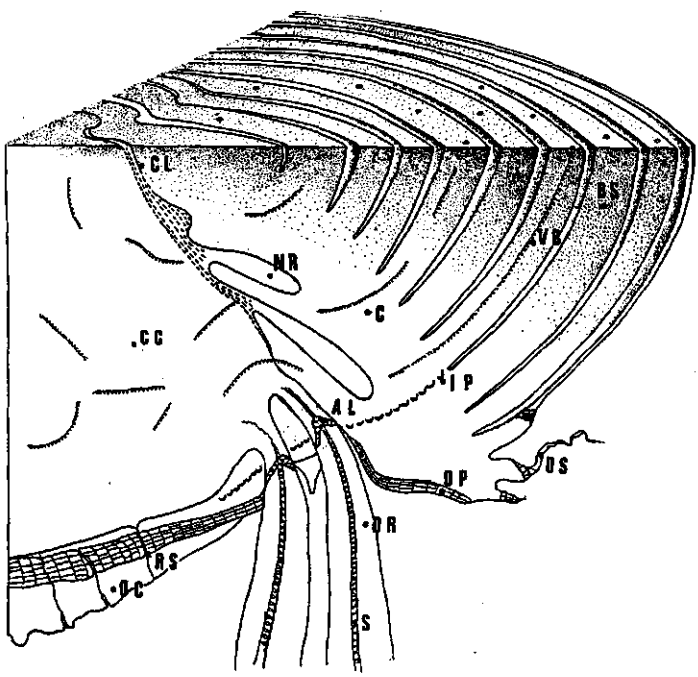

Fig. 2.1. Segment of the basal plate of a narcissus bulb during storage in August. AL, abscission layer; BS, mesophyl of the tunic (scale); C, cortex outer parenchyma with vessels; CC, central cylinder inner parenchyma with pith and vessels; CL, 'cambium', meristematic cambium-like layer; IP, inner periderm; NR, adventitious root, new; OC, cortex tissue, old; OP, outer periderm; OR, adventitious root, old; OS, rest of old tunic (scale); RS, old root scar; S, stele of the root; VB, vascular bundle of the scale.

Changes in the bulb anatomy were observed during the growing season (Fig. 2.2.). Post-dormancy development of bulbs starts with formation of adventitious roots on the 'cambial' periphery of the central cylinder (CC). These roots grow through the parenchymatous cortex (C) and rupture the protecting outer periderm if the external humidity is sufficiently high. After rupturing the outer periderm, the parenchymatous cells become exposed at once to the environment outside the bulb.

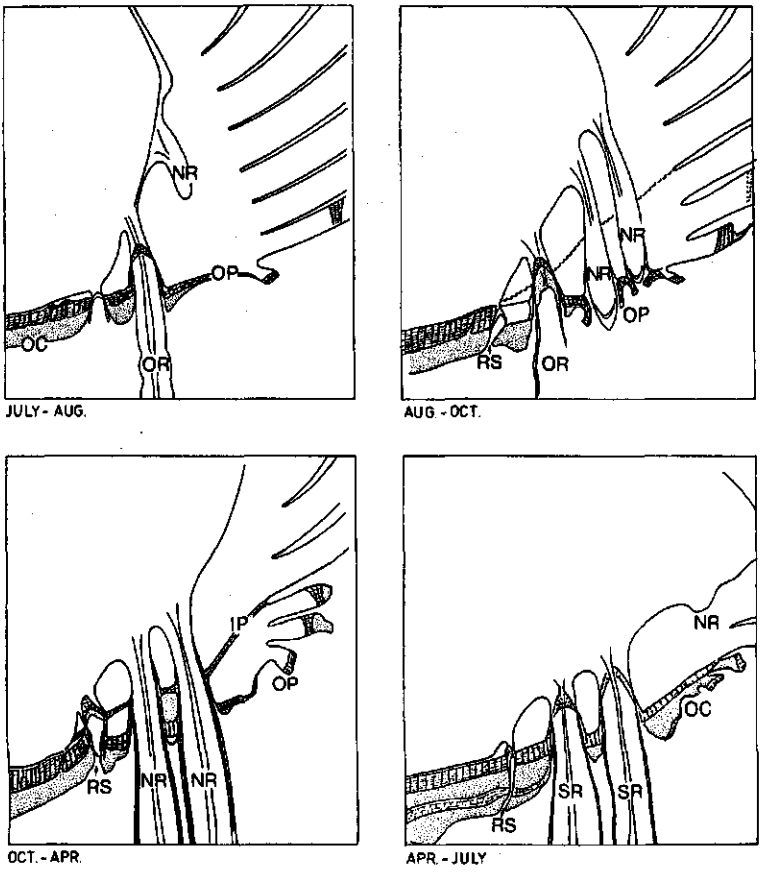

Fig. 2.2. Sequential stages of periderm formation of basal plate and roots of a rooting and developing bulb. Drawings based on histological studies during the growing season. AL, abscission layer; C, cortex outer parenchyma with vessels; CC, central cyclinder inner parenchyma with pith and vessels; IP, inner periderm; NR, new root; OC, old cortex tissue; OP, outer periderm; OR, old root; RS, scar of old root; SR, senescing root.

Some marked changes in the anatomy of the roots and bulbs could be observed during further plant development. Suberized barriers, formed in different plant parts, could be detected microscopically in S- and CB--stained tissue.

Suberin is produced within seven days in the cell walls of the basal plate which surround the emerging roots. This wound periderm was observed when bulbs had been incubated at temperatures around 10 °C (Fig. 2.3C.: WP). It is absent or cannot be observed anymore when the wounded tissue has been infected by the pathogen at 17 °C or above (Fig. 2.3B).

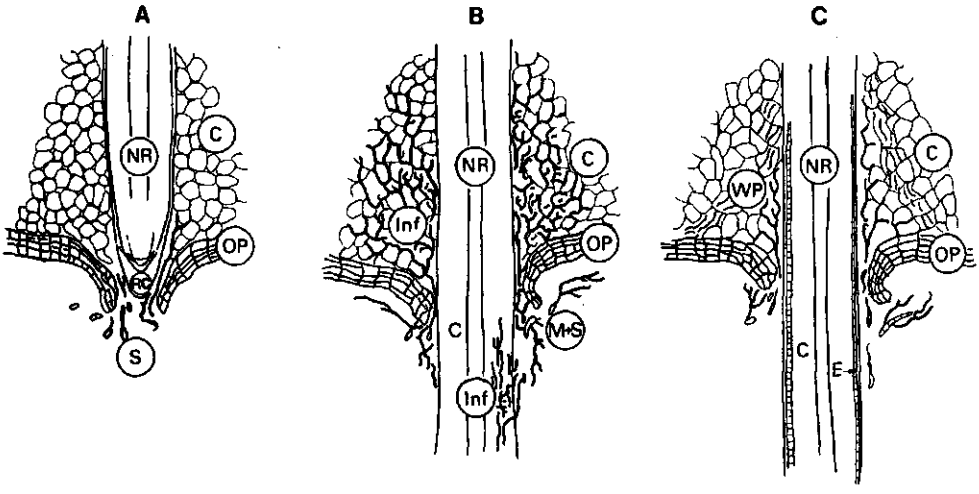

Fig. 2.3. Infection of bulb tissue and root (Inf), formation of wound periderm in the bulb cortex (WP) and suberization of exodermis cell walls in the root (E) after rupture of the outer periderm (OP) by the calyptra (RC), of an emerging new root (NR) in presence of conidia (S) and mycelium (M) of *F. oxysporum* f. sp. *narcissi*. A: initial situation at 10 °C and 17 °C, RC ruptures OP, conidia germinate, fastly at 17 °C, slowly at 10 °C; B: establishment of infection in the cortex of bulb and root after incubation for six days at 17 °C; C: effective wound healing in the cortex after incubation for six days at 10 °C.

Suberin formation is also detectable in the cell walls of the exodermis of the outgrowing roots within 10 days, firstly at the root base, thereafter extending to some cells behind the root tip. A perceptible

suberization of the exodermis has been observed in the roots incubated at 10 °C (Fig. 2.4A.). The roots become less fragile with the progress of cork production, while new invasion by fungal hyphae becomes impossible.

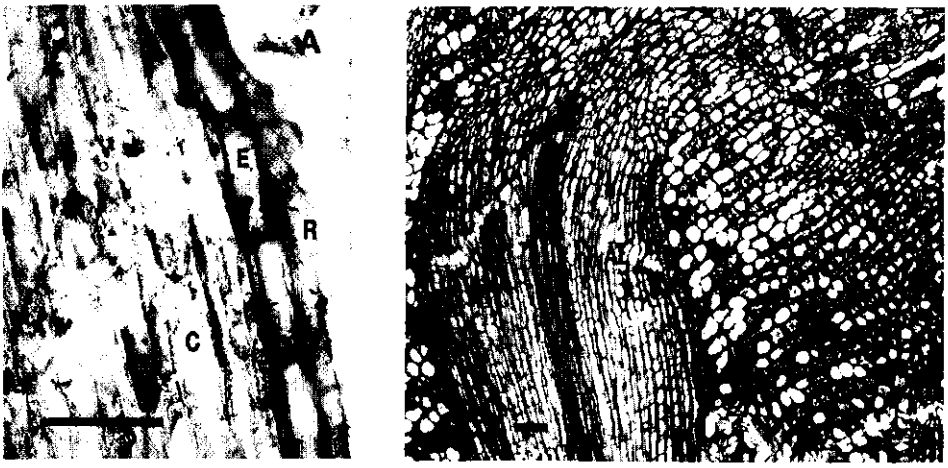

Fig. 2.4. Details of tissues in emerging and dying roots as sketched in Fig. 2. A: stained suberized cell wall in exodermis (E) of emerged root in September, cortex cell (C), rhizodermis cell (R); B: stained lignified abscission layer (AL) in senescing root in June (fourfold staining procedure of Johansen). Bar represents 100 µm.

Nutrient reserves in the outer bulb scales are used for shoot and root development from the first moment of development, leading to senescence of the two or three outer scales, while a new inner periderm is formed inside the basal plate tissue extending parallel to the outer periderm (Figs 2.2., 2.5.). The tissue excluded by the new periderm dies during the following period of shoot development.

Sometimes, a few adventitious roots grow out at the end of the growing period when leaves start yellowing. The previously formed roots are cut off from the central cyclinder, become translucent and decay. Delimitation of the roots appears to be facilitated by an abscission layer (AL) across the root cortex and stele (Fig. 2.2.). The abscission layers develop 2-4 mm from the periphery of the central cylinder. They consist of thin-walled cells. Lignification of the cell walls has been demonstrated by staining with crystalline violet (Fig. 2.4B.). Fungal invasion of the root cortex between abscission layer and the periphery of the central

Fig. 2.5. Detail of internal suberization of the basal plate of a narcissus bulb. Tissue of stained cortex showing cell walls of the inner periderm (IP) and cross-walls (CW) parallel to the bulb base. Location of tissue as indicated in Fig. 2.2. Bar represents 20 µm.

cylinder, has never been observed microscopically in decaying roots.

Similar abscission layers were found at the bases of the outer senescent bulb scales, leaves and flower stems during normal yellowing of the plants at the end of the growing season (Fig. 2.2.).

2.4. Survival of *F. oxysporum* f. sp. *narcissi* on hot-water treated bulbs

The effects of storage conditions after hot-water treatment (HWT) for 2 h at 43.5 °C on the viability of inoculum of the pathogen were determined over a period of three years. Three types of inoculum were examined:

a. naturally bulb-borne conidia, chlamydospores and mycelial fragments of different ages released during treatment in the bath;
b. artificially cultured young micro- and macroconidia, added to the bath; and
c. mycelial resting structures present in old dead roots.

Table 2.1. shows that all kinds of inoculum, independent of origin or age, are only partly killed by a HWT. Young conidia, however, have little resistance to such a treatment. Dry storage after HWT decreases the viability more than the treatment itself does, especially with respect to young conidia.

Table 2.1. Survival of three types of *F. oxysporum* f. sp. *narcissi* inoculum after a hot-water treatment (2 h at 43.5 °C) and a subsequent storage (24 h at 15 °C) under humid (RH 99%) and dry conditions (RH 70%). Percentage survival, given as the lowest and highest values observed in four different experiments.

Inoculum tested	% survival	
	RH 99%	RH 70%
Bulb-borne conidia and mycelial fragments	60-85	10-15
Conidia (artificial culture)	23-36	3- 5
Mycelium in old roots	18-35	4-28

From these experiments it is evident that inoculum released during HWT in the absence of a fungicide can contaminate the healthy bulbs in the bath. The older the inoculum, the heavier the contamination will be. Quick drying of the bulbs after HWT always reduces survival. Living mycelium in tissue of the cortex (Fig. 2.1.: C) and old cortex (Fig. 2.1.: OC) was affected by HWT in a similar way, but the effect of drying appeared to be less.

2.5. Aetiology of root rot

Infection of emerged roots was studied in laboratory and field experiments. Hot-water treated bulbs were contaminated with conidia of *F. oxysporum* f. sp. *narcissi* and/or saprophytes, and planted under various conditions. Assessments were made of: a. infection of the roots; b. the significance of root age for infection; c. the influence of inoculum potential and incubation temperature at planting time on infection and symptom development; d. the influence of the soil microflora on the extent of root rot.

a. Root infection. Contaminated and non-contaminated bulbs were planted at 15 °C in a natural soil, on which ornamental bulbs had never been grown. Healthy looking and visibly infected roots from both treatments were

selected after 14 days. Transverse and longitudinal sections were made to study mycelial growth inside the roots.

Roots without symptoms did not show hyphal growth in the cortex. Symptoms were only found on roots of contaminated bulbs. They showed reddish-brown stripes of various lengths on different parts of the roots. Penetrating hyphae could not be found in any section through young and older infections, but hyphal growth in the cortex was obvious. Hyphae initially grew intercellularly parallel to the stele. Radial spread was limited during the third and fourth week when the soil temperature varied between 14-20 ^{o}C. Later on, growth was also visible within the cell walls (Fig. 2.6A.) and the infected tissue turned dark reddish-brown to black. When the stele was invaded, the root became translucent. Further mycelial growth towards the basal plate seemed to be inhibited by bacteria and saprophytic fungi from the soil, because the pathogen was never isolated from the translucent tissue.

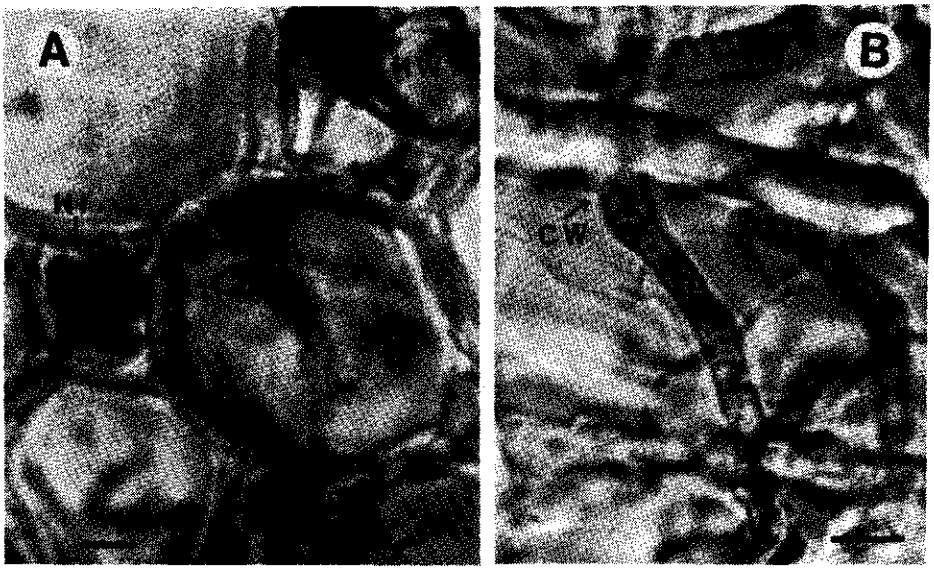

Fig. 2.6. Mycelial growth of *F. oxysporum* f. sp. *narcissi* in cortical tissue of narcissus. Hyphae stained with Cotton Blue. A: hyphae in intercellular spaces (HI) and within cell walls of cortical cells (HW) in a cross-sectioned root; B: hyphae penetrating the cell wall (CW) in the cortex of the bulb *discus*. Bar represents 10 µm.

When bulbs were planted in the field at temperatures varying between 9 °C and 14 °C, the course of the infection was similar to that observed in the laboratory at 15 °C, but mycelial growth was slower, resulting in shorter infected areas, which were mostly located near the bulb base. In general, the development of visible root rot was delayed for some weeks.

b. Root age. The significance of the root age for penetration by the pathogen was determined for bulbs, which were pre-rooted for 0-16 days, contaminated with conidia of the pathogen and incubated in sterile soil at 17 °C. Results are given in Fig. 2.7. The chance of infection obviously decreased with the age of the roots. The highest percentages of infected roots were found when recently emerged roots came in contact with pathogenic inoculum. Approximately two weeks after emergence, infection became almost impossible.

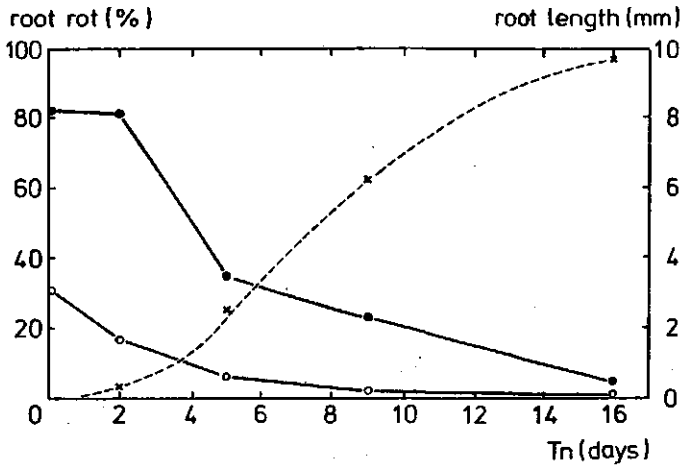

Fig. 2.7. Effect of root age (Tn, days of pre-rooting at 10 °C) on development of root rot caused by *F. oxysporum* f. sp. *narcissi*. Pre-rooted bulbs were dipped in a conidial suspension of 500 con/ml for 30 sec, planted in sterile (-●-) or natural soil (-o-), and incubated at 17 °C for 14 days. The average root length (x- -x) was also measured. Root rot is expressed as the average percentage of visibly infected roots per bulb (n = 30 bulbs).

c. Inoculum density and temperature. The combined effects of inoculum density and incubation temperature were tested under laboratory and field conditions. In experiment A, lots of 6 bulbs, cleaned and treated like those in the pre-rooting experiment, were dipped in conidial suspensions

of the pathogen, planted in sterile soil at constant temperatures and examined two weeks later. In experiment B and C, lots of 64 bulbs were treated in warm water (2 h at 43.5 °C) to which conidia of the pathogen at various concentrations had been added, and stored thereafter at 17 °C (RH 70%) until planting in the field was possible at the artificially achieved temperatures of 13-15 °C (C) and 8-12 °C (B) respectively. Bulbs from both lots were digged and examined six weeks after planting when rooting was almost complete and the soil temperature had dropped to 5 °C.

The temperature appears to be important for establishment of an infection (Table 2.2.). In natural soil, no infection was observed when temperature at the time of root formation remained below 13 °C. A high inoculum density ($\geq 5x10^4$ con/ml) did not overcome this negative temperature effect, neither at incubation in sterile soil (2 w at 10 °C) nor at incubation in natural soil (6 w at 5-12 °C). In sterile soil, however, a very low concentration (500 con/ml) already gave high percentages of infection (> 77%) when the temperature exceeded 14 °C.

Table 2.2. Influence of inoculum density of *F. oxysporum* f. sp. *narcissi* and soil temperature during the first two weeks of rooting on the development of root rot. Root rot is expressed as the average of visible infected roots per bulb (n_A=6, $n_{B,C}$=64).

Experiment	°C in soil	Concentration of viable conidia per ml applied to bulbs after HWT							
		0	10^2	$5x10^2$	$5x10^3$	10^4	$2x10^4$	$5x10^4$	$5x10^5$
A (sterile soil)	10	0	0	0	0	0	-	-	0
A (sterile soil)	15	0	2	77	94	100	-	-	*
A (sterile soil)	20	0	6	94	100	*	-	-	*
B (field)	8-12	0	-	0	0	0	0	0	-
C (field)	13-15	0	-	3	13	15	20	24	-

* no rooting, roots completely rotten inside basal plate
\- effect of conidia concentration not tested

d. soil microflora. The influence of the soil microflora on infection of roots was studied in different ways. The effect of root age on infection was determined both in natural soil, taken from fields on which ornamental bulbs had often been grown, and in sterilized soil of the same origin.

Fig. 2.7. clearly shows that the soil microflora reduces the final infection percentage to a much lower level. It was also found in the same experiment that planting in sterilized soil of bulbs additionally contami-

nated with *Penicillium corymbiferum*, isolated from roots which grew in natural soil, resulted in infection percentages intermediate to those found with sterile and natural soils. Analogous effects of the soil microflora on infection are evident from the figures given in Table 2.2. More root rot developed on bulbs incubated in sterile soil (experiment A) than on those planted in the field (experiment C).

2.6. Infection of the basal plate

Over three years detailed information was collected about the penetration of the basal plate by *F. oxysporum* f. sp. *narcissi* and the mycelial growth inside the bulb base. Histological studies were made to elucidate the infection mechanism.

Active penetration of the basal plate of 'dormant' bulbs was never observed. When internally developed roots have reached the outer periderm, the first and sole opportunity to enter the bulb tissue arises (Fig. 2.3A.). The pathogen enters the underlying parenchymatous cells when the outer periderm is ruptured by the root tips. Intercellular growth follows at sufficiently high temperatures (12 ^{o}C) and quickly becomes 'intracellular' (Figs 2.3B., 2.6B.) at 17 ^{o}C or higher. Further mycelial extension is limited when walls of the cells surrounding the emerged roots become suberized and the inner periderm is formed (Figs 2.2., 2.3C., 2.5., 2.8A.). Depending on the balance between speed of fungal growth and rate of wound periderm formation, infection can establish itself and leads to rotting of the basal plate in autumn. Rotting will prohibit formation of wound periderm in the bulb cortex (Fig. 2.3B.).

In case part of this rotten cortex tissue, the so-called 'rupture rot', is not excluded from the healthy tissue by the inner periderm at completement of its formation, symptoms will also be expressed in the foliage in early spring. 'Dwarf plants' and small plants with bended or twisted leaves may develop, but, more often bulbs will show basal rot at lifting or during subsequent storage (see chapter 3).

Generally, a first infection is not followed by development of bulb symptoms in autumn, because soil temperatures are mostly too low (< 15 ^{o}C). Symptom expression fails to appear if the infection inside the cortex is completely excluded by the inner periderm. Remnants of such infection can be seen as brown or black pits consisting of disintegrated tissue, situated in old non-functional bulb tissue. They are termed necrotrophic contaminations for this reason.

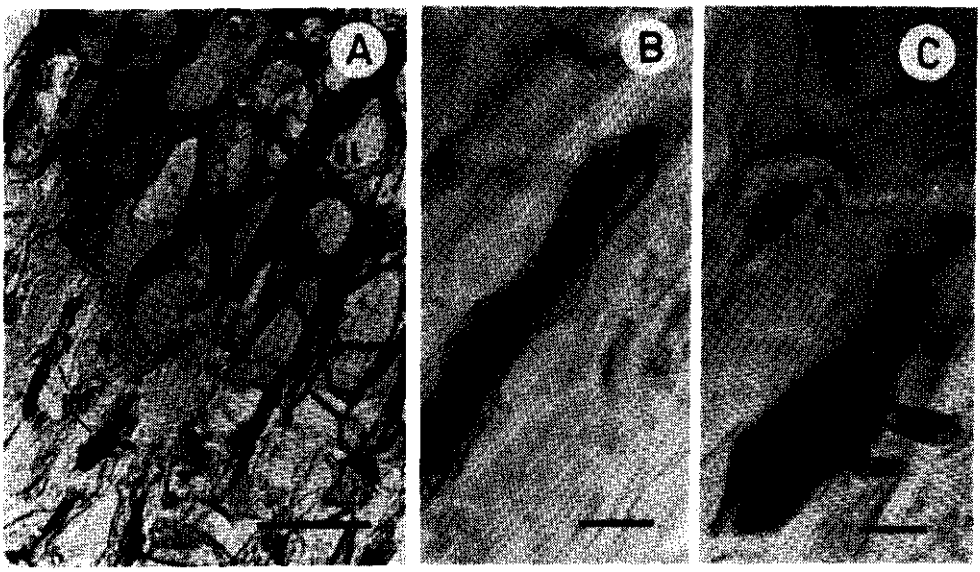

Fig. 2.8. Latent infections of *F. oxysporum* f. sp. *narcissi* in the cortex of a narcissus bulb. Tissue stained with CB-S method (see text). A: decaying tissue (DC) separated by the inner periderm (IP) from the still intact tissue of the cortex (C) with stained hyphe--like structures indicating presence of latent infections (HL and HD). B and C: detail of HD-infections in A. Bars represent 100 µm in A and 10 µm in B and C.

Sometimes, hyphae have already reached or passed the inner periderm, mycelial growth does not continue and active rotting cannot be observed in autumn. Such infections are defined as latent (Verhoeff, 1974). Two types of latent infections are distinguished here (Fig. 2.8.). The first type (HD = hyphe dormant) concerns an infection which has passed the narrow zone in which suberization of cell wall will result in formation of the inner periderm. It becomes latent with decreasing temperatures in autumn. Furthermore, biochemical and physiological changes, due to suberization near the infection site, may affect the growth activity of the mycelium as well. The still resting and viable mycelium will be reactivated during the next summer, when temperatures usually exceed 15 °C. In addition, the metabolism of the plant will also undergo a change and might influence reactivation of the pathogen as well. Leaf tips of full-grown leaves show often in May or June a premature yellowing, which indicates breaking of the latency. The other infection type (HL = hyphe latent) is

also called latent. The mycelium has been enclosed inside the cells of the inner periderm itself. Symptoms will never develop, unless this layer is ruptured, and the still living mycelium is able to penetrate the parenchymatous tissue lying behind the periderm.

The storage after HWT appears to be very critical, especially when bulbs showing the first signs of root emergence are wetted and contaminated with inoculum of the pathogen. If rooting is not prevented after wetting, normal penetration and infection occurs as described. When, on the other hand, bulbs are slowly dried and stored thereafter, the periderm will slightly be ruptured, although root emergence does not take place and consequently, formation of wound periderm fails to appear. If penetration takes place at this time, infection of the basal plate occurs, resulting in early development of basal rot. Quick drying within 24 h after wetting may prevent penetration completely.

2.7. Relation between root and basal plate infection

Judging from the histological evidence obtained it is possible that *F. oxysporum* f. sp. *narcissi* enters the basal plate via infected roots (Figs 2.3B., 2.3C.). Under circumstances where roots become infected in autumn, an infection of the basal plate may take place as well. A field experiment in which the conditions for autumn root infection were met provided further evidence for this possibility (Langerak, 1977). At that time attention was mainly paid to the effects of fungicides in the control of root rot, basal rot and 'skin disease', and to the influence of the pathogen and saprophytic fungi on the colonization of the roots. The data of Langerak (1977), supplemented with some new information are represented in Table 2.3. Both the degree of root colonization by the pathogen and the disease incidence in October and December correlate positively with the final percentage of basal rot at harvest, whereas the data obtained in June do not. Note, however, that in autumn infections of the bulb are visible, but those of the cortex covering the central cylinder of the bulb are not, unless the bulbs are sectioned.

Increase of the root decay indices between two sampling dates in June was not accompanied by increased colonization of the roots with *F. oxysporum* f. sp. *narcissi*. On the contrary, colonization of the roots by other formae speciales of *F. oxysporum* decreased in June, but relatively less for the saprophytic ones. A significant positive correlation ($P < 0.01$) was found

Table 2.3. Seasonal colonization of healthy roots by *F. oxysporum* f. sp. *narcissi* and development of symptoms in roots and bulbs. Hot-water treated bulbs (HWT), either contaminated in the bath with natural inoculum (25,000 propagules per ml) or not, and untreated bulbs were planted in September (9-14 °C) and lifted in July. Intermediate disease assessments were made on samples of 20 bulbs per treatment and a final one in July on the remaining 600.

Treatment	% roots colonized with the pathogen (n=5x100)				Disease incidence in roots and bulbs (indices 0-100)				% bulbs with basal rot
					root rot	root decay		cortex rot	
	October		June		December	June		December	July
	14th	28th	16th	30th	9th	16th	30th	9th	31st
HWT+pathogen	45	58	14	18	5.4	85	90	20	27
HWT-pathogen	1	3	5	6	1.5	75	95	6	2
Untreated	2	7	14	17	1.7	78	90	6	1

between increase of root decay indices and an increased colonization by *Trichoderma spp.* for bulbs not treated with fungicides and for bulbs treated with formalin which has no residual activity against fungi at all.

2.8. Discussion

Basal rot of narcissus can develop after infection of bulbs by *F. oxysporum* f. sp. *narcissi*, which often ensues from wounds inflicted at lifting and transport, or by cleavage of daughter bulbs from the mother bulb. Temperatures above 17 °C and high humidity promote symptom expression both during storage and already earlier in the soil (Hawker, 1935; McClellan, 1952). The disease also starts from infection through natural wounds in the basal plate which are caused by emergence of the roots. In this study more exact information was obtained about the natural course of penetration and infection of roots and basal plate.

Penetration and infection of roots is possible from the first moment of their emergence through the outer periderm layer of the basal plate (Figs 2.2., 2.7.). At this time, inoculum potential of the pathogen must be sufficiently high (Price, 1977b; Table 2.2.), and suberization of the exodermis must not have started (Fig. 2.4A.). In practice, this situation

presents itself only at the time new roots are produced during the first weeks of rooting in late summer as assumed by Hawker (1943b) and McClellan (1952). The root rot observed by them in early summer might be the result of penetration and infection in the preceding autumn or late summer. However, mistaking root rot caused by the pathogen for root decay caused by other formae speciales of *F. oxysporum* or saprophytes cannot be excluded (Table 2.3.).

The assumption that the pathogen is able to penetrate and infect the basal plate is probably correct. Penetration of the basal plate from senescent roots seems to be unlikely, since prior to senescence, the cortex and stele of the roots are cut off by a lignified abscission layer (Chan, 1952; Figs 2.2., 2.4B.). The roots become a substrate for many kinds of saprophytes besides the pathogen, and will consequently decay. Whether various saprophytic fungi and bacteria may cause and stimulate the process of root decay in early summer is largely influenced by the treatment given in autumn. In practice it is of little importance whether the basal plate is infected via roots or not. The chance of direct infection of the basal plate in autumn is always greater than that of root infection.

A prerequisite for direct penetration and infection of the tissue is the rupturing of the outer periderm by the calyptra of emerging roots (Figs 2.2., 2.3.). Furthermore, temperature and humidity have to be high enough to allow activation of and penetration by pathogenic inoculum. A decrease in temperature in autumn retards mycelium growth in the basal plate tissue. Progress of infection into the bulb can also be limited by wound periderm, and by the formation of an inner periderm across the cortex (Figs 2.2., 2.5., 2.8.).

This study concerning the pathogenesis of *F. oxysporum* f. sp. *narcissi* gave sufficient information to define the various types of infection more exactly than it was done in the past. This appeared to be essential in the evaluation of the experimental data from further investigations (see chapter 3 and 5). The existence of the invisible basal plate infections in autumn (Fig. 2.8.), called latent here in accordance with the description by Verhoeff (1974), was not demonstrated earlier. Hence, experimental data could often not be evaluated properly. For example, the sudden appearance of basal rot after lifting, especially at the point where mother and daughter bulbs are connected has always been a common but unexplained phenomenon. Gregory (1932) supposed that sand-grains

between the bulb scales might cause wounds in the basal plate tissue, so that penetration by the pathogen could follow. Our experience is, that a successful wound infection requires respectively: deeper wounds, a high inoculum potential of the pathogen, and above mentioned incubation conditions, factors, which will rarely occur simultaneously in practice. It is more likely that the latent infections inside the basal plate tissue play a role. Especially in full-grown more-nosed bulbs, the daughter bulbs will start separating from the mother bulb at the drying of the outer scales. A result might be the formation of small cracks within the cortex tissue and in the protecting periderm through which the pathogen can easily penetrate the most sensitive tissue of the bulbs.

Ruptures in the periderm and inside the tissue protected by it, can also arise in other ways, e.g. by rough handling during the period from lifting till planting. The most obvious ruptures, however, will originate when new roots emerge in late summer or autumn after a period of dormancy of the bulb. Further invasion of the basal plate by the pathogen will be inhibited then if temperatures remain below 15 ^{o}C.

Commercial growing of narcissus should involve careful handling of bulbs. Cutting off the not fully dead foliage, early lifting and cleaning of bulbs give rise to wounds in leaves, flower stems, roots and basal plates. Even when wound healing follows, it does not occur at places where periderm would be formed as a means of natural abscission. Furthermore, spread of inoculum is favoured by all handling of the bulb material, especially by the application of a hot-water bath.

In order to control basal rot, particular cultural measures have to be taken, which will be discussed in chapter 3 and 5. Chemicals should be used during HWT since in the absence of fungicides the pathogen is not fully killed by this treatment (Table 2.1.). Fungicides have to be added to avoid further spread of inoculum. Curing of visibly of latently infected bulbs is difficult and almost impossible in practice.

Residual activity of fungicides against *F. oxysporum* f. sp. *narcissi* is only required for the short period in which roots emerge. Very persistent fungicides, active against *Fusarium spp.*, or fungicides which selectively favour soil-borne or bulb-borne antagonistic saprophytic fungi have to be used to inhibit root decay in early summer as well as 'skin disease'. Certain formulations of pimaricin and combinations of formalin and thiram appear to meet these requirements as do mercurials (Langerak, 1977).

3 THE RELATION BETWEEN AUTUMNAL INFECTION OF NARCISSUS BULBS BY *FUSARIUM OXYSPORUM* F. SP. *NARCISSI* AND DISEASE DEVELOPMENT IN THE FOLLOWING SEASON

3.1. Introduction

Basal rot of narcissus is caused by *Fusarium oxysporum* Schlecht. f. sp. *narcissi* (Cooke & Massee) Snyder & Hansen. Healthy bulbs may become contaminated via contact with diseased bulbs during storage, through air-borne inoculum and/or by spread of inoculum during a hot-water treatment (HWT), which is recommended for control of parasitic insects, mites and nematodes before planting (Van Slogteren, 1931). Spread of the pathogen in the bath can be prevented by addition of a suitable fungicide (Hawker, 1940; Miller & Gould, 1967; Langerak, 1977).

Survival of the pathogen during HWT, storage conditions after treatment, soil temperatures at planting, and the physiological condition of the bulbs affect infection of the roots and basal plate, as well as the development of symptoms. Infection occurs at emergence of the roots in late summer or autumn. Development of rot in roots and basal plate is only possible in autumn if circumstances are favourable for the pathogen (Langerak, 1977; see chapter 2). The first signs of such infections can be noticed during the flowering period (Gregory, 1932; see chapter 2), although bulbs which have been infected in autumn do not always give development of symptoms in the foliage during the subsequent growing season. Infections may become latent under conditions being not favourable for the pathogen and result in a delay of symptom expression until next summer (see chapter 2). Presence of symptoms is usually most obvious at the lifting of the bulbs in summer or during the following storage period (Gregory, 1932).

It is of economic importance, that symptomless bulbs from a crop looking healthy in spring and early summer often show a severe development of basal rot during the following storage period. Such symptom development is stimulated by a relatively high soil temperature before lifting and by humid and warm storage thereafter (Hawker, 1940, 1943b; McClellan, 1952; Price, 1975). Unaware of the presence of latent infections, growers lift their bulbs often too late and even store them under conditions which are conducive to disease development. When bulbs are graded early, hot-water treated and disinfected, it is usually not realized that development of basal rot will continue in spite of such treatments, because deeply located

dormant infections are not eliminated (Hawker, 1935). Furthermore, early planting at high soil temperature of a bulb lot with such hidden infections will put off the disease problem till the next harvest period (see chapter 2).

Prediction of the disease incidence in the following harvest opens the possibility to make recommendations about preventive cultural measures in addition to chemical disinfection. The aim of this study was to investigate whether there is a relationship between autumnal infection and subsequent disease development, and to contribute to a scheme of preventive cultural recommendations. All experiments were carried out in the field so as to achieve conditions that approximated the practical situation. For the same reason, bulbs were either contaminated with different concentrations of conidia of the pathogen, or with one concentration of conidia in combination with a fungicide in order to achieve the same. In addition to standard fungicides, experimental formulations of pimaricin were used, an antibiotic which had given promising results for replacement of mercurials (Langerak, 1977).

3.2. Materials and methods

Plant material. 'Round bulbs' were obtained from one commercial stock of narcissus bulbs (*Narcissus pseudonarcissus* cv. 'Carlton'), which were stored outdoors in open trays at 14 - 20 °C until they were hot-water treated. Visibly infected bulbs were discarded before use.

The fungus. One highly virulent single-conidium culture of *Fusarium oxysporum* f. sp. *narcissi* (collection code F_9) was used in all experiments. The fungus was isolated from roots of a narcissus bulb, tested for its pathogenicity and maintained as described in chapter 2. Conidia needed in the field experiments were cultured in large quantities on autoclaved bulb scales of narcissus (Langerak, 1977).

Soil. Experiments were carried out on the experimental garden of the Bulb Research Centre in Lisse, The Netherlands (in sandy soil on which no flower bulbs had been grown for two years).

Fungicides. 'Aretan Rood' (Hg), a formulated product containing methoxy mercury chloride (3%) was purchased from Bayer-Agrochemie N.V., Arnhem, The Netherlands. Experimental formulations of pimaricin were kindly provided by Gist-Brocades N.V., Delft, The Netherlands. One formulation with the code name Pim-N concerned a wettable powder which only partly dissolved in water;

the others with code names BAM-B, BAM-C and BAM-D were liquid formulations, each containing pimaricin in a different concentration; miscible with water in the ratio of 1 : 50 (v/v) so that the antibiotic dissolved completely up to 200 µg/ml.

Treatment and storage of bulbs in field experiments. Series of two similar portions A and B of 96 bulbs each, packed in nylon rope nettings, were simultaneously hot-water treated (2 h at 43.5 °C) in one bath. Conidia of the pathogen were suspended in the bath at the start of the HWT (T_s) and fungicides were added depending on the intention of the treatment. Each treatment was repeated four times. Treated bulbs were stored outdoors under cover of a black polyethylene sheet for 4 days at about 17 °C and RH 95%. Thereafter, bulbs of lot A were planted in the field at a depth of 10 cm and at soil temperatures, which varied between 13 and 15 °C (method A). Bulbs of lot B were stored at 17 °C (RH 70%) for 14 days and then planted at a soil temperature of 8 - 12 °C (Method B).

Assessment of conidial viability after HWT. Viability of conidia was measured at the start (T_s) and the end (T_e) of the HWT.

When no fungicides had been added, duplicate samples of 5 ml were taken from the water in the bath at T_s and T_e, diluted with sterile water so that 1 ml did not contain more than 200 living conidia. Five samples of 1 ml were mixed each with 20 ml of PDA containing 50 µg/ml vendarcin (PDA-V) and poured into petri dishes of 14 cm diameter. Colonies were counted after 2 and 5 days incubation at 25 °C.

In the presence of fully dissolved fungicides, 2 samples of 25 ml were taken from the bath at T_s and T_e, and centrifuged at 3,000 g for 15 min in a Home-F centrifuge. The pellet containing the conidia was washed several times with sterile water, followed by centrifuging, diluted and plated with PDA-V as described.

3.3. Symptom development in roots and bulbs contaminated with conidia of *F. oxysporum* f. sp. *narcissi* before planting

Hot-water treated bulbs were contaminated with conidia and planted according Methods A and B. Assessments were made for: a. root rot in December, b. bulb rot in December, c. disease symptoms in the foliage and bulbs in April and d. advanced basal rot at lifting in July and after storage in August.

a. Root rot in December. Samples of bulbs from both lots A and B were lifted in the first week of December and stored at 5 - 10 °C until examination took place within 2 days after lifting.

Bulbs planted at 8 - 12 °C did not show root rot. Roots of bulbs planted at 13 - 15 °C, however, showed reddish-brown longitudinal lesions of 0.5 - 4 cm. Lesions were usually located near the bulb base when bulbs were contaminated with conidia at low dosages (Fig. 3.1B.). When heavily contaminated, lesions could be observed at any distance of the bulb base (Fig. 3.1A.). Very small lesions at the root base (Fig. 3.1C.) were sometimes found when fungicides had been added during HWT.

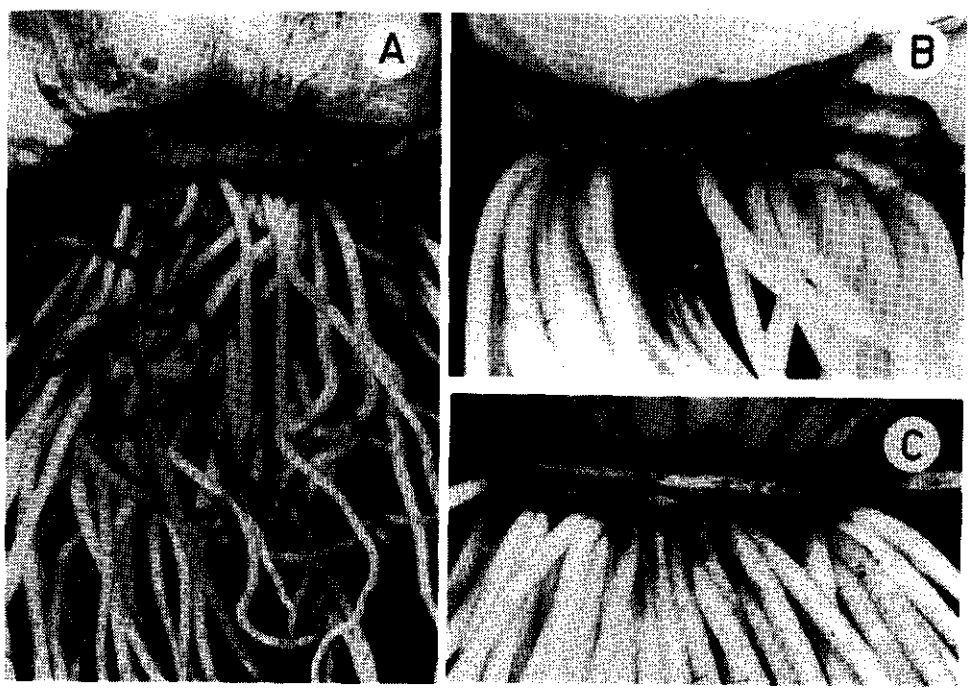

Fig. 3.1. Root rot of narcissus caused by *F. oxysporum* f. sp. *narcissi* at 13 - 15 °C during root emergence at heavy (A) and at moderate contamination with the pathogen, either in absence (B) or in presence of a fungicide (C).

The root rot incidence in December (y_{RD}) obviously depends on the inoculum density of the pathogen (x_e) in the hot-water bath on T_e (Table 3.1., Fig. 3.2A.). Regression of the y_{RD}-values on the logarithms of x_e results

in a linear relationship being statistically significant at P = 0.01. The following regression equation was obtained: $\bar{y}_{RD} = 15.8 \log x_e + 19$. Extrapolation of x_e to a 'zero-level' of contamination (e.g. 1 con/ml), testing the intercept on the y-axis for significance (P = 0.05) indicates that about 20 percent of the bulbs would have shown root rot without any free inoculum in the bath water. Thus an inoculum source different from that added to the bath must have contributed to infection of the roots. It was

Table 3.1. Influence of the inoculum density during HWT, the storage conditions after HWT and soil temperature at planting time (methods A and B) on disease development during the growing season and after harvest. Bulbs exposed during HWT either to two different dosages of conidia (without pimaricin) or to 75,000 con/ml at two different dosages of pimaricin, stored subsequently and planted according to method A and B. Disease development expressed in average percentages of the number of bulbs of plants examined per treatment.

Date (n): symptoms	Percentage of visibly infected bulbs or plants							
	Survival of conidia after HWT (con/ml)							
	without pimaricin				with pimaricin[a]			
	method A		method B		method A		method B	
	50	3,900	50	3,900	50	3,900	50	3,900
December (4 x 16 bulbs):								
bulbs with root rot	44	81	0	0	3	44	0	0
rupture rot, normal sprout	2	5	3	19	0	6	2	14
basal rot, normal sprout	0	2	2	10	0	3	0	7
rupture rot, abnormal sprout	0	0	0	0	0	0	0	0
basal rot, abnormal sprout	0	0	0	8	0	0	0	0
advanced basal rot, no sprout	0	0	4	11	0	0	0	12
April (4 x 80 plants):								
sickle plants	2	5	2	8	0	5	1	8
dwarf plants	0	3	2	14	0	2	1	3
blind plants	0	0	3	14	0	0	1	8
July (4 x 72 bulbs):								
advanced basal rot	32	65	8	33	2	53	1	21
advanced basal rot, mummified	5	6	3	31	1	7	0	7
August:								
new advanced basal rot (extra)	15	16	9	10	0	13	1	9

a) Survival of conidia after HWT at the pimaricin concentrations of respectively 159 and 28 µg/ml.

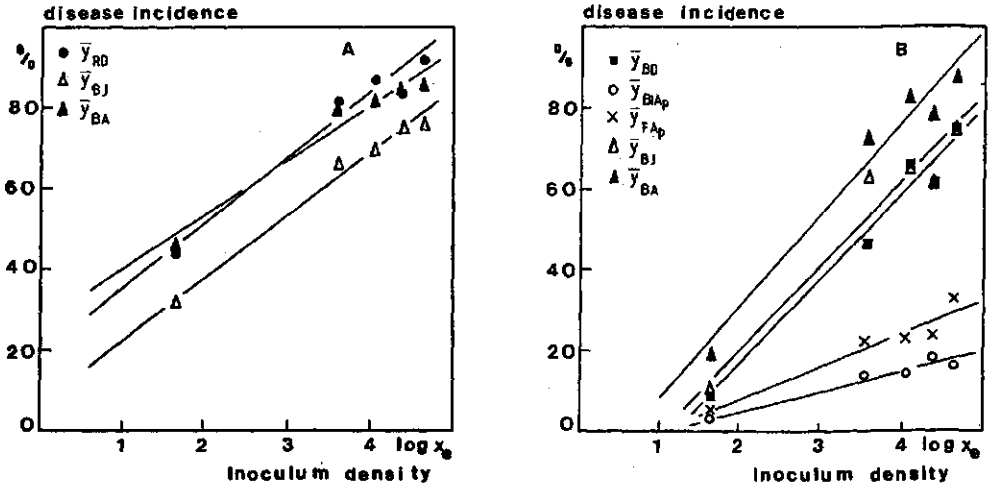

Fig. 3.2. Relation between concentration of viable conidia of *F. oxysporum* f. sp. *narcissi* on hot-water treated bulbs and disease development during the growing season. Bulbs after contamination during HWT stored and planted at different soil temperatures according to method A (A, 13 - 15 °C) and method B (B, 8 - 12 °C). Disease incidences expressed as average percentages of diseased bulbs or plants with $\bar{y}_{RD}$, bulbs with root rot in December; $\bar{y}_{BD}$, bulbs with bulb rot in December; $\bar{y}_{BlAp}$, blinds in April; $\bar{y}_{FAp}$, total of sickle and dwarf plants in April; $\bar{y}_{BJ}$, basal rot in July and $\bar{y}_{BA}$, basal rot in August. Inoculum density as log x_e, the logarithm of the conidial survival (con/ml) at the end of the HWT.

found that bulbs from the same stock, which were similarly treated in presence of formalin, and planted under the same conditions did not show any root rot at all. Knowing that formalin does kill almost all inoculum in the bath, but does not have a residual effect thereafter (Langerak, 1977), it may be concluded therefore, that the bulbs themselves had carried some inoculum internally, and the soil can be excluded as the inoculum source for the extra root rot.

b. Bulb rot in December. After examination of the roots for the presence of root rot, all bulbs were longitudinally cut across the basal plates. Few of the heavily contaminated bulbs of the lots planted at 13 - 15 °C showed infections of the basal plate. Much more bulb rot was found in bulbs, which were stored after HWT according to method B prior to planting at 8 - 12 °C. Various types of symptoms could be observed (Fig. 3.3.).

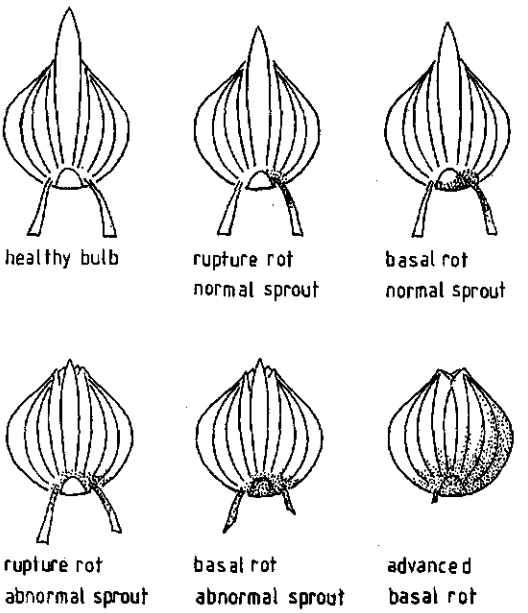

Fig. 3.3. Disease symptoms in December of bulbs, which were hot-water treated and contaminated with *F. oxysporum* f. sp. *narcissi* in September, subsequently stored and planted at 8 - 12 °C according to method B.

Occurrence and severity of the symptoms in December (y_{BD}) strongly depend on the inoculum density (x_e) after HWT as follows from Table 3.1. and Fig. 3.2B. The significant linear relationship ($P = 0.01$, obtained by regression of the $\bar{y}_{BD}$-values on the logarithms of the x_e-values ($\bar{y}_{BD} = 21.6 \log x_e - 3$) does not indicate a contribution of bulb-borne inoculum in the development of bulb rot as it was found for the root rot development under planting conditions of method A.

c. Disease symptoms in the foliage in April. All remaining plants were inspected in the field for emergence of the sprout and symptom development in the foliage. Additionally, 8 plants of a single row in each plot were lifted in order to compare symptoms in the sprouts with those inside the bulb base.

Four degrees of disease development could be distinguished: 1. apparently healthy, 2. basal rot without sprout development ('blinds'), 3. basal rot and poor sprout formation ('dwarf plants') and 4. basal rot and some curved leaves ('sickle plants') (Fig. 3.4.). Abnormal plants always originated from visibly infected bulbs. Healthy looking plants had sometimes grown from

bulbs with a slight degree of 'rupture-rot' in the basal plate. Clear symptoms in the foliage were mainly noticed in bulbs which were stored according to method B prior to planting at 8 - 12 °C and were rarely seen in those stored according to method A.

Symptom expression appears to be positively correlated with the degree of contamination with viable conidia after HWT (Table 3.1.). For both the percentages of 'blinds' and the percentages of 'dwarf and sickle plants', a linear relationship, being statistically significant at P = 0.01, was found with the logarithms of x_e (Fig. 3.2B.). With the equations $\bar{y}_{BlAp} = 4.9 \log x_e - 4$ and $\bar{y}_{FAp} = 8.5 \log x_e - 10$, it is illustrated once more that bulb-borne inoculum plays a negligible role in the attack of the bulbs stored and planted according to method B.

d. Basal rot in July and August. In the second week of July all bulbs, including the mummified remnants of heavily diseased bulbs, were examined for the presence of basal rot and stored in open trays outdoors at temperatures varying between 20 °C and 30 °C. The final percentages of bulbs showing basal rot were determined at the end of August.

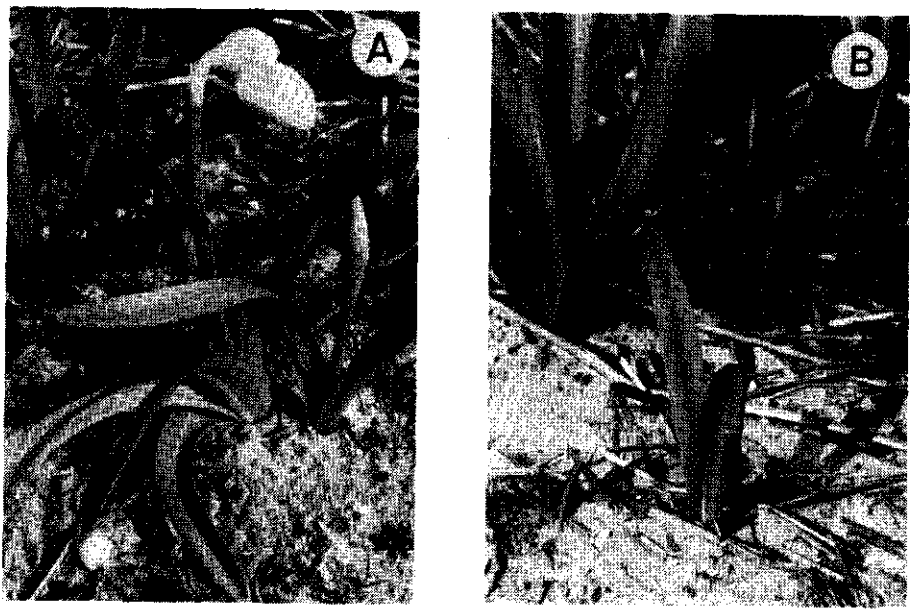

Fig. 3.4. Symptoms in the foliage of narcissus in April, caused by *F. oxysporum* f. sp. *narcissi*. A, sickle plant; B, dwarf plant.

In July all stages of basal rot development could be observed; from small externally visible brown lesions in the basal ring tissue between the root ring and the outer brown bulb skins, to fully decayed bulbs composed solely of shriveled scales. These mummified bulbs were counted separately. During further storage new basal rot development was observed in originally healthy looking bulbs. The new symptoms were mainly found at the connection points between mother and daughter bulbs.

Table 3.1. gives final data about basal rot development in July and August for two levels of x_e. Linear graphs with $\bar{y}_{BJ} = 15.2 \log x_e + 7$ and $\bar{y}_{BA} = 13.3 \log x_e + 27$ in Fig. 3.2A. and $\bar{y}_{BJ} = 20.5 \log x_e - 20$ respectively $\bar{y}_{BA} = 23.9 \log x_e - 16$ in Fig. 3.2B. show the relationship between the inoculum density at the end of the HWT (x_e) and the extent of basal rot which finally developed. Symptom development was most advanced in bulbs which were stored according to method B and planted at 8 - 12 °C. The total disease incidence, however, appeared to be the highest for the bulbs stored according to method A and planted at 13 - 15 °C.

The conclusion is drawn here again that storage and planting conditions of method A provoked better circumstances for internal bulb infections at planting time than those of method B did.

3.4. The influence of bulb treatment with fungicides on disease development

A study of the relation between autumnal infections and disease development in the following season also required evaluation of data which originated from a more practical situation than those in the previous paragraph did. Differentiation in disease levels and infection types was achieved therefore by contamination of bulbs with conidia of *F. oxysporum* f. sp. *narcissi* at the beginning of the HWT (75,000 con/ml) and a simultaneous treatment with various fungicides. Standard chemicals added to the bath were an organic mercury compound (Hg) and formalin (Form). In addition experimental formulations of pimaricin were used, (BAM-B, BAM-C, BAM-D and Pim-N). The effect of the pimaricin concentration was measured in BAM-B and Pim-N series. Bulbs were stored after HWT, and planted according to methods A and B. Conidial survival was determined at the end of the HWT (T_e) and assessments for disease symptoms were made in December, April, July and August.

a. Conidial survival at HWT. Both the standard fungicides and the experimental formulations of pimaricin with dosages higher than 150 µg/ml reduced the

concentration of living conidia to 50 con/ml or less. Therefore, very low disease incidences were found for these treatments. The treatment objects of the BAM-B and Pim-N concentration series with dosages of dissolved antibiotic less than 150 µg/ml permitted enough survival of pathogenic conidia that reasonable levels of root and bulb attack could develop.

b. Root rot in December. Bulbs planted at 8 - 12°C (method B) did not show root rot at all, those planted at 13 - 15 °C (method A) gave symptoms as shown in Fig. 3.1C., provided that more than 50 viable conidia were found at T_e (Table 3.1., Fig. 3.5A.). The relationship between conidial survival and disease incidence (y_{RD}) in pimaricin treated bulbs appeared to be linear (P = 0.01) at regression of the disease incidences on the conidial survival, x_e, transformed in its square roots ($\bar{y}_{RD} = 0.7 \surd x_e - 2.3$).

c. Bulb rot in December. Symptoms in the basal plate were rare when bulbs were planted at 13 - 15°C (method A). Visible infections were found in bulbs which were planted at 8 - 12 °C (method B), provided that the bath water contained at least 50 viable conidia per ml at T_e (Table 3.1., Fig. 3.5B.). At a significant correlation between the extent of conidial survival and disease incidence (y_{BD}), a significant linear relationship (P = 0.01) between both variables was found provided that the x_e-values had been transformed in their square roots ($\bar{y}_{BD} = 0.51 \surd x_e - 2.3$).

d. Disease symptoms in the foliage in April. Malformations in the foliage could mainly be observed when the treated bulbs were stored and planted according to method B. Severe development of symptoms in the foliage (y_{FAp}) and non-emergence of 'blind plants' (y_{BlAp}) were only found for treatments with pimaricin at dosages of 100 µg/ml or less resulting in a conidial survival of 400 con/ml or more (Table 3.1., Fig. 3.5B.). The same kind of relationship between disease development and $\surd x_e$ is noticed here as found for the root and bulb rot in December; with $\bar{y}_{FAp} = 0.17 \surd x_e + 0.1$ and $\bar{y}_{BlAp} = 0.49 \surd x_e - 2.3$.

e. Basal rot in July and August. Basal rot was found in bulbs of both lots A and B whether they were planted at 13 - 15 °C or at 8 - 12 °C. The final disease incidence was higher in the former than in the latter case, especially at the higher levels of contamination in the hot-water bath, viz. on T_e. Treatment with the standard fungicides or the pimaricin formulations at higher dosages of the antibiotic dissolved in water (> 150 µg/ml) reduced the development of basal rot strongly to levels below 5%. A relatively good effect against basal rot was obtained at low concentrations

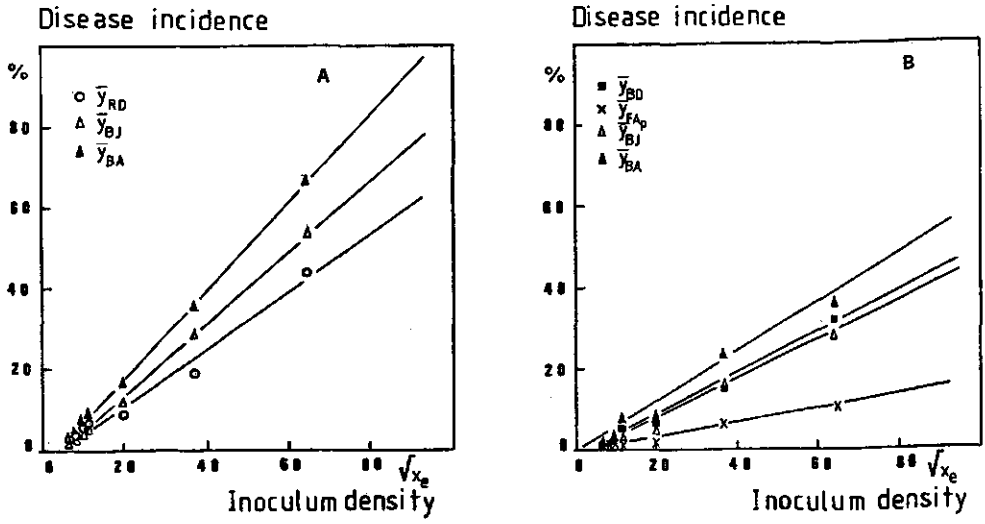

Fig. 3.5. Relation between concentration of viable conidia of *F. oxysporum* f. sp. *narcissi* on bulbs hot-water treated in presence of various dosages of pimaricin and disease development during the growing season. Bulbs after contamination and treatment during HWT stored and planted at different soil temperatures according to method A (A, 13 - 15 °C) and method B (B, 8 - 12 °C). Disease incidences expressed as average percentages of diseased bulbs or plants with $\bar{y}_{RD}$, bulbs with root rot in December; $\bar{y}_{BD}$, bulbs with bulb rot in December; $\bar{y}_{FAp}$, total of sickle and dwarf plants $\bar{y}_{BJ}$, basal rot in July and $\bar{y}_{BA}$, basal rot in August. Inoculum density as $\sqrt{x_e}$, the square root of the conidial survival (con/ml) at the end of the HWT.

of pimaricin (<100 µg/ml). Table 3.1. and Figs 3.2. and 3.5. demonstrate this when the various data are compared with each other. A particularly good response was obtained when bulbs were planted at 8 - 12 °C. In that situation a conidial survival of approximately 4,000 con/ml results in 40% basal rot, but when planted at 13 - 15 °C, 65% is found. The response at low soil temperatures at planting time is less pronounced at the same level of contamination in absence of pimaricin. Then, an infection of 75% is reduced to 65%. For lot A, $\bar{y}_{BJ} = 0.9 \sqrt{x_e} - 5.1$ and $\bar{y}_{BA} = 1.1 \sqrt{x_e} - 5.1$ is found, for lot B respectively $\bar{y}_{BJ} = 0.49 \sqrt{x_e} - 2.3$ and $\bar{y}_{BA} = 0.61 \sqrt{x_e} - 1.1$.

3.5. Relationship between presence of root rot in December and basal rot in summer, with or without fungicide treatment

Bulbs contaminated with conidia of *F. oxysporum* f. sp. *narcissi* during HWT, stored for 4 days at 17 °C, and planted at 13 - 15 °C in the field (method A) showed root rot in December (Fig. 3.1.) and basal rot after lifting in summer. Although there is no direct causal relationship between root rot and development of basal rot (see chapter 2), a mathematical relationship might exist between the appearance of symptoms on roots and the basal plate, because in both cases infection takes place at almost the same time and originates from the same sources of inoculum, viz., bulb-borne mycelium, spores, conidia and mycelial fragments dispersed in the hot-water bath. The existence of a relationship between symptom development in autumn and disease incidence in summer was therefore investigated, using data from field experiments, obtained with 16 different kinds of treatments, involving hot-water treatments with or without addition of fungicides and with addition of conidia of the pathogen. A part of these data has already been presented in the previous paragraphs to demonstrate the dependence of the disease incidences on the degree of contamination with the pathogen during the HWT.

A first evaluation of the data showed that those of two pimaricin treated objects (encircled in Fig. 3.6.) deviated obviously from the data of the other objects. Calculation of the ratio's for the average incidences of root rot ($\bar{y}_{BJ}$) and of basal rot in August ($\bar{y}_{BA}$) learned that the $\bar{y}_{BA}/\bar{y}_{BJ}$ values did not differ significantly from each other for any of the 16 objects. Thus, development of symptoms after lifting is neither depending on the fungicide used during the previous HWT, nor on the degree of contamination with the pathogen thereafter. In contrast with this, the ratio's $\bar{y}_{BJ}/\bar{y}_{RD}$ exceeded the value of 1.0 significantly for the two pimaricin objects mentioned above, whereas those for the other objects did not. This may implicate that at low dosages of the antibiotic (<100 µg/ml) either symptom expression has been suppressed in autumn or that roots have been penetrated by the pathogen some time after the basal plates were infected. For this reason, the data of these pimaricin treated objects were omitted in a statistical analysis of the data. It was found that the basal rot incidences determined in July and August correlate positively with the incidences of root rot found in samples drawn in December.

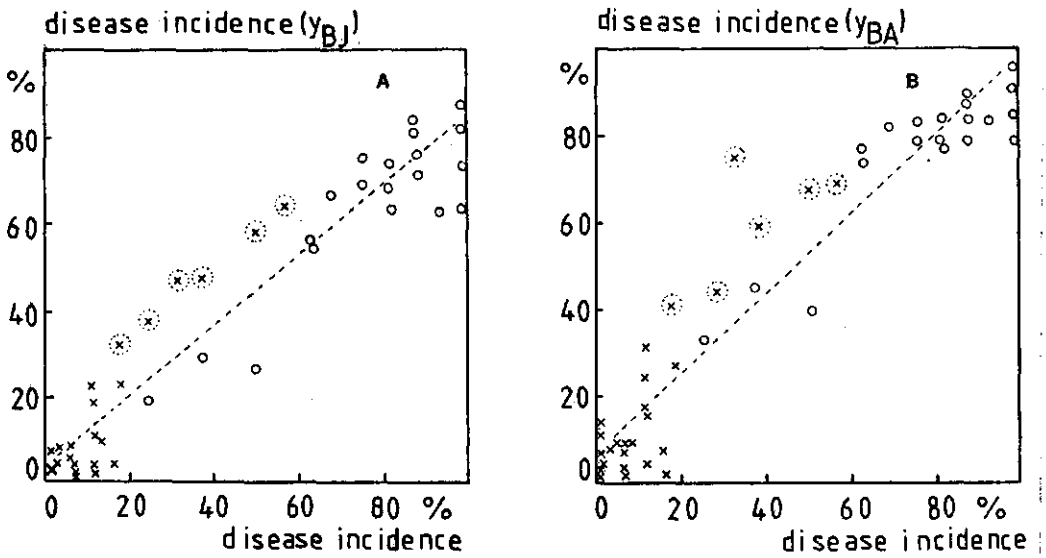

Fig. 3.6. Relation between the presence of root rot in December and the basal rot incidences in July and August. Bulbs were hot-water treated and simultaneously contaminated either with various concentrations of *F. oxysporum* f. sp. *narcissi* conidia only (o) or with 75,000 con/ml of the pathogen in presence of various dosages of pimaricin (x), stored and planted at 13 - 15 °C (method A). A: percentages of bulbs with basal rot in July (y_{BJ}) plotted against the percentages of bulbs with visible root rot in December (y_{RD}), and B: percentages of bulbs with basal rot in August (y_{BA}) against y_{RD}. Encircled points concern data of treatment objects for which 28 and 56 µg/ml of pimaricin was measured and the end of the HWT.

The circumstances under which the experiment was carried out were optimal for disease development in roots and bulbs. Less than optimal conditions, however, would have given lower coefficients of regression. Development of new basal rot was not observed after the final assessment in August.

It is concluded therefore, that the percentage of bulbs showing any root rot in December will give a reasonable estimation of the maximum percentage of bulbs which can show basal rot at lifting or during storage in the subsequent summer, provided the circumstances after planting in autumn are suitable for infection of the roots and of the basal plate tissue. Absence of root rot in December, when bulbs are planted early at soil temperatures exceeding 12 °C, will normally be followed by a very low disease incidence in spring and next summer. When planted at lower temperatures, however, one

has to consider the presence of bulb infections only. The relevance of such infections for disease development later in the season will be evaluated in the next paragraph.

3.6. Relationship between presence of bulb rot in December, disease symptoms in the foliage in spring and basal rot in summer, with or without fungicide treatment

Hot-water treated bulbs, contaminated with conidia of *F. oxysporum* f. sp. *narcissi*, stored for 18 days at 17 °C, and planted at 8 - 12 °C in the field (method B), showed different types of basal plate infections in December (Fig. 3.3.). Various malformations in the emerged sprouts in spring (Fig. 3.4.) and basal rot at lifting in summer were noticed. The occurrence of all kinds of symptoms at different times during the growing period was directly related to the level of contamination with the pathogen during the HWT whether fungicides were added to the bath or not (Figs 3.2B. and 3.5B.).

An attempt was made to predict the extent and nature of further disease development in spring both in bulbs and in the foliage based on the occurrence of symptoms in December. A scheme was elaborated empirically for the data obtained in one control object to enable an estimation of the disease development in April for the other treated objects (Table 3.2.). The usefulness and accuracy of this table became evident when it was tested afterwards against the data obtained for the other control objects and different fungicide treatments (Tables 3.3. and 3.4.). Data in Table 3.4. show that crop inspection in spring (April) may not give full information about the actual disease incidence. Especially missing plants ('blinds') will be overlooked. Such blinds will undoubtedly contaminate the other bulbs at

Table 3.2. Relationship between infection of bulbs in December and disease symptoms on above ground plant parts in April.

Symptoms in bulbs in December	% of plants with visible symptoms in the foliage			
	none	sickle plant	dwarf plant	blind plant
none, normal sprout	100	0	0	0
rupture-rot, normal sprout	75	15	10	0
basal rot, normal sprout	10	30	60	0
rupture-rot, abnormal sprout	0	10	80	10
basal rot, abnormal sprout	0	0	60	40
advanced basal rot	0	0	0	100

Table 3.3. Relationship between the various disease symptoms developed during the growing season. Bulbs were subsequently soaked in hot-water (2 h at 43.5 °C) in which 23,500 con/ml survived, stored and planted according to method B. Figures between parentheses represent the actual values determined, the others are deduced from those by means of Table 3.2. and by using information obtained at crop and sample inspection on several dates in spring.

Date (n)	Symptom		Symptom expression for 64 bulbs in December (%)						
			no symptom		normal sprout		abnormal sprout		no sprout
			healthy	infected	rupture rot	basal rot	rupture rot	basal rot	advanced basal rot
		A/B [a]	(23)	(16)	(25)	(12)	(2)	(2)	(20)
April	none	(59)	23	16	19	1	0	0	0
(320)	sickle plant	(7)	0	0	3	4	<1	0	0
	dwarf plant	(15)	0	0	3	7	1	1	3
	blind plant	(19)	0	0	0	<1	<1	1	17
July	none	(39)	23	16	0	0	0	0	0
(288)	basal rot	(34)	0	0	25	8	1	0	0
	basal rot mummified	(27)	0	0	0	4	1	2	20
Aug.	none	(23)	23	0	0	0	0	0	0
(288)	basal rot	(50)	0	16	25	8	1	0	0
	basal rot mummified	(27	0	0	0	4	1	2	20

[a] A, actual percentages determined in December; B, actual percentages in April, July and August.

Table 3.4. Symptom development on narcissus bulbs and plants in April. Bulbs contaminated with conidia of *F. oxysporum* f. sp. *narcissi* during HWT, treated simultaneously by addition of fungicides to the bath (except in controls, see foot note), stored and planted according to method B. Disease incidences expressed in percentages of bulbs or plants with symptoms either determined at inspection of the crop and of samples drawn in April, or estimated on the basis of data obtained for samples drawn in December by means of the scheme presented in Table 3.2.

Treatment	Average percentage diseased in April (n_{crop} = 320, n_{sample} = 32)											
	healthy plants			sickle plants			dwarf plants			blind plants		
	inspection		estimated	inspection		estimated	inspection		estimated	inspection		estimated
	crop	sample		crop	sample		crop	sample		crop	sample	
Control B[a]	96	93	94	2	2	1	2	2	1	0	3	4
Control C	74	64	67	9	8	6	17	14	13	0	14	14
Control D	73	57	48	9	8	8	18	18	19	0	17	25
Control E	60	50	46	10	9	9	30	25	19	0	16	26
Mean of controls	*75.8*	*66.0*	*63.8*	*7.5*	*6.7*	*6.0*	*16.7*	*14.8*	*13.0*	*0*	*12.5*	*17.2*
Bam-B-1[b]	87	81	79	9	8	4	4	3	5	0	8	12
Bam-B-2	93	92	95	3	3	2	4	4	1	0	1	2
Bam-B-3	97	95	98	2	2	1	1	1	1	0	2	0
Bam-B-4	98	97	98	1	1	1	1	1	0	0	1	1
Mean of BAM-B treatments	*93.7*	*91.2*	*92.5*	*3.8*	*3.5*	*2.0*	*2.5*	*2.3*	*1.8*	*0*	*4*	*3.7*
Aretan[b]	100	100	100	0	0	0	0	0	0	0	0	0
Formalin	99	99	98	1	1	1	0	0	0	0	0	1
Mean of standards	*99.5*	*99.5*	*99.0*	*0.5*	*0.5*	*0.5*	*0*	*0*	*0*	*0*	*0*	*0.5*

[a] on T_e : concentration of viable conidia for B, C, D and E respectively 50, 3,900, 12,500 and 75,000 con/ml.

[b] on T_s : concentration of viable conidia for all fungicide treatments 75,000 con/ml; dosages of pimaricin in BAM-B-(1-4) respectively 28, 56, 94 and 156 µg/ml.

lifting while part of the disintegrated tissue will remain in soil, so that such particular field will need special attention when used again for narcissus growing. The estimation made in December gives this information about the blinds and will indicate in addition whether roguing is desirable to remove, for example, dwarf and/or sickle plants.

Application of Table 3.2. on data obtained from samples of 60 bulbs, randomly taken from about one thousand bulbs harvested in December, underscored the accuracy and usefulness of this table once more. The disease incidences to be expected in April were in good agreement with the actual average values determined by crop and sample inspection. It was calculated from this that the disease incidence for about 100 kg of bulbs randomly sampled per hectare in December may predict with good accuracy the number of missing plants, the visible symptom development in the crop and the percentage of healthy bulbs.

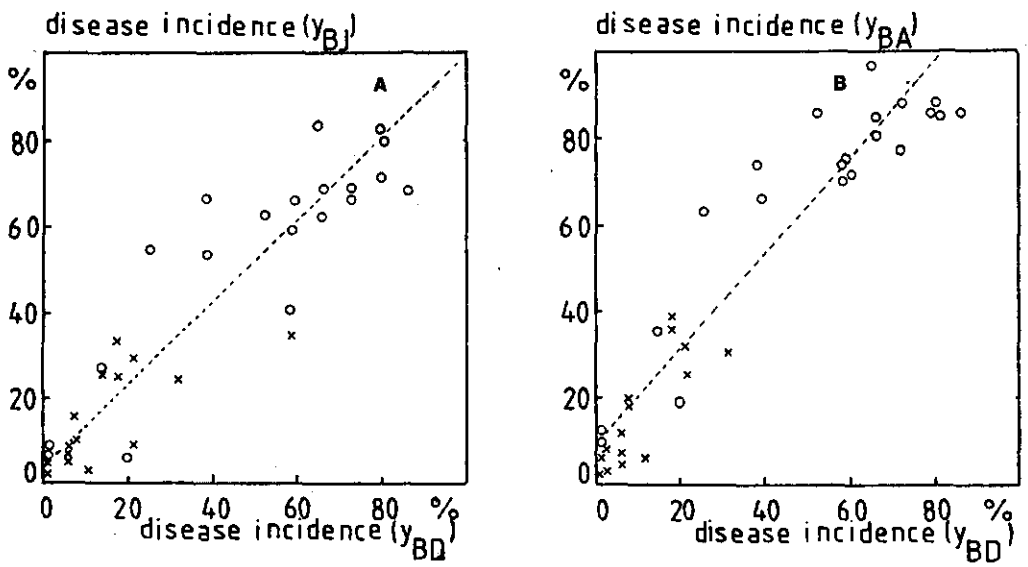

Fig. 3.7. Relation between the presence of bulb rot in December and the observed disease incidences in July and August. Bulbs hot-water treated and simultaneously contaminated either with various concentrations of *F. oxysporum* f. sp. *narcissi* conidia only (o) or with 75,000 con/ml of the pathogen in presence of various dosages of pimaricin (x), stored and planted at 8 - 12 °C (Method B). A: percentages of bulbs with basal rot in July (y_{BJ}) plotted against percentages of bulbs with visible rot in December (y_{BD}) and B: percentages of bulbs with basal rot in August (y_{BA}) against y_{BD}.

The relationship between percentages of bulbs showing bulb rot in December, disease symptoms in the foliage in April and basal rot in July and August is obvious (Table 3.1., Figs 3.2B. and 3.5B.). High positive correlations were found when data obtained in December were compared with those determined later in July and August (Fig. 3.7.) for the control and some pimaricin treated objects. The finally obtained percentages of diseased bulbs in July were about equal and in August higher than those determined in December. Similar results were obtained for other fungicide treatments.

It can be concluded that the first signs of basal rot development in summer will already be present in December, provided that the circumstances for bulb penetration are favourable at the time root emergence, after planting, starts. Furthermore, information obtained in December about visible bulb infections gives a more reliable prognose on the extent of basal rot in summer than the field observations in April will do.

3.7. Discussion

Basal rot of narcissus caused by *F. oxysporum* f. sp. *narcissi* constitutes an ever-recurring problem, which is difficult to understand and to deal with. Poor emergence, occurrence of sickle and dwarf plants in spring (Fig. 3.4.), an early yellowing of leaf tips followed by premature dying off of the foliage indicate infection of bulbs. Intensive roguing is rarely carried out at the present-day very large areas with narcissus cultivation. As a consequence, increased contamination of soil and healthy bulbs can be observed at harvest time (Price, 1977b). The most important problem might be the late development of basal rot when previously only few plants did show symptoms in the foliage (Tables 3.3. and 3.4.).

Experiments, described in this article show that a reliable impression of the extent of basal rot in summer can be obtained a considerable time before harvest. A high positive correlation was found between the incidence of root rot in December and of basal rot at the end of the season (Fig. 3.6.). Similar results were obtained for the incidence of bulb rot in December and the subsequent development of symptoms in the following summer (Fig. 3.7.).

These relationships appear to hold for various situations in practice, independent of disinfection of the basic plant material or the planting of bulbs at high or at low soil temperatures. Although the experiments do not cover all possible field situations they clearly illustrate that the maximum

disease incidence in summer may already be estimated some months after planting.

Furthermore, the proposition, that root rot and basal rot mainly originate from autumnal infections (see chapter 2), has been confirmed. That severe disease development in harvested bulbs may be favoured by high soil temperatures in early summer (McClellan, 1952), was also found in our experiments. Thus, disease incidence would have been less in a cooler summer, coefficients of regression would have been lower also, and the risk of a late symptom expression in store higher.

Presenting data about effectiveness of chemicals has not been the first aim of this article. Nevertheless, application during HWT of the standard fungicides and some experimental pimaricin formulations has demonstrated a direct fungicidal action of these compounds on conidia, chlamydospores and bulb-borne mycelium of *F. oxysporum* f. sp. *narcissi*, which resulted in a sufficient control of basal rot under circumstances being optimal for the pathogen. The mode of action of the antibiotic pimaricin, however, could not fully be understood on the basis of the data presented (Tables 3.1. and 3.4. and Fig. 3.6.). More information is needed about the contribution by antagonistic saprophytes in the control of *Fusarium* (Langerak, 1977). It might explain why low concentrations of pimaricin gave less disease development than expected from the survival of the conidia at the end of the HWT. It might also become clear why planting at 8 - 12 ^{o}C finally resulted in a lower basal rot incidence than planting at 13 - 15 ^{o}C did in spite of a more severe symptom expression in the first situation at a similar treatment of bulbs (Table 3.1.; chapter 5).

The aetiology of the disease has been studied by many investigators. Recommendations about cultural measures to be employed for optimal control of the disease have been given (Gregory, 1932; Hawker, 1935, 1940, 1943a; McClellan, 1952; Price, 1975, 1977a, b). These measures, including a hot-water treatment and disinfection of bulbs did not always appear to be appropriate because essential knowledge about the aetiology was lacking. Furthermore, bulb-growers often take the hot-water treatment and disinfection for a method by which bulbs can be cured. As a consequence too little attention is paid then afterwards to factors favouring the pathogen, which can still be present in latent infections (see chapter 2) or in the soil.

A HWT and disinfection of bulbs will undeniably remain important measures in the control of diseases and pests of narcissus. The best recommendations for control of basal rot deal with measures which avoid favouring of

the pathogen in the stages that bulbs or plants are susceptible for penetration and/or symptom development, and that effects of previous treatments cannot be expected anymore.

4 THE ROLE OF ANTAGONISTS IN THE CHEMICAL CONTROL OF *FUSARIUM OXYSPORUM* F. SP. *NARCISSI*

4.1. Introduction

In the past, basal rot and primary root rot of narcissus, caused by *Fusarium oxysporum* f. sp. *narcissi*, were controlled predominantly by organic mercury treatment. Secondary root rot and 'skin disease', in which several formae speciales of *F. oxysporum* play a role, were also controlled in this way. Because organic mercury is no longer acceptable for toxicological and environmental reasons, alternative methods of control have been investigated. In recent years a combination of formalin with thiram has been used successfully (De Rooy, 1976), but formalin is rather irritating for those, who have to carry out the treatments. Screening of a number of potential agents showed the antibiotic pimaricin, a polyene macrolide, to be active against the diseases mentioned (Langerak, 1975). In subsequent field experiments with bulbs treated either with methoxy ethyl mercury chloride ('Aretan Rood'), pimaricin or thiram, no colonization of the rhizosphere near the basal plate by *F. oxysporum* f. sp. *narcissi* and other ff. spp. of *F. oxysporum* could be observed, although the amounts of fungicide present on the bulb surface decreased below the level toxic to these *Fusarium spp.* The newly formed roots of treated bulbs were mainly colonized by non-pathogenic *Penicillium* and *Trichoderma spp.*

The aim of this study was to investigate whether these fungi, by antagonistic action towards the pathogen, might contribute to disease control by the fungicide. Thus, special attention was also paid to several aspects of the pathogenesis of the diseases concerned.

4.2. Materials and methods

Plant material. 'Round bulbs' were obtained from a commercial stock of narcissus (cv. 'Carlton'); in order to inhibit the development of roots, the bulbs were stored under dry conditions at 17 °C from 31 August onwards.

Fungi. All fungi used, viz. *Fusarium oxysporum* Schlecht. f. sp. *narcissi* (Cooke & Massee) Snyder and Hansen, *Fusarium oxysporum* ff. spp., *Cylindrocarpon destructans* (Zins.) Scholten, *Penicillium corymbiferum* Westling, *Penicillium janthinellum* Biourge, *Trichoderma hamatum* (Bon.) Bain. aggr. and *Trichoderma viride* Pers. ex S.F. Gray aggr., were isolated from the roots of narcissus bulbs.

Soil. Sandy soil was obtained from the experimental fields of the Bulb Research Centre in Lisse, The Netherlands.

Fungicides. 'Aretan Rood', a formulated product containing methoxy ethyl mercury chloride (3% Hg) was purchased from Bayer-Agrochemie N.V., Arnhem, the Netherlands. Formalin, containing 37% formaldehyde was obtained from E. Merck Nederland N.V., Haarlem, The Netherlands. Liro-thiram 50W, containing 50% a.i. thiram, was supplied by Ligtermoet Chemie N.V., Roosendaal. An experimental formulation of pimaricin (BAM-A) was kindly provided by Gist-Brocades N.V., Delft, The Netherlands and vendarcin by Mycofarm, Delft, the Netherlands.

Cellulose membrane. Uncoated cellophane PT 300 was obtained from N.V. Papierindustrie Van Straten en Boon, Den Dolder, The Netherlands.

Isolation and culturing of fungi. Root pieces (3 - 5 mm) were cut from healthy roots of narcissus, close to the juncture of the basal plate. The pieces were rinsed in tap water to remove soil particles and spores, and plated on a medium containing quintozene and oxgall according to Papavizas (1967), which favours mycelial growth of *Fusarium spp.* to that of *Penicillium* and *Trichoderma spp.* The plates were incubated at 25 °C in the dark for 4 days, followed by 10 °C and artificial light during 2 days, a.o. to avoid reproduction of saprophytic nematodes. After subculturing on Oxoid PDA, containing 50 µg/ml of vendarcin to suppress development of bacteria, the fungi were transferred to a mixture of peat soil and oatmeal (19 : 1 w/w) at 4 °C. Fungi to be used for the experiments were again transferred to PDA.

Harvest of conidia. Erlenmeyer flasks, containing autoclaved bulb scales of narcissus were inoculated with pieces of mycelium and incubated for 4 days at 25 °C in the dark followed by 2 days at 10 °C in artificial light to stimulate sporulation. The cultures were then washed with sterile water containing one drop of Tween 20 per 100 ml and subsequently filtered through two layers of Kleenex tissue in order to remove hyphal fragments and starch grains.

Assay of fungitoxicity. A solution or suspension of the fungicide was added to molten PDA at 50 °C, which was then poured into Petri dishes. After solidification 5-mm agar discs with young mycelium were placed on the surface; radial growth of the colonies was measured after various periods of time.

Residue analysis. To estimate the persistence of fungicides after planting of treated bulbs, an experiment was carried out with stored root material.

Portions of 0.25 g of roots were submerged in 5 ml of a fungicide solution at 43.5 °C for 2 h, dried overnight, and placed in PVC pots filled with 500 ml sandy soil at 12 - 15 °C, and covered with glass to avoid evaporation. Weekly, 25 ml water was added to simulate rainfall. Fungicide present on the roots was bioassayed by plating out 0.5-cm pieces on PDA seeded with conidia of *F. oxysporum* f. sp. *narcissi* (10^4 per ml) and measuring the inhibition zones.

Assessment of antagonism *in vitro*. Slightly modified versions of the double-layer technique (DLT) of Herr (1959) and Etheridge & Craig (1973) were used to determine the antagonism between the pathogen and other fungi. In this technique use is made of two agar layers, separated by a cellulose membrane. The top layer is seeded with conidia of the potential antagonist; metabolites secreted by this fungus may pass the membrane and diffuse into the bottom layer; an agar disc with mycelium of the test fungus is placed on top of this layer; after various periods of time the diameter of the colony is assessed and compared with that on agar without fungal metabolites (Fig. 4.1., DLT-A). A modified version of this technique allows to test the combined effect of the metabolites secreted by the antagonist and a fungicide (Fig. 4.1., DLT-B). In a third version the conidia of the antagonist to be tested are sown in the bottom layer, which afterwards is covered by the membrane and a top layer, containing a fungicide. After 2 days, when the top layer in addition to the fungicide contains the metabolites secreted from the bottom layer, the fungitoxicity is assessed with the test fungus in the way described above (Fig. 4.1., DLT-C).

Assessment of antagonism *in vivo*. Bulbs were soaked in 0.25% formalin at 43.5 °C for 2 h to reduce superficial contaminants, then contaminated with the pathogen and the potential antagonist by a 30-sec dip in the appropriate suspensions with conidia, and planted in sandy soil. Fungicide was added to the condial suspensions when the combined effect of fungicide and antagonist had to be studied. Bulbs were planted in the field or, for laboratory experiments, in PVC trays with sandy soil, which was autoclaved twice for 1 h at 110 °C with an interval of 24 h; the trays were placed in a climate room at 17 °C. Disease severity was assessed 8 weeks after planting.

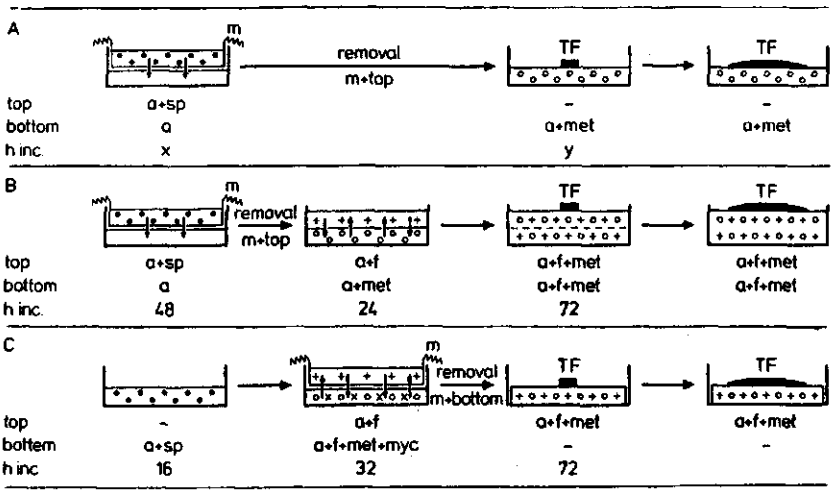

Fig. 4.1. Experimental stages in versions A, B and C of the double-layer technique (DLT). The different components of the layers are represented by: a, 10 ml PDA; m, sterile cellulose membrane; met, fungal metabolites (o); myc, mycelium (x); f, fungicides (+); sp, fungal conidia (•) and TF, agar disc + mycelium of the test fungus. h inc., hours of incubation.

Mathematical treatment of fungitoxicity data for two variables. Interaction between a fungicide and either antifungal metabolites or conidia of an antagonist was tested by statistical analysis of variance at the P = 0.05 level. The type of interaction was determined according to the graphical method of Tammes (1964).

4.3. Pathogenesis

Literature data. Basal rot and primary root rot of narcissus are caused by *F. oxysporum* f. sp. *narcissi* (Gregory, 1932; Hawker, 1935, 1943b; McClellan, 1952). Hot-water treatment of bulbs (usually a 2-h dip at 43.5 °C) for control of parasitic insects, mites and nematodes favours spread of the pathogen from diseased to healthy bulbs in the bath (Van Slogteren, 1931). This may be avoided by addition of a fungicide. Effective chemicals are mercurials (Hawker, 1940; McClellan, 1948; Miller & Gould, 1967), formalin (Gregory, 1932; Hawker, 1935, 1940, 1943a, b) and pimaricin (Langerak, 1975). Suppression of basal rot by thiram has been described by McClellan & Stuart (1947). These fungicides are similarly active against spread of the weakly

parasitic *F. oxysporum* ff. spp., which cause secondary root rot and the 'skin-disease' of narcissus (Langerak, 1975).

Hawker (1935, 1943b) and McClellan (1952) concluded that basal rot originates from basal plate infections, which take place in early summer, when the average soil temperature exceeds 13 °C. At this time, the bulb roots die; they assumed that the pathogen penetrates into the basal plate via the dying roots.

Own observations. In contrast to the generally accepted view outlined above, it was found that after planting of bulbs in the late summer or autumn, infection of the basal plate does take place already during the first weeks of rooting. Since soil temperatures are low in autumn (<10 °C), development of basal rot symptoms will be delayed until the following summer (see chapters 2 and 3). In the presence of growing mycelium of the pathogen infection of new roots will occur, after the disruption of the basal plate starts and when the soil temperature for a period of two weeks exceeds 12 °C. The disrupted parenchymatous tissue of the basal plate is more readily infected than the fast developing new roots. Therefore, primary root rot in winter indicates that also primary infection of basal plate tissue has taken place. On the other hand, when no root rot is observed during a period of four weeks even though temperatures are suitable for the development of the pathogen (not below 13 °C), it may be assumed that infection of the basal plate did not take place. Hence, root rot was used as a criterion for bulb infection in all our experiments carried out at temperatures higher than 12 °C.

4.4. Fungitoxicity of 'Aretan', pimaricin and thiram

From the ED_{50}- and ED_{95}-values of the active ingredient of 'Aretan', and of pimaricin and thiram, which were measured for the pathogens and a few frequently isolated rhizosphere fungi, it appears that the pathogens and *Cylindrocarpon destructans* were more sensitive to the three fungicides tested than the *Penicillium* and *Trichoderma spp.* (Table 4.1.).

Table 4.1. ED_{50}- and ED_{95}-values (µg/ml) for inhibition of mycelial growth of fungi from the rhizosphere of narcissus as measured on PDA containing 'Aretan', pimaricin and thiram after incubation for 7 days at 25 °C.

Fungi tested	'Aretan'		Pimaricin		Thiram	
	ED_{50}	ED_{95}	ED_{50}	ED_{95}	ED_{50}	ED_{95}
F. oxysporum f. sp. *narc.*	1.5	3	2	4	18	90
F. oxysporum ff. spp.	2	3	1.5	3.5	20	100
P. corymbiferum	8	16	4.6	8	40	300
P. janthinellum	3	8	4	6	50	200
T. hamatum	7	9	8	10	>100	>>100
T. viride	2.5	3.5	8	10	>100	>>100
C. destructans	2	4	1.5	4.5	30	250

4.5. Antagonism of rhizosphere fungi

In vitro. The antagonistic action of various fungi isolated from narcissus roots towards the pathogens was assessed according to the DLT-A method. The results, presented in Table 4.2., show that the *Penicillium spp.* and *C. destructans* were more strongly antagonistic to the *Fusarium* pathogens than the *Trichoderma spp.*

Table 4.2. Antagonistic action of fungi isolated from narcissus roots towards *F. oxysporum* f. sp. *narcissi* ('narc.'), *F. oxysporum* f. sp. ('f. sp.'), *P. corymbiferum* (P. cor.) and *T. viride* (T. vir.). Fungitoxicity of metabolites was determined in the DLT-A for 10^4 conidia per ml in the initial top layer, which was removed from the test layer after 4 days.

Fungi isolated	Mycelial growth in % of control (= 100)			
	'narc.'	'f. sp.'	P. cor.	T. vir.
P. corymbiferum	25	18	100	40
P. janthinellum	5	17	5	20
T. hamatum	90	-	45	60
T. viride	80	-	35	30
C. destructans	26	7	-	-

In vivo. Antagonism *in vivo* of a number of saprophytic fungi towards *F. oxysporum* f. sp. *narcissi* was investigated in natural or sterilized soil. Bulbs were dipped in conidial suspensions either of the pathogen alone or of the pathogen with one of the fungi to be tested. Data on root rot in both

treatments are given in Table 4.3. They show that artificial contamination with conidia of *Penicillium spp.* and *C. destructans* reduced the incidence of root rot considerably. *T. viride* had a moderate effect in natural soil. The antagonistic effects were less pronounced in sterilized soil, where *T. viride* at high conidial densities even stimulated root rot. In that case the infected roots decayed completely and infections of the basal plate were more frequent and had penetrated more deeply.

Table 4.3. Reduction of narcissus root rot by artificial contamination with antagonists, isolated from the rhizosphere. Bulbs were dipped in a conidial suspension containing $25x10^3$ con/ml (natural soil) or $5x10^3$ con/ml (sterilized soil) of *F. oxysporum* f. sp. *narcissi*, and conidia of the potential antagonist. Root rot is expressed as percentage of the control. Per treatment 24 (natural soil) or 20 bulbs (sterilized soil) were used. In the controls in natural soil 25% of the roots were infected, in those in sterilized soil 80%.

Additional contaminant	con/ml	Root rot in % of control	
		natural soil	sterilized soil
P. corymbiferum	$2x10^4$	-	56
P. corymbiferum	$2x10^5$	29	61
P. janthinellum	$4x10^4$	40	60
P. janthinellum	$2x10^5$	30	54
T. viride	10^3	-	75
T. viride	$2x10^5$	59	120
C. destructans	$5x10^3$	-	50
C. destructans	$2x10^5$	28	-

4.6. Effect of fungicides on antagonism

In vitro. The effect of organic mercury, pimaricin and thiram on antagonism of various fungi towards *F. oxysporum* f. sp. *narcissi* and *F. oxysporum* ff. spp. was tested in the double-layer technique, B- and C-variants, as described under Materials and methods. From Fig. 4.2. it appears, that pimaricin and the fungitoxic metabolites produced by *P. janthinellum* towards *F. oxysporum* ff. spp. were slightly synergistic. The antibiotic might also stimulate the secretion of toxic metabolites, as shown for *C. destructans* (Fig. 4.4.). Thiram did not interact with the metabolites of *P. janthinellum* (Fig. 4.2.). This fungicide reduced antagonism (Fig. 4.3.) at low doses (<1 µg/ml), but at high doses it enhanced antagonism of *P. janthinellum*. Organic mercury stimulated the secretion of metabolites of *P. janthinellum*, toxic towards *P. corymbiferum* (Fig. 4.5.).

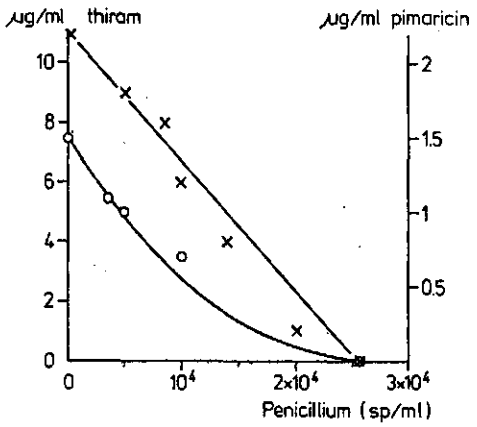

Fig. 4.2. Effect of thiram (x) and pimaricin (o), in combination with fungitoxic metabolites from *P. janthinellum*, on mycelial growth of *F. oxysporum* f. sp. *narcissi* on PDA; data expressed as so-called ED_{50}-isoboles. Metabolites produced in absence of fungicide, in agar containing various conidial concentrations.

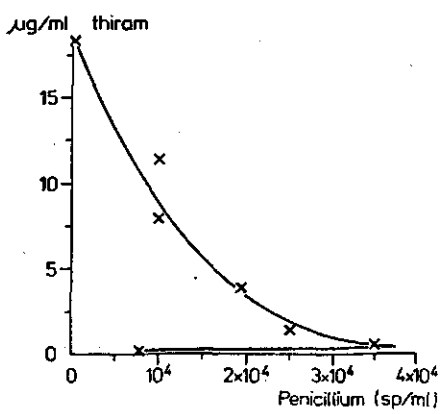

Fig. 4.3. Effect of thiram, in combination with fungitoxic metabolites from *P. janthinellum*, on mycelial growth of *F. oxysporum* f. sp. *narcissi* on PDA; data expressed as so-called ED_{50}-isobole. Metabolites produced in presence of fungicide, in agar containing various conidial concentrations.

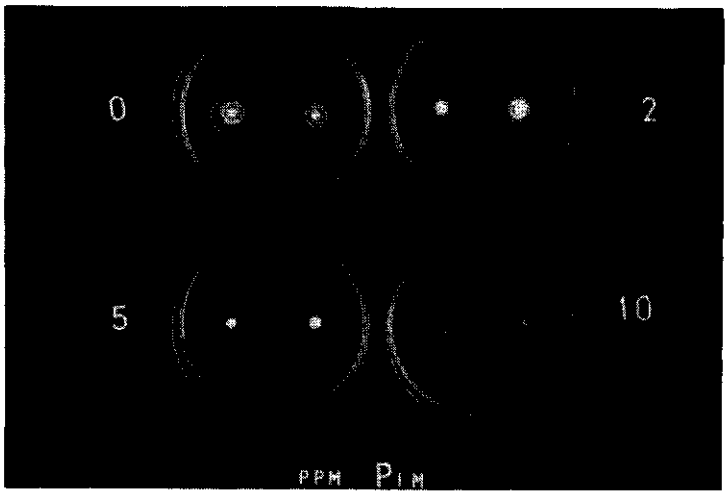

Fig. 4.4. Effect of pimaricin (µg/ml) on secretion in PDA of metabolites of *C. destructans*, toxic towards *Bacillus sp.*

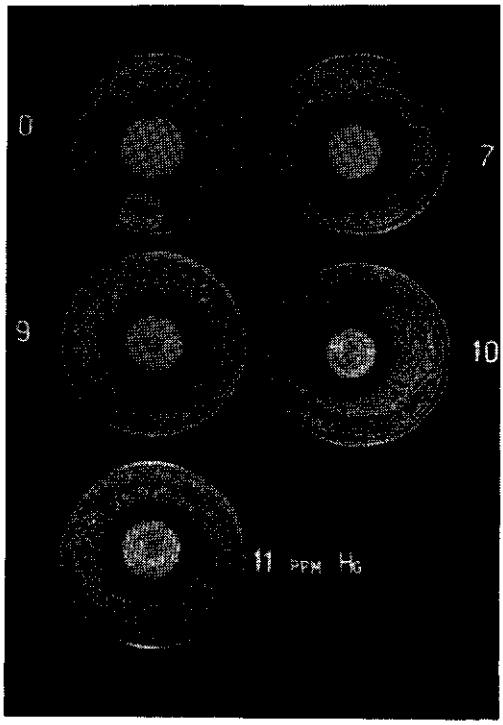

Fig. 4.5. Effect of methoxy ethyl mercury chloride (µg/ml) on secretion in PDA of metabolites of *P. janthinellum*, toxic towards *P. corymbiferum*.

In vivo. Interference of thiram and pimaricin with antagonism of various rhizosphere fungi towards the pathogen was studied in experiments in natural and sterilized soil. Fig. 4.6. and Table 4.4. show that conidia of *P. janthinellum* and thiram were synergistic against root rot and basal rot. Similarly, the antibiotic pimaricin and conidia of *C. destructans* appeared to act synergistically in the control of basal rot (Table 4.4.).

Table 4.4. Control of narcissus basal rot, caused by *F. oxysporum* f. sp. *narcissi*, by thiram and conidia of *P. janthinellum* and by pimaricin and conidia of *C. destructans*. Bulbs (n = 240) before planting dipped in a suspension containing the fungicide and conidia of the pathogen ($25x10^3$ con/ml) and of the antagonist. Percentage of bulbs showing basal rot, one month after lifting in July.

Thiram (µg/ml)	*P. janthinellum* (con/ml)			Pimaricin (µg/ml)	*C. destructans* (con/ml)	
	0	$4x10^4$	$2x10^5$		0	$2x10^5$
0	90	93.5	90	0	90	90
1000	38	28	27.5	100	70	40
2500	30	19.5	19	200	50	20

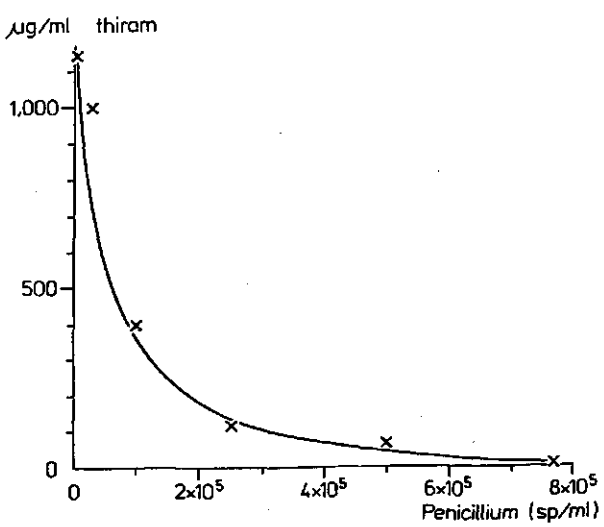

Fig. 4.6. Effect of thiram and *P. janthinellum* on root rot caused by *F. oxysporum* f. sp. *narcissi* in sterilized soil. Bulbs dipped in conidial suspensions of the pathogen ($2x10^4$ con/ml), to which thiram and conidia of *P. janthinellum* had been added. Data expressed as ED_{50}-isobole.

4.7. Field experiments

Control of basal rot, root rot and 'skin disease' of narcissus of methoxy ethyl mercury chloride ('Aretan'), formalin, pimaricin or thiram was studied in field experiments. Bulbs were dipped in hot water (2 h at 43.5 °C) which contained conidia of *F. oxysporum* f. sp. *narcissi*, and planted at initial soil temperatures ranging between 9 and 14 °C.

The following assessments were made: a. survival of *Fusarium* on the bulbs after treatment in the bath, b. fungicide residues on bulbs after planting, c. development of symptoms from the time of planting until one month after lifting, d. degree of colonization of the rhizosphere by pathogenic and saprophytic fungi during the season and e. soil temperature.

a. Survival of *Fusarium*. The data, represented in Table 4.5., show that only bulbs of Control A became heavily contaminated with conidia of *F. oxysporum* f. sp. *narcissi*. All fungicides tested, with exception of thiram, completely eliminated the pathogen in the bath. Contamination with conidia of *F. oxysporum* ff. spp. did hardly occur. Shortly after planting the old roots were heavily colonized by *F. oxysporum* f. sp. *narcissi* in Control A, but only slightly in Controls B and C. *F. oxysporum* ff. spp., originating from dead bulb tissue and/or the surrounding soil, strongly colonized the old roots of the Controls B and C, and moderately those of the formalin treatment.

Table 4.5. Effect of fungicides on survival of hot-water treated (2 h at 43.5 °C) conidia of *F. oxysporum* f. sp. *narcissi* and of *F. oxysporum* ff. spp., and on colonization of old roots by these fungi, one week after planting. 25,000 con/ml of the pathogens added in Control A and in fungicide treatments. No conidia were added in Control B (hot water) and Control C (cold water).

Treatment	Surviving conidia per ml		% colonized old roots (n = 500)	
	'narc.'	'ff. spp.'	'narc.'	'ff. spp.'
Control A	10,000	3	75	8[a)]
Control B	<1	3	14	58
Control C	<1	13	16	75
Formalin, 1%	<1	<1	0	17
'Aretan', 0.25%	<1	<1	0	<1
Pimaricin, 0.03%	<1	<1	0	<1
Thiram, 0.5%	200	<1	0	<1

a) Absence of colonization as a result of high degree of contamination of bulbs with 'narc.'.

Table 4.6. Toxicity of fungicide residues towards *F. oxysporum* f. sp. *narcissi* on old roots of narcissus (dipped 2 h at 43.5 °C), put into soil (12 - 15 °C), and plated on PDA, seeded with conidia of the test fungus. -, no inhibition zone observed; ±, + or ++, zones of <5mm, 5 - 10 mm or >10 mm in diameter.

Treatment	Incubation time in weeks			
	0	1	3	7
Water	-	-	-	-
Formalin, 1%	-	-	-	-
'Aretan', 0.25%	±	-	-	-
Pimaricin, 0.03%	+	±	-	-
Thiram, 0.5%	++	+	±	-

b. Fungicide residues. Old roots of narcissus were treated with fungicides, as in the field experiment (Table 4.5.), put into soil and biologically assayed for the presence of fungicide residues. Table 4.6. shows that 3 weeks after putting the roots into the soil residues were no longer present with fungitoxicity towards *F. oxysporum* f. sp. *narcissi*.

Table 4.7. Effect of fungicides on disease symptoms of hot-water treated (2 h at 43.5 °C) bulbs, caused by *F. oxysporum* f. sp. *narcissi* ('narc.') and other ff. spp. of *F. oxysporum* ('ff. spp.'). 25,000 con/ml of the pathogens added in Control A and in the fungicide treatments. No conidia were added in Control B (hot water) and in Control C (cold water). Root rot and root decay: 0 = no visible symptoms, 100 = symptoms on all roots of 20 bulbs. Basal rot expressed as a percentage of visibly infected bulbs (n = 600). 'Skin disease' expressed as a percentage of bulbs with moderate and heavy symptoms (n = 600).

Treatment	'narc.' + 'ff. spp.'		'narc.'	'ff. spp.'
	root rot Dec.	root decay May	basal rot July	'skin disease' July
Control A	26	85	27	50
Control B	26	50	2	40
Control C	32	75	3	90
Formalin, 1%	0	25	0.6	50
'Aretan', 0.25%	0	4	0.8	25
Pimaricin, 0.03%	0	9	0.3	20
Thiram	0	12	1.3	20

c. Development of symptoms. Data about the symptoms on roots and bulbs of narcissus are summarized in Table 4.7. During the first months after planting, early root rot caused by *F. oxysporum* f. sp. *narcissi* and by the other ff. spp. of *F. oxysporum* was found only in the controls. This result reflects the initial high degree of colonization (Table 4.5.) by these pathogens. Controls A, B, and C showed a fast decay of roots, mainly caused by the same *Fusarium spp.* as mentioned above, at the time of maturation of the bulbs in May. Upon fungicide treatment the highest degree of root decay was found for bulbs treated with formalin. This fact is connected with the moderate colonization of the old roots, shortly after planting. The results on 'skin disease' caused by *F. oxysporum* ff. spp. were similar to those found for root decay.

After the bulbs were lifted, 27% basal rot, caused by *F. oxysporum* f. sp. *narcissi*, was found for Control A, which finding agrees with the initial high level of contamination of the bulbs in the first two weeks after planting (Table 4.5.). Low percentages of such infected bulbs were obtained for other controls and fungicide treatments; they reflect the initial low degree of contamination.

d. Colonization of the rhizosphere. Initially, colonization of young roots by *F. oxysporum* f. sp. *narcissi* was high for control A, but decreased gradually to the same low level as found for Controls B and C. Absence of this fungus on bulbs treated with fungicides resulted in lack of colonization of the roots after planting (Fig. 4.7A.). In the controls, *F. oxysporum* ff. spp. behaved quite similarly to *F. oxysporum* f. sp. *narcissi* (Fig. 4.7B.). The strongest colonization by these fungi could be observed for the cold-water control. Roots of bulbs treated with formalin showed the same colonization pattern as found for those of Control B. Roots of bulbs, treated with 'Aretan', pimaricin or thiram, remained almost free from *Fusarium spp.* until the end of the season.

Colonization by *P. corymbiferum* during the first 6 weeks was higher when bulbs were hot-water treated (Fig. 4.7C.). Treatment with fungicides resulted in a slow colonization by this fungus. Finally, the same levels of colonization were reached as in control B (Fig. 4.7D.).

With respect to the controls and the formalin treatment, dips in either 'Aretan', pimaricin or thiram favoured *T. viride* (Figs 4.7E. and 4.7F.) and *P. janthinellum* (Fig. 4.7G.).

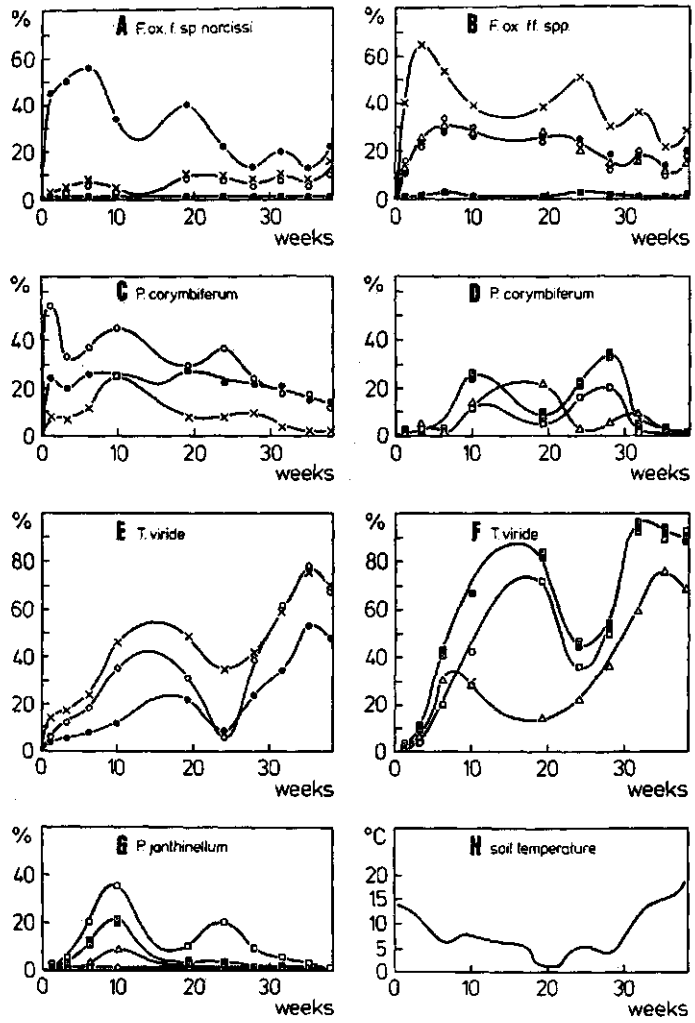

Fig. 4.7. Effect of various bulb treatments on colonization of narcissus roots in natural soil by various fungi during the growing season (A, B, C, D, E, F and G). 25,000 con/ml were added in Control A (●) and in the fungicide treatments (2 h at 43.5 °C): formalin (Δ), 'Aretan' (■), pimaricin (▨) and thiram (□). No conidia were added in the hot-water Control B (o) and the cold-water Control C (x). Graphs represent percentages of root pieces colonized (n = 5x100). Average daily soil temperatures at a depth of 10 cm during the season (H).

e. Soil temperature. Soil temperatures were continuously recorded during the growing season at a depth of 10 cm. The start of colonization by the *Penicillium spp.* (Figs 4.7C., D and G) coincided with low temperatures around the 6th and the 18th week after planting (Fig. 4.7H.).

4.8. Discussion

Narcissus bulbs are routinely dipped in a fungicide-containing water bath in order to disinfect them and to protect them after planting against infection by pathogens in the soil. Formalin, organic mercury, pimaricin and thiram all provided satisfactory disinfection, although after thiram treatment some pathogenic conidia survived (Table 4.5.). The last three compounds also protected the roots during the growing season (Table 4.7.) against *F. oxysporum* f. sp. *narcissi* and other ff. spp. of *F. oxysporum*. Protection occurred even though, within three weeks after planting, the amount of fungicide on the treated bulbs decreased below a level toxic to the pathogens (Table 4.6.), probably due to leaching or detoxification. Colonization of the rhizosphere of newly formed roots by these pathogens did not occur (Fig. 4.7.) and disease control was significant (Table 4.7.). Treatment with formalin, although a good disinfectant, did not result in such a long-lasting protection.

After treatment of bulbs with organic mercury, pimaricin or thiram, the newly developing roots were colonized by non-pathogenic *Penicillium* and *Trichoderma spp.* during the growing season (Fig. 4.7.). These fungi were less sensitive to the fungicides than the *Fusarium spp.* (Table 4.1.), and thus more or less selectively favoured by the treatment. On agar plates several of these non-pathogenic rhizosphere fungi were antagonistic to the pathogenic *Fusarium spp.* (Table 4.2.). Furthermore, contamination of bulbs with conidial suspensions of these antagonists did provide some protection against infection by the pathogens (Table 4.3.). From these data it seems plausible that these antagonists may contribute to disease control. Antagonism may be based on competition for nutrients or on fungitoxicity of secreted metabolites. *P. janthinellum* e.g., one of the most active antagonists, has been reported to produce the antibiotic janthinellin (Bekker et al., 1963), which is strongly fungistatic *in vitro* (Lisina & Bekker, 1964).

Besides exerting a selective action, which favours development of antagonists, fungicides at sublethal concentrations may enhance the production

and secretion of antibiotics, or may act synergistically with the secreted antibiotics. Such a synergistic effect was found for pimaricin and the metabolites of *P. janthinellum* (Fig. 4.2.). An increase of fungitoxic metabolite production by this fungus was obtained at sublethal doses of thiram (Fig. 4.3.) or organic mercury (Fig. 4.5.). Pimaricin stimulated the production of bacteriostatic metabolites by *C. destructans* (Fig. 4.4.).

The data obtained indicate that fungicide treatment of bulbs may not only provide disease control by direct action on the pathogenic organisms, but also indirectly via antagonists. Analogous results have been obtained by Richardson (1954) and Chinn (1971). After treatment of soil with thiram or organic mercury, growth of bacteria and *Penicillium spp.* was stimulated which was assumed to contribute to control of *Pythium ultimum* and *Cochliobolus sativus* on pea and wheat seedlings, respectively. However, by soil treatment with selective fungicides also other pathogens, tolerant to the particular fungicide concerned, may become predominant, as was shown for sharp eye spot on rye, treated with benomyl (Prew & McIntosh, 1971; Van der Hoeven & Bollen, 1972).

Our own experiments indicate that the use of fungicides, which interfere selectively with the pathogen and the rhizosphere microflora, may contribute to an efficient disease control in practice. Use of a tolerant antagonist together with a selective fungicide might also offer a possibility for disease control. Failure of biological control of plant diseases in the past often appeared to be due to a non-permanent shift in the microflora in favour of these antagonists.

5 THE INFLUENCE OF INCUBATION CONDITIONS AFTER LIFTING ON DEVELOPMENT OF BASAL ROT AND SKIN DISEASE AND ON EFFECTIVENESS OF CHEMICAL CONTROL MEASURES

5.1. Introduction

Several fungal disease problems may occur when narcissus bulbs are grown commercially. Most striking are those cases where the underground parts of the plant become infected by *Fusarium oxysporum* Schlecht. f. sp. *narcissi* (Cooke & Massee) Snyder & Hansen, and basal rot develops in the bulbs (Van Slogteren, 1931; Gregory, 1932). Basal rot can be visible at the time of digging in the summer. Very often, however, healthy looking but infected bulbs may show the symptoms in store 6-8 weeks later. An early dying-back of the foliage prior to lifting may indicate the existence of such latent infections. However, similar information would have been obtained much earlier, when the occurrence of root rot was assessed in the previous winter (see chapter 3).

The presence of root rot, which indicates infection by *F. oxysporum* f. sp. *narcissi*, will both reduce yield and affect quality. It is not yet clear whether the pathogenic *F. oxysporum* f. sp. *narcissi* or other *F. oxysporum* ff. spp. contribute to the development of the so-called skin disease, a dry rot in the fleshy outer scales. Such diseased bulbs become crumpled in store, loose weight easily and may transmit the pathogen to the next crop. It was observed earlier (Langerak, 1977) that several formae speciales of *F. oxysporum* are able to colonize the roots especially when the pathogen has penetrated them before. So, a causal relationship may also exist between the level of contamination of roots with all *F. oxysporum* ff. spp. at the beginning of the season and the incidence of skin disease at the end.

Various recommendations to control basal rot have been presented during the last five decades. A chemical treatment, usually given in combination with a hot-water treatment (HWT), is the principal measure taken by bulb growers. Roguing during the growing season and discarding diseased bulbs after lifting often do not get the attention needed. Underestimation of the importance of the latter measures has led several times to unexpected outbreaks of basal rot. Ignorance of the present-day knowledge of the aetiology of basal rot (Price, 1977; see chapter 2) may be the reason

that the present control measures do not fully take into account the quality of the bulbs to be used for further propagation. Sometimes, however, disease control may fail in spite of the fact that the right precautions have been taken. It remained unexplained for instance why in some cases less basal rot than expected develops from the level of contamination determined after treatments with pimaricin (see chapter 3).

As storage conditions often differ in practice, it would be important to know how and to what extent such conditions directly or indirectly influence disease development in the following season. Moreover, information about the effectiveness of fungicides may also help to make an up-to-date set of recommendations for control of *Fusarium* diseases in narcissus.

For that purpose laboratory studies, followed by field experiments were carried out to investigate the influence of several incubation variables on disease development. Formaldehyde, thiram, mercury and especially the antibiotic pimaricin were used to provide different inoculum levels in the material planted. Special attention was also paid to the role other bulb-borne saprophytic fungi played in these experiments.

5.2. Materials and methods

Plant material. 'Round bulbs' were obtained from five commercial stocks of narcissus bulbs (*Narcissus pseudonarcissus* cv. Carlton). One stock (I), providing all bulbs for the field experiments A, B, C and D, was stored outdoors in open trays at 14-20 °C until grading took place. After discarding bulbs with visible basal rot, those remaining were treated and stored again according to the experimental design presented in Table 5.1. Bulbs used in laboratory experiments originated from other stocks, namely I, II, III, IV and V and were always kept in store at 17 °C (RH 70%) unless otherwise indicated.

Fungi. All fungi used, viz., *Fusarium oxysporum* f. sp. *narcissi* (F_9), *Cylindrocarpon destructans* (C_1), *Penicillium corymbiferum* (P_1), *Penicillium janthinellum* (P_4) and *Trichoderma viride* (T_3) were isolated from the roots of narcissus bulbs, cultured, and maintained as described by Langerak (1977). Pathogenicity of F_9 was tested according to the method described in chapter 2. Large quantities of conidia were cultured on autoclaved bulb scales of narcissus bulbs (Langerak, 1977).

Soil. Experiments were carried out in the experimental garden of the Bulb

Research Centre in Lisse, The Netherlands. The sandy soil of this garden where no flower bulbs had been grown for two years was also used in the laboratory experiments.

Fungicides. Aretan Rood (Hg), a formulated product containing methoxy ethyl mercury chloride (3%) was purchased from Bayer-Agrochemie N.V., Arnhem, The Netherlands. Formalin, containing 37% formaldehyde was obtained from E. Merck Nederland N.V., Haarlem, The Netherlands, Liro-thiram 50W, containing 50% thiram was supplied by Ligtermoet Chemie N.V., Roosendaal, The Netherlands. Standard dosages of these fungicides were respectively 0.25%, 1% and 0.5%. Experimental formulations of pimaricin were kindly provided by Gist Brocades N.V., Delft, The Netherlands. One formulation with the code name Pim-N was a wettable powder which partly dissolved in water; the others with code names BAM-A and BAM-B were liquid formulations containing pimaricin in different concentrations and were miscible with water in the ratio of 1:20 and 1:50 (v/v) respectively.

Dissecting of bulbs. A certain sequence was followed in preparing various tissue parts for plating in order to avoid cross-contaminations as much as possible. The nomenclature used here is similar to that introduced in chapter 2. Old roots (OR) were pulled out from the basal plate and cut into pieces of 3-5 mm. Next, the paper-like outer scales (OS) were separated from the bulb to punch out disks of 5 mm diameter. Then, all old and corked cortex material (OC) including the outer periderm (OP) was carefully disconnected from the bulb base and divided into pieces of about 3x3x3 mm. The remnants of implanted root parts, then exposed, were cut out around the dark coloured abscission layers (AL). After the removal of respectively OR, OS, OC and AL, the ring of parenchymatous cortex tissue (C) was excised from the central cylinder (CC) and scales (BS) and cut into blocks of 3x3x3 mm.

After excision, pieces of living tissue (AL and C) were immediately transferred to moist filter paper in petri dishes and incubated at 5 °C to prevent drying before plating. Other dead tissue parts such as OR, OS and OC were stored at that time at 20 °C in closed test tubes.

Dry an humid incubation after soaking. Bulb tissue parts, which had to be dried quickly after wetting, e.g. after a hot-water treatment (HWT), were drained off between flamed filter paper and transferred to sterile conical flasks or test tubes, which together were connected by means of polyethylene tubing to one central compression unit supplying a continuous

and equal dust-free airstream. The pressure maintained was adjusted in such a way that the wet tissue dried back to its original moisture content within 6 hours.

Where humid incubation was required, the material was also transferred to flasks or tubes. These were closed with rubber stoppers and kept at 20 °C until further steps in the experiment were carried out.

Assessment of viability of fungi in treated tissue. Bulb tissue parts were, mostly after treatment, plated on a medium containing quintozene and oxgall (Papavizas, 1967), which favours mycelial growth of *Fusarium spp.*, *F. oxysporum* ff. spp. and *Cylindrocarpon spp.* over that of other bulb-borne fungi such as *Penicillium* and *Trichoderma spp.* The plates were incubated at 25 °C in the dark for 4 days and then transferred to 10 °C where artificial light was given for 2 days (18 h per day) to stimulate condidial formation and to omit excessive reproduction of saprophytic nematodes.

Experienced analysts were able to differentiate between the outgrowing fungi at microscopical inspection (x 12-50 magnification). Even the pathogenic *F. oxysporum* f. sp. *narcissi* could be recognized among other formae speciales of *F. oxysporum*, common on narcissus tissue as confirmed by testing isolates for their pathogenicity (see chapter 2).

Assessment of the colonization of roots. In order to determine the degree of colonization of new roots (NR) with fungi, bulbs were lifted from soil, washed thoroughly under running tap water to remove soil particles and adhering contaminants. Then, pieces of 3-5 mm were cut from healthy looking roots close to the juncture of the basal plate, mixed, rinsed in sterile water three times, plated on Papavizas medium, and incubated as decribed above.

Assessment of relative growth rates of bulb-borne fungi *in vitro*. Growth of some bulb-borne fungi, viz., F_9, T_3, P_1 and P_4 was measured on potato dextrose agar (PDA) at temperatures ranging from 5 °C to 40 °C with intervals of 5 °C. Agar discs of 5 mm diameter with 1-day old mycelium were transferred to the centre of agar plates (14 cm diameter) and incubated. Growth of three colonies per fungus and incubation temperature was measured daily over a period of 9 days.

Relative growth rates at 10 °, 15 ° and 20 °C were obtained by: a. plotting the colony diameters at the 9th day against the different incubation temperatures; b. estimating the optimum growth temperature from curves obtained, and c. expressing per temperature level the observed growth on each particular day as the percentage of the growth at the optimum

temperature on the same day.

Field experiments. A general outline for four different experiments is given in Table 5.1. Details of the HWT, including determination of conidial survival and of fungicide concentration at the end of the treatment have been described earlier in chapter 3. Treatment of bulbs in experiments A, B and D was followed by storage for 4 days at 14-20 ^{o}C under humid conditions. Mutual differences for these experiments mainly concern variation in storage conditions before HWT and from 4-days humid storage onwards. Further, three replicates of 100 bulbs per treatment were used in experiment D while four times 96 bulbs per treatment were used in experiments A and B.

Experiment C, carried out on 3x100 bulbs per treatment stands out from the other experiments with respect to the nature of the treatments applied. First, all bulbs were soaked together at the same time in a big basin filled with 0.25% formalin and heated for two hours at 43.5 ^{o}C in order to reduce superficial contamination with fungi. After this HWT and draining for one day, the second treatment followed, including artificial contamination with conidia of the pathogen and/or conidia of potential antagonists using a 30-sec dip in the appropriate suspensions.

Criteria used at inspections in December, July and August to assess disease incidence were similar to those described in chapter 3.

5.3. Influence of incubation conditions on viability of bulb-borne fungi

Experiments were carried out to study effects of hot water treatments and subsequent storage conditions on survival of bulb-borne fungi in and on various bulb parts. For hot-water treatment for 2 hours at 43.5 ^{o}C, complete bulbs were mostly used, which were dissected afterwards. Effects of the following variables were studied: a. treatment with hot water, b. drying of soaked material, and c. repeated wetting through dips in cold or warm water. In some of the experiments the effect of fungicides on fungal survival was also studied. In that case either Aretan (methoxy ethyl mercury chloride), Pim-N (pimaricin) and/or Liro-thiram (thiram) were added to the water in which the bulbs were treated. Effects on survival were expressed as percentages of bulb tissue parts showing outgrowth of mycelium after plating out and incubation.

Table 5.1. Design of some field experiments with recordings of average daily temperatures in soil (°C) at a depth of 10 cm during the growing season, and in a shaded place during storage outdoors; (*) means incubation in soil.

Date		Temperature		Experiment A	Experiment B	Experiment C	Experiment D
month	week	store	soil				
September	1	17	-	storage	storage	grading	grading[a]
	2	17	-	grading	grading	storage	storage[a]
	3	17	-	HWT+storage	HWT+storage	HWT(formalin)	storage[a]
	4	17	15.0	planting	storage[a]	2nd treatment	storage[a]
October	1	17	13.0	*	storage[a]	planting	storage[a]
	2	17	10.0	*	planting	*	storage[a]
	3	17	8.0	*	*	*	storage[a]
	4	17	7.5	*	*	*	storage
November	1	17	8.0	*	*	*	grading
	2	17	5.0	*	*	*	HWT+storage
	3	-	6.5	*	*	*	planting
	4	-	4.5	*	*	*	*
December	1	-	4.0	sampling	*	*	*
	2	-	1.0	inspection	sampling	sampling	*
	3	-	2.5	*	inspection	inspection	*
	4	-	4.5	*	*	*	*
June	1	-	12.5	*	*	*	*
	2	-	18.5	*	*	*	*
	3	-	17.0	*	*	*	*
	4	24	20.0	lifting	*	*	*
July	1	26	22.0	inspection	lifting	lifting	
	2	26	19.0	storage	inspection	inspection	lifting
	3	20	19.0	storage	storage	storage	inspection
	4	21	18.0	storage	storage	storage	storage
August	1	19	-	storage	storage	storage	storage
	2	17	-	storage	storage	storage	storage
	3	20	-	inspection	storage	storage	storage
	4	18	-	-	inspection	inspection	storage
September	1	-	-	-	-	-	inspection

a) storage indoors at RH of 70%

a. Treatment with hot water. The effect of a HWT on survival of fungal inoculum in narcissus bulb tissue is demonstrated in Table 5.2. Though bulbs of stock II were healthy it appeared that not only old roots and corked cortex tissue were colonized by *F. oxysporum* ff. spp., *Penicillium* and *Trichoderma spp.* but also the internal cortex tissue (C), which is protected by the outer periderm (OP). Besides this, the implanted root parts showed often more outgrowth of *F. oxysporum* ff. spp. than the old roots.

Heating bulbs affects inoculum of *F. oxysporum* ff. spp. and of *Trichoderma spp.* more than that of *Penicillium spp.* It may be concluded therefore that a treatment with hot water will contribute more to control of Fusarial diseases than a cold water soak.

Table 5.2. Influence of hot-water treatment on the survival of bulb-borne inoculum located in the cortex and old roots of narcissus bulbs. Three healthy bulbs, either left untreated (-HWT) or soaked in water for 2 h at 43.5 °C (+HWT), were stored at 17 °C for two days (RH, 80%), and dissected into tissue parts. Pieces of tissue were incubated on Papavizas medium according to standard procedures and analized for colonization with various fungal species (n=3x100 for roots; n=3x50 for cortex tissue).

Type of bulb tissue	Percentage of tissue pieces colonized					
	F. oxysporum		*Penicillium*		*Trichoderma*	
	- HWT	+ HWT	- HWT	+ HWT	- HWT	+ HWT
old roots (OR, external part)	11	1	87	21	4	1
old roots (AL, implanted part)	43	2	61	31	30	3
cortex (OC, corked part)	24	1	17	36	67	10
cortex (C, outer parenchyma)	28	3	33	38	34	4

b. Drying after soaking. The effect of a forced drying process on survival of fungi in hot-water treated old roots (OR), corked cortex (OC) and old scales (OS) was studied. Healthy bulbs (stock III) were soaked either in hot water or hot dilutions of Aretan and then dried quickly or stored under humid conditions until dissecting and plating out of tissue parts followed.

The symptomless bulbs still carried inoculum of respectively *F. oxysporum* ff. spp., *Penicillium* and *Trichoderma spp.* after HWT, as shown in Table 5.3. Most inoculum of *F. oxysporum* ff. spp. was found in the old roots, less in the corked cortex and nothing in the old scales. Forced

Table 5.3. Effect of forced drying on the survival of some bulb-borne fungi in various tissues of narcissus bulbs. Bulbs soaked for 2 h at 43.5 °C either in water or in solutions of a mercury fungicide at various dosages, dissected and tissue parts either dried or kept moist for 24 h. Presence of viable inoculum of *Fusarium spp.* (Fus), *Penicillium spp.* (Pen) and *Trichoderma spp.* (Tri) determined after plating tissue pieces on PDA and incubation for 5 days at 25 °C.

Inoculum source	Storage conditions	Percentages of tissue pieces with outgrowth on PDA (n=40)											
		water			methoxy ethyl mercury chloride (µg/ml)								
					1			10			50		
		Fus	Pen	Tri	Fus	Pen	Tri	Fus	Pen	Tri	Fus	Pen	Tri
old roots	dry	0	90	48	3	98	13	0	25	0	0	5	0
old roots	humid	45	100	42	13	98	55	3	85	0	0	50	0
old cortex	dry	5	100	30	0	100	85	0	88	8	0	50	0
old cortex	humid	13	100	45	8	100	100	0	65	10	0	35	0
old scales	dry	0	95	58	0	98	10	0	80	0	0	5	0
old scales	humid	0	100	0	0	100	30	0	95	0	0	20	0

drying affects these fungi considerably in different tissues whereas effects on *Penicillium* and *Trichoderma spp.* are negligible.

Addition of the mercury to the hot-water bath reduces the survival of all fungi. Inoculum of *F. oxysporum* ff. spp. is almost completely killed at concentrations of 10 µg/ml, that of *Trichiderma* somewhere between 10 and 50 µg/ml. *Penicillium spp.* survived a dose of 50 µg/ml rather well unless forced drying followed.

c. Repeated wetting. Effects of a second wetting on survival of *F. oxysporum* ff. spp. and *Penicillium spp.* was studied on old roots from bulbs of stock IV from which 50% was discarded due to basal rot. Roots were collected, cut into pieces and subsequently treated with hot water, dried, soaked again during various periods of time in cold or warm baths containing either water or solutions of fungicides, and dried for a second time prior to plating out. It should be mentioned that a dip for 1 minute in a cold bath was not long enough to saturate the dry tissue whereas 1 minute at 43.5 °C was.

Data in Table 5.4. illustrate that both *Fusarium* and *Penicillium spp.* survived the HWT and drying process to which the high level of inoculum has contributed. Viability of *F. oxysporum* remained unaffected when roots were dipped in cold water for 1 minute, but survival was reduced considerably when they were soaked for a longer period or treated with warm water. No negative effects were found with regard to *Penicillium*.

Addition of the organic mercury fungicide, pimaricin or thiram to the second bath enhanced the effects against *F. oxysporum* ff. spp. at both the low and higher temperature. The inoculum of *Penicillium*, however, was not affected at all by a cold-water treatment. In contrast with this, all fungicides, especially thiram, reduced viability of both *Penicillium* and *Fusarium* when the root material underwent a warm re-soaking.

5.4. Influence of temperature on growth of bulb-borne fungi *in vitro*

Response of mycelium growth to temperature was determined for several bulb-borne fungi using PDA as the substrate. Relative growth rates at 10 °C, 15 °C and 20 °C are presented in Fig. 5.1. These data provide an indication of how inoculum will respond to temperature when subjected on bulbs to conditions suitable for growth, viz., in store or after planting in soil.

Table 5.4. Effect of cold (15 °C) and warm (43.5 °C) fungicide dips on viability of *Fusarium* and *Penicillium* inoculum in old root pieces, which had survived an earlier HWT and subsequent drying process. Root pieces dipped for a second time either in water or in fungicide solutions for respectively 1, 15 and 60 minutes; then dried again, and plate out on Papavizas medium.

Treatment	Percentage of root pieces with outgrowth on Papavizas medium (n=100)											
	Fusarium oxysporum ff. spp.						*Penicillium spp.*					
	cold dip			warm dip			cold dip			warm dip		
	1 m	15 m	60 m	1 m	15 m	60 m	1 m	15 m	60 m	1 m	15 m	60 m
water	85	30	28	33	34	11	85	96	93	84	82	98
pimaricin (75 µg/ml)	31	19	13	26	15	7	94	96	99	92	99	84
mercurial (10 µg/ml)	23	10	2	11	4	2	96	82	100	97	79	44
thiram (75 µg/ml)	17	15	10	8	2	0	92	97	99	96	41	2

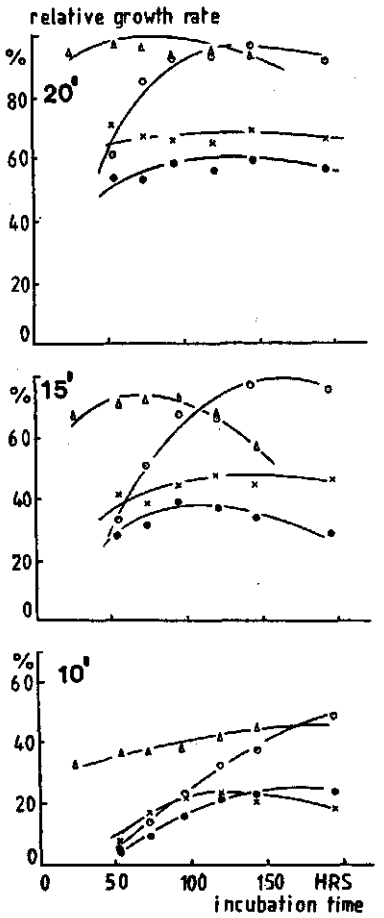

Fig. 5.1. Growth *in vitro* of some bulb-borne fungi at respectively 10 °C, 15 °C and 20 °C. Agar discs with mycelium of *Fusarium oxysporum* f. sp. *narcissi* (-x-), *Penicillium corymbiferum* (-o-), *Penicillium janthinellum* (-●-) and *Trichoderma viride* (-Δ-) plated on PDA and incubated at various temperatures ranging from 5 °C to 40 °C with intervals of 5 °C. Radial growth measured daily in mm and expressed as the percentage of potential growth at the optimum temperature.

P. corymbiferum in particular appears to be the most 'cold-responsive' among the fungi tested. This means that its relative growth rate at lower temperatures increases for a longer period of time than it does for *F. oxysporum* f. sp. *narcissi*, *P. janthinellum* and *T. viride*. The response to temperature is more constant for the latter group of fungi.

It was of interest to observe that *P. janthinellum* with an optimum temperature of 28 °C reacts by secreting a red dye at higher temperatures.

5.5. Influence of soil temperature on colonization of new roots

Two kinds of experiments were carried out to evaluate the influence of soil temperature on colonization of young narcissus roots by the common bulbe-borne fungi such as *F. oxysporum* ff. spp., *Penicillium*, *Trichoderma spp.* and *Cylindrocarpon destructans*. The first experiment (a) was performed under well-conditioned laboratory circumstances. Bulbs of stock I, surface-sterilized with 0.25% formalin, were planted in trays filled with natural bulb soil and incubated at 10 ° and 15°C. A number of these trays incubated at 10 °C were transferred to 15 °C after 19 days, while the opposite was done with trays incubated at first at 15 °C. In the other experiment (b), bulbs of stock IV soaked for two hours either in cold water (CWT, 15 °C) or in warm water (HWT, 43.5 °C) were stored for 4 days at 14-20 °C outdoors and planted in the field. Temperatures at plant depth were recorded continuously.

In both experiments four bulbs per replicate were lifted at various intervals in order to analyse the newly formed roots for colonization by the fungi mentioned.

a. Colonization at fixed soil temperatures. The bulb-borne fungi found on the bases of roots react differently to the soil temperature, as illustrated in Fig. 5.2. *Penicillium spp.* are obviously favoured at 10 °C while *C. destructans* and *T. viride* grew faster at 15 °C. The response of *F. oxysporum* ff. spp. is not so clear, as a low increase in colonization both at 10 ° and 15 °C is followed by a slight decrease at 15 °C, Changing the temperature from 10 °C to 15 °C or the reverse results for *P. corymbiferum*, *C. destructans* and *T. viride* in the same kind of response on temperature as outlined above. *F. oxysporum* ff. spp. react in a less distinct way. One might conclude from this that its response to temperature depends more on the capacity of the other fungi to colonize the roots than the other way around.

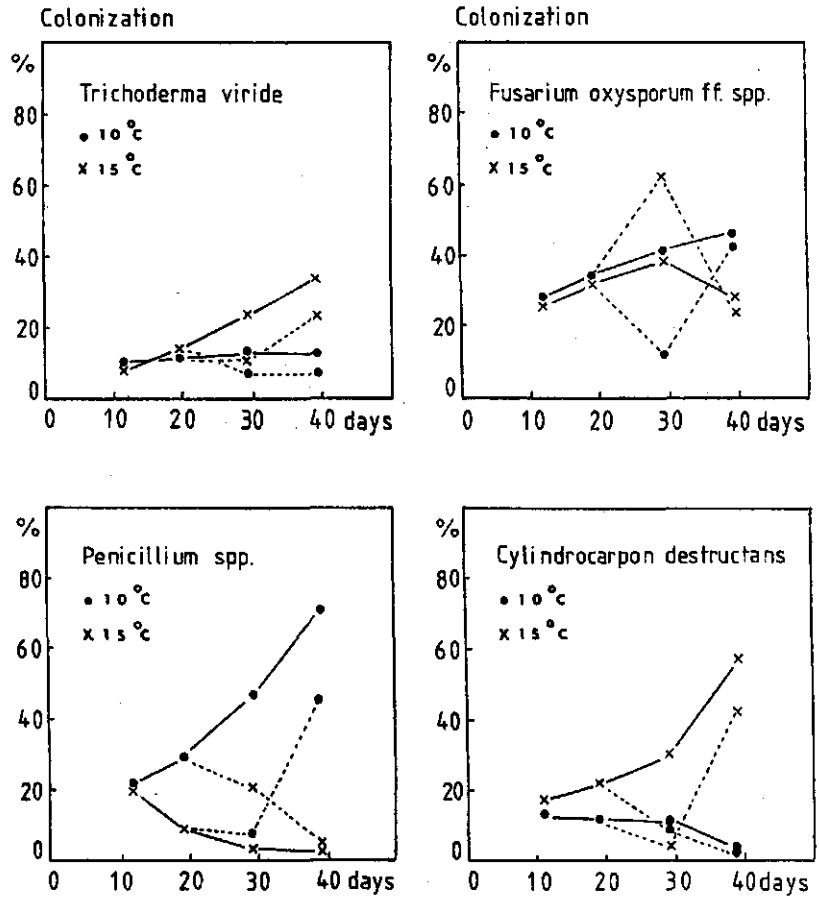

Fig. 5.2. Colonization of narcissus roots by bulb-borne fungi at different soil temperatures. Bulbs surface-sterilized with formalin and planted in natural soil at 10 °C and 15 °C. Temperatures either maintained at respectively 10 °C and 15 °C (straight lines) or changed after 19 days from 10 °C to 15 °C or from 15 °C to 10 °C (dotted lines). Four bulbs were lifted at intervals and roots analyzed on Papavizas medium for presence of respectively *Cylindrocarpon destructans*, *F. oxysporum* ff. spp., *Penicillium spp.* and *Trichoderma viride*. Colonization expressed as the percentage of root pieces showing outgrowth (n=3x50 root pieces per analysis).

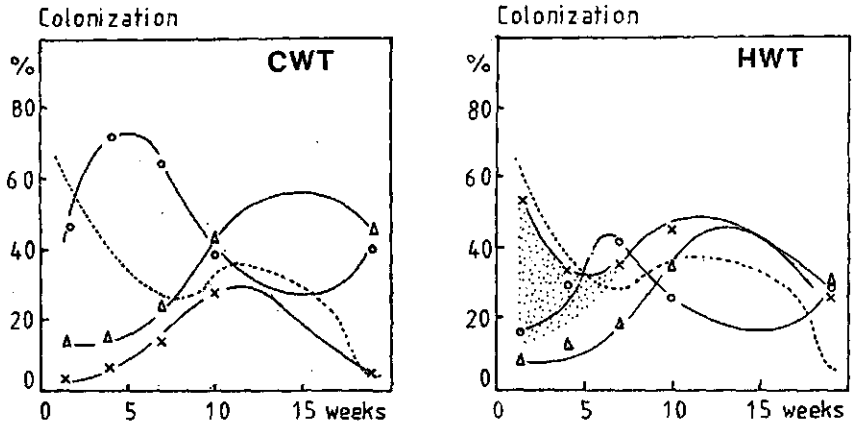

Fig. 5.3. Effect of variable soil temperatures on colonization of young roots of narcissus bulbs planted in the field. Bulbs soaked for 2 hours either in water at 15 °C (CWT) or in water at 43.5 °C (HWT). Degree of colonization expressed as the average percentages of root pieces (n=5x100) showing outgrowth on Papavizas medium of: *F. oxysporum* ff. spp. (-o-), *Penicillium corymbiferum* (-x-), and *Trichoderma viride* (-Δ-). Dotted lines represent average daily soil temperature at plant depth. Shaded area (see text)

b. Colonization at variable temperatures. When comparing the progress in root colonization of bulbs which underwent either a CWT or a HWT it appears that a HWT favours *P. corymbiferum* and *T. viride* to lesser extent not only during storage, but also over the whole periode of 20 weeks in natural soil (Fig. 5.3.). *P. corymbiferum* in particular demonstrates this effect for bulbs from the HWT-series during the first 6 weeks of planting. A great part of its capacity to colonize was lost during that period as indicated by the shaded area in Fig. 5.3., bounded by the dashed artificial line. The high activity of this *Penicillium sp.* probably suppressed *F. oxysporum* ff. spp. Using the artificial line, it becomes easier to interpret the graphs representing the progress in colonization and changes in temperature. Similarity for both types of bulbs (CWT and HWT) can be observed with regard to the individual response of the fungi to the change

in soil temperature. A gradual decrease in temperature from 14 ^{o} to 5 ^{o}C during the sixth week is accompanied by an increase in colonization by all fungi. Such increase continues for *P. corymbiferum* and *T. viride* after 6 weeks, whereas *F. oxysporum* ff. spp. have reached an optimal level at that time, and is followed by a gradual decrease thereafter. Colonization by *P. corymbiferum* and *T. viride* becomes optimal after respectively 12 and 14 weeks.

Knowing the further course of the graphs from Langerak (1977) it is assumed here that *P. corymbiferum* and *P. janthinellum*, react more directly in a positive sense to temperatures below 10 ^{o}C than *F. oxysporum* ff. spp. and *T. viride*. The negative reaction to low temperatures by the latter two fungi is more indirect and depends probably on the high biological activity of the *Penicillium spp.* It was also observed that later in the season when temperatures exceed 15 ^{o}C *Penicillium spp.* gradually lost their capacity to colonize the roots whereas *Trichoderma* and *Fusarium oxysporum* ff. spp. where then strongly favoured.

5.6. Influence of external contamination on development of bulb and root diseases

Attention was paid to the role some common bulb-borne fungi might play in the control of basal rot and root rot, both primarily caused by *F. oxysporum* f. sp. *narcissi*, and of skin-disease caused by *F. oxysporum* ff. spp. In one experiment (a) the significance of natural external contamination for disease development was studied by analyzing the bath water at the end of the HWT. In a second experiment (b) the final effect of artificial contamination on disease development was determined according to the procedures described for experiment C (Table 5.1.). In this case superficially disinfected bulbs were contaminated by dipping bulbs either in suspensions of conidia of a test fungus only (F_9) or in suspensions containing conidia of the test fungus and of the pathogen ($+F_9$).

a. Influence of natural contamination. A causal relation between contamination of the bath water with *F. oxysporum* inoculum and the development of basal rot c.q. root rot was not found since the internal inoculum was more important. However, a significant linear relation (P=0.01) does exist with regard to the development of skin disease, as assessed in four duplicate control lots of experiments A and B (Fig. 5.4.). A positive correlation was obtained with regard to the contamination with *F. oxysporum*

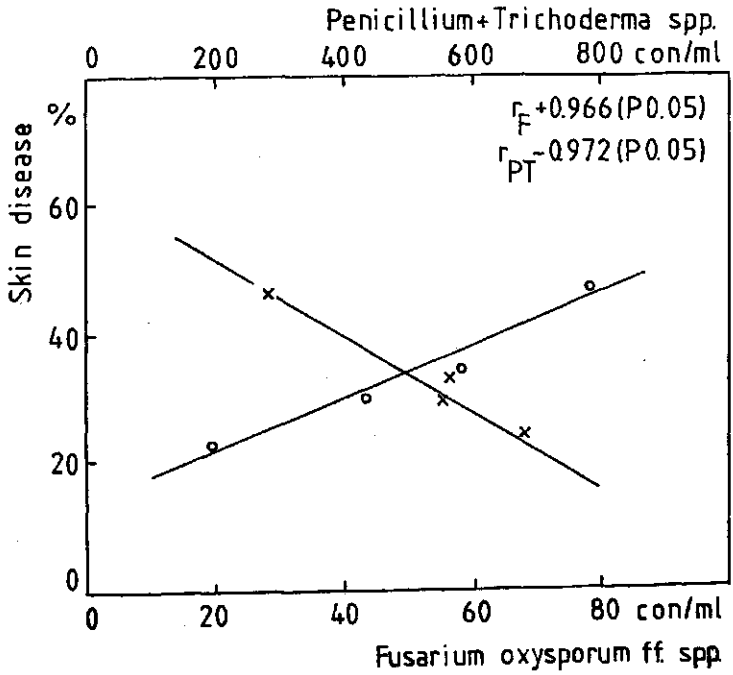

Fig. 5.4. Relation between external contamination of bulbs with *F. oxysporum* ff. spp. (-o-) or *Penicillium spp.* plus *Trichoderma spp.* (-x-), and the development of skin disease in the following season. Duplicate lots (n=4x96 bulbs per lot) soaked two by two in warm water (2 h at 43.5 °C), and planted separately according to procedures described for the field experiments A and B. Survival of inoculum released by bulbs during HWT determined in platings on PDA. Skin disease development for the mean of two duplicates assessed 6 weeks after lifting.

ff. spp. (r= +0.966) and a negative one with regard to the contamination with *Penicillium* plus *Trichoderma* inoculum (r= -0.972). Correlation appeared to be significant at P = 0.05.

b. Influence of artificial contamination. In $-F_9$ objects, where no pathogen had been added, only *T. viride* influenced disease development, as basal rot incidence slightly increased (Table 5.5.). Contamination of the disinfected bulbs with the pathogen strongly stimulated disease development both in bulbs and in roots. None of the test fungi added reduced basal rot incidences in that case. This indicates that antagonism against F_9 can not be built up so quickly that infection of the basal plate is prevented at emergence of the roots directly after planting. That antago-

Table 5.5. Effect of bulb-borne fungi of narcissus on development of basal rot, root rot caused by *F. oxysporum* f. sp. *narcissi* and on skin disease caused by *F. oxysporum* ff. spp. Before planting at 10-13 °C bulbs were soaked in 0.25% formalin (2 h at 43.5 °C), and dipped thereafter either in a conidial suspension of the test fungus only (- F_9) or in suspensions containing conidia of the test fungus and of the pathogen (+ F_9, 25,000 con/ml). Root rot assessment on samples collected in December; basal rot and skin-disease on bulbs lifted in July and stored until August.

Test fungus		Average percentages of bulbs showing symptoms					
species	con/ml	basal rot(n=3x80)		root rot(n=3x20)		skin disease[a]	
		- F_9	+ F_9	- F_9	+ F_9	- F_9	+ F_9
none (control	-	3.6	94.5	2	15	29	62
C. destructans	$2x10^5$	0.8	91.0	3	5	32	30
T. viride	$2x10^5$	9.5	90.8	3	11	30	34
P. janthinellum	$4x10^4$	-	93.9	-	8	-	46
P. janthinellum	$2x10^5$	5.7	90.4	4	7	38	30
P. corymbiferum	$2x10^5$	-	92.0	-	7	-	70

a) percentages based on number of bulbs which remained free of basal rot in store

nism against the pathogen is possible follows from the effects on development of root rot and skin disease. This leads to the conclusion that infection of roots and basal plates does not occur simultaneously.

The rather strong reduction of root rot incidence by the 'cold-responsive' *P. corymbiferum* and the minor effect by *T. viride* is plausible as the average soil temperature during the first two weeks after planting varied between 10 °C and 13 °C. Less easy to explain in such a way is the strong antagonism by *C. destructans* and by the less 'cold-responsive' *P. janthinellum* which like *T. viride* are favoured more at higher temperatures.

Figures for skin disease development in the + F_9 series also illustrate that all test fungi with the exception of *P. corymbiferum* are able to eliminate the extra disease development induced by *F. oxysporum* f. sp. *narcissi*. The ineffectiveness of *P. corymbiferum* here fits well with the observation that infection of the scales takes place at the end of the season when temperatures exceed 15 °C and with the knowledge that the activity of this fungus will be relatively low under such conditions.

5.7. Influence of soil temperature on effectiveness of standard fungicides

The influence of soil temperature on the effectiveness of formaldehyde, methoxy mercury chloride, pimaricin and thiram was determined with respect to control of basal rot and skin disease. The responses on three different planting dates were compared in the field experiments A, B and D (Table 5.1.) and evaluated. It is mentioned here that apart from differences in initial soil temperatures after planting differences in time of duration of HWT and kind of storage have to be taken into account of evaluating the data presented in Table 5.6. Distinction has also to be made between external and internal sources of inoculum of *F. oxysporum* f. sp. *narcissi* and other formae speciales of *F. oxysporum*.

Table 5.6. Influence of soil temperature at planting on the effectiveness of standard fungicides, previously applied during a HWT, on the control of basal rot and skin disease. With the exception of the controls, standard dosages of the fungicides were added to the bath which had been contaminated with 75,000 con/ml of *F. oxysporum* f. sp. *narcissi*. Treated bulbs stored and planted according to the procedures described for the experiments A, B and D. Basal rot incidence expressed as the percentage of bulbs showing visible symptoms after post harvest storage; skin disease incidence as the percentage of basal rot-free bulbs with typical dry scale rot.

Fungicides added during HWT	Survival conidia after HWT (con/ml)	Average percentage of diseases bulbs soil temperature during the first two weeks					
		Exp A:13-15°C		Exp B:8-10°C		Exp D:4.5-6.5°C	
		basal rot	scale rot	basal rot	scale rot	basal rot	scale rot
None (control I)[a]	100	47.2	41	19.7	34	2.7	24
None (control II)	23,500	84.4	61	76.7	48	4.3	30
'Aretan' (0.5% v/v)	50	4.2	28	0.3	16	-	-
Formalin (1% v/v)	50	1.9	41	0.3	18	-	-
Liro-thiram (0.5% v/v)	1,000	17.5	27	18.8	15	-	-
BAM-A (5% v/v)	50	2.2	21	1.7	13	1.3	5

a) no conidia of the pathogen added

The 20% of bulbs in control I showing basal rot (experiment B) probably originated from internal inoculum as the temperature of 8-10 °C at planting would have been too low to allow infection of the basal plates

from outside. Such diseased bulbs might have originated from infections which took place during the dry storage at 17 °C for 14 days. It may therefore be concluded, that among the fungicides tested only thiram was unable to overcome this kind of infections in either experiment A or B. The other fungicides however did, and beyond that they controlled those infections caused by externally located inoculum. Planting bulbs from the Control I and II series at temperatures below 8 °C in late November resulted in very low incidences of basal rot notwithstanding the increase of temperature to above 18 °C in the following summer. This emphasizes once more that infection of basal plates is only possible when the roots emerge.

Soil temperature at planting time influences both the initial colonization of bulbs with *F. oxysporum* ff. spp., including f. sp. *narcissi*, which causes basal rot, and the colonization of the bulbs during the rest of the season (Figs 5.2., 5.3., Table 5.6.). This is also confirmed by the incidences of skin disease found for the bulbs of the two control series (Table 5.6.): the later they were planted the lower was the disease incidence.

Another conclusion pertains to the indirect effects of the mercury fungicide, pimaricin and thiram on the development of skin disease. Existence of such effects in which *Penicillium* and *Trichoderma* play an active role, is indicated by the fact that formalin does eliminate basal rot to the same extent as the mercury compound in experiment A, whereas no reduction of skin disease is found.

5.8. Influence of incubation conditions on the mode of action of pimaricin in the control of *Fusarium* diseases of narcissus

The influence of storage conditions after a HWT and soil temperature after planting on the effectiveness of pimaricin previously applied during a HWT was investigated. The antibiotic formulated as BAM-B and Pim-N was applied therefore in various dosages on old roots from stock IV (a) and on complete bulbs of stock I which were internally and externally contaminated with *F. oxysporum* f. sp. *narcissi* and other formae speciales of *F. oxysporum* (b). Roots were plated out after HWT to determine the effects of pimaricin on survival of the bulb-borne inoculum. Bulbs were stored after HWT and then planted following the procedures described for experiments A and B (Table 5.1.). Routine assessments were made to collect data relating to the survival of inoculum in the bath, the indices of respectively root rot

in December, advanced basal rot at lifting in July, new basal rot developed thereafter, skin disease in August and increase in weight. All data were expressed as percentages of the values obtained for the corresponding control objects, and plotted against the concentration of the antibiotic dissolved in the bath water at the end of the HWT.

a. Effect on survival in old roots. The survival of *F. oxysporum* inoculum in old roots decreased strongly at dosages in the range of 20-40 µg/ml as shown in Fig. 5.5. *Penicillium spp.*, however, were favoured in that range as the frequency in which outgrowth from root pieces occurred increased considerably. An optimal response has been found at 35-40 µg/ml.

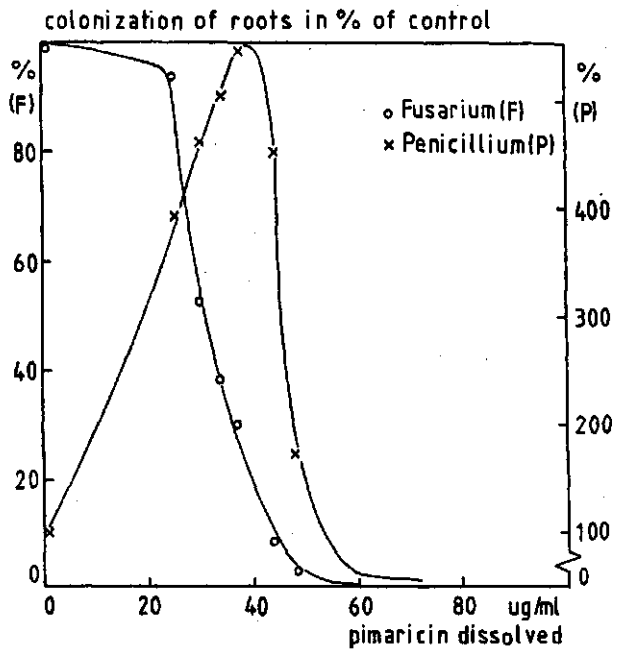

Fig. 5.5. Effect of pimaricin on survival of some bulb-borne fungi in old narcissus roots. Root pieces were treated in hot water (4 h at 43 °C) in presence of various dosages of Pim-N; stored after HWT for 24 h at 20 °C (RH 90%); plated out on Papavizas medium; incubated for 6 days at 25 °C and examined for outgrowth of *F. oxysporum* ff. spp. (-o-) and *Penicillium spp.* (-x-). Survival of fungi expressed as the average percentages of colonization found in the control (n=2x100).

b. Effects on diseases in complete bulbs. From analysis of the bath water it appeared that at final dosages of 28, 56 and 94 µg/ml 3,900, 1,300 and 400 conidia/ml, respectively, survived against 23,500 conidia/ml in the control.

Bulbs from experiment B did not show any root rot in December as a result of the low temperature at planting time, whereas those from experiment A did for initial soil temperatures of 13-15 °C. In the latter case a sigmoid dosage-response curve was obtained (Fig. 5.6B.). A slight interruption in its course at dosages between 30 and 60 µg/ml might indicate that a second factor interfered with the activity exerted by pimaricin.

Although bulbs from both experiments (A and B) were treated under equal circumstances, a clear difference in reaction has been found with regard to the development of basal rot assessed at lifting in July (Figs 5.6A.). The average values were connected by the best fitting curve, since similarity in response on concentration was found for all four replicates per treated object. Apart from an almost constant difference in disease development, probably the result of lower initial soil temperatures, an extra reduction of basal rot is found then for the bulbs of lot B in the range of 30-60 µg/ml with an optimal effect at about 40 µg/ml of the antibiotic in the bath.

Curves presented for new basal rot developed after lifting (Fig. 5.6B.) also demonstrate a difference in response to treatment procedures and to pimaricin concentration in the hot-water bath at less than 60 µg/ml. Bulbs from experiment B show a gradual decrease in disease incidence with respect to these in the control samples. Bulbs from experiment A, however, reacted quite differently, since, especially in the range 30-50 µg/ml, much higher levels of rotten bulbs were found than in the control.

Comparison of the curves in Fig. 5.7., representing response of skin disease development to concentration of pimaricin, shows that bulbs from both experiments A and B, stored and planted under different circumstances, react in a similar way on addition of pimaricin to the bath. An extra reduction of skin disease is found again in the range of 20-50 µg/ml. Procedure B yielded more healthy bulbs with respect to the control sample than procedure A, especially at concentrations of 40 µg/ml or more. The nature of the response to concentration is identical for both experiments which deviates from the findings for basal rot development. This strenghtened the opinion that the moment that infection of the basal plate occurs does not coincide with the time at which invasion of the bulb scales takes place.

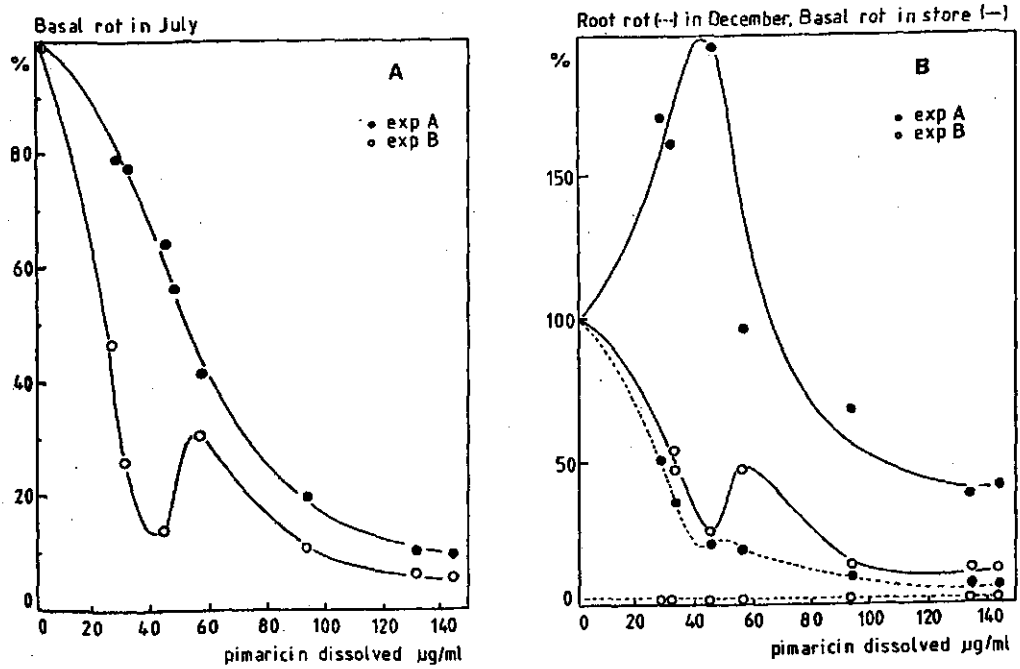

Fig. 5.6. Effects of pimaricin on basal rot and root rot of narcissus caused by *F. oxysporum* f. sp. *narcissi*. Bulbs soaked for 2 h at 43.5 °C in conidial suspensions of the pathogen (75,000 con/ml) to which had been added either pimaricin in various dosages or nothing (control). Bulbs after HWT stored and planted according to procedures for experiments A (-●-) and B (-o-). Assessments were made for the incidence of advanced basal rot in July (Fig. A), and of root rot in December (dashed line) and new basal rot developed in store (Fig. B). Disease incidences expressed as the percentage of disease development in the control (n=4x16 for root rot; n=4x80 for basal rot).

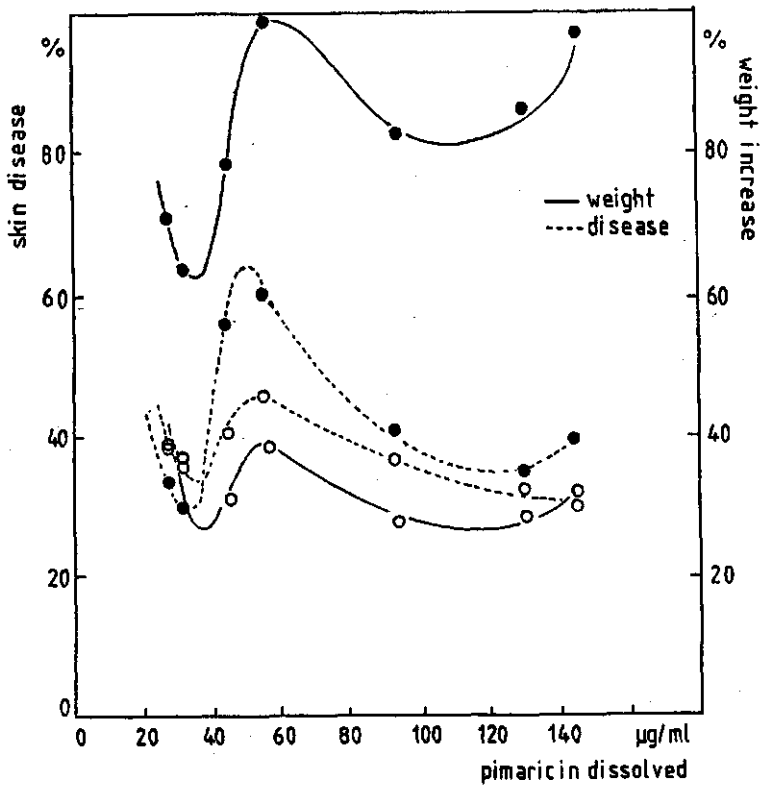

Fig. 5.7. Effects of pimaricin on weight increase and development of skin disease of narcissus bulbs caused by *F. oxysporum* ff. spp. Bulbs soaked for 2 h at 43.5 °C in conidial suspensions of the pathogen (75,000 con/ml) to which had been added either pimaricin in various dosages or nothing (control). Bulbs after HWT stored and planted according to procedures for experiments A (-●-) and B (-o-). Incidence of skin disease and weight increase determined on bulbs showing no basal rot 6 weeks after lifting. Disease incidence expressed as the percentage of bulbs showing symptoms in the control; increase in weight as percentage of weight increase of control bulbs.

A certain degree of parallelism in reaction to pimaricin dosages was observed with regard to yield increase and skin disease development although an opposite response would have been more logical. The relatively higher levels of skin disease, when bulb growth has apparently not been limited by *Fusarium* root attack, can be explained by the formation of cracks in the fleshy outer scales as a result of excessive bulb growth. Contamination with higher levels of inoculum will occur then at lifting when bulbs are mixed thoroughly with the soil in which they have grown. The relatively high yield increase for bulbs from the A-series can be explained by the very low weight of the control bulbs in this series.

The similarity in response at 20-50 µg/ml, at which concentration the effectiveness of pimaricin looks better than at higher dosages, may have the same background for all different examples presented, viz., an increased antagonism through *Penicillium spp.*, which is favoured at that concentration range (Fig. 5.5.).

5.9. Discussion

Standard hot-water treatments (HWT) of narcissus bulbs or parts of bulbs reduced the viability of bulb-borne *Fusarium oxysporum* ff. spp. Saprophytic fungi such as *Penicillium* and *Trichoderma spp.* were relatively less affected by this kind of treatment (Table 5.2.). Drying back of bulb material, which had been soaked in hot water, also caused a shift in the bulb microflora. Inoculum of the saprophytes appeared to be more resistant to quick drying than *F. oxysporum* ff. spp. (Table 5.3.). The shift in favour of *Penicillium* and *Trichoderma spp.* was reinforced when thiram, pimaricin or methoxy ethyl mercury chloride was added to the hot water (Table 5.4.). Very pronounced changes in colonization with *F. oxysporum* ff. spp. and *Penicillium spp.* were particularly observed when root pieces were treated in hot water, containing various dosages of pimaricin. *Penicillium spp.* were favoured between 20 and 50 µg/ml with a maximal effect at 35-40 µg/ml whereas the viability of *F. oxysporum* ff. spp. was affected most severely at such concentration (Fig. 5.5.). After a HWT, the temperature in store also influenced the balance between the various bulb-borne fungi. Low temperatures (< 15 °C) are known to be favourable for development of *Penicillium spp.* (Saaltink, 1968), whereas higher temperatures stimulate *Fusarium oxysporum* f. sp. *narcissi* (McClellan, 1952). Tempera-

ture in soil will also influence differently the fungi which are already present in the bulb and those which will colonize the bulb from the soil itself (Langerak, 1977). The data presented show that *P. corymbiferum* and to a lesser extent *P. janthinellum* are fungi favoured by cold (Figs 5.2., 5.3). *Trichoderma viride*, *Cylindrocarpon destructans* and *F. oxysporum*, however, grow much better at higher temperatures (> 15 °C). The difference in response to temperature (Fig. 5.1.) also explains why, prior to planting, the addition of *P. corymbiferum* inoculum to bulbs resulted in a significant reduction of root rot in autumn (10-13 °C), and why almost there was no activity against development of skin disease in summer (18-20 °C) (Tables 5.1. and 5.5.). The opposite effects obtained for bulbs treated with *T. viride* conidia can be explained in a similar way. However, the rather strong antagonistic action of *P. janthinellum* and *C. destructans* against root infections caused by *F. oxysporum* f. sp. *narcissi* are more difficult to understand since these fungi are favoured at temperatures higher than those measured at planting time (Table 5.1.). Their action against the pathogen might have been the result of a production of antifungal metabolites induced at and accelerated by the minimal growing conditions for these fungi. Such a phenomenon has been observed earlier for the same fungi when they were maintained *in vitro* under stress conditions created by sub-lethal dosages of fungicides (Langerak, 1977).

It is obvious now in view of the influence environmental factors may have on the bulb microflora that the effectiveness of fungicides applied to bulbs cannot be explained by the intrinsic activity of the fungicide alone. The results of Table 5.6. clearly demonstrate that a HWT late in the season followed by planting at soil temperatures below 8 °C can reduce disease development to the same extent as the addition of a fungicide to the bath. It is also interesting to observe that heavy external contamination with *F. oxysporum* f. sp. *narcissi* enhanced the incidence of skin disease, and that reduction of basal rot due to late planting was more pronounced than the suppression of skin disease. Furthermore, it is of practical value to know that a delayed planting of bulbs treated with formaldehyde can result in a significantly lower incidence of skin disease although no selective stimulation of antagonistic fungi can be expected from the fungicide itself.

An exact determination of the optimal dose of pimaricin for control of both basal rot and skin disease in narcissus encountered some problems. The disease figures obtained did not fit in ordinary linear dosage-response

curves when the data were treated according to standard procedures for regression. It was obvious that in addition to the concentration of pimaricin dissolved in the warm-water bath, at least one other factor was involved. Therefore, the values for four replicates per treatment were used to fit curves through the mean values which made interpretation of the data possible (Fig. 5.6.). Conspicious effects on disease development can especially be observed in the range of 20 to 60 µg/ml. Skin disease velopment was remarkably low at such concentrations. An optimal effect was reached then at approximately 35 µg/ml independently of whether bulbs had been treated and planted according to method A or B. Bulbs treated according to method B showed similar effects with regard to the development of advanced basal rot in July. This phenomenon might be attributed to an increased development of *Penicillium spp.* antagonistic to *F. oxysporum* ff. spp. This inference is most plausible since such a specific effect of pimaricin on *Penicillium spp.* had been found earlier *in vitro* for the same concentration range (Fig. 5.5.). Such an indirect contribution in disease control does not seem to exist with regard to the development of advanced basal rot in experiment A (Fig. 5.6.). However, the effect might be there, considering that in both experiments the differences in response to new basal rot developed in store. One should accept then that a considerable number of the basal plate infections in experiment A were very slight as a result of antagonistic action by *Penicillium spp.* at 13-15 ^{o}C, whereas in experiment B these infections did not occur at all due to the low soil temperature (8-12 ^{o}C), and the positive effect of that temperature on antagonism through *Penicillium spp.*

6 GENERAL DISCUSSION AND CONCLUSIONS

The data obtained from laboratory and field experiments lead to a revised view on the aetiology of basal rot of narcissus. Natural infection of narcissus bulbs by *F. oxysporum* f. sp. *narcissi* occurs at emergence of the roots, and not as accepted earlier at the moment bulbs mature and die off. Although also the basal plate may be infected at that time, basal rot mostly develops only at the end of the growing season or in store after lifting. This new view on the aetiology of the disease, will obviously have consequences for disease control.

Infection of narcissus bulbs only occurs when living tissue of a bulb has been wounded and sufficient inoculum of the pathogen is present at the infection site. Wounding of the bulb base and scales will often be the result of mechanical damage at respectively lifting, transport, grading and planting. However, injuries can also be caused by active growth of the bulb itself. One might think then of emergence of the roots by which the biggest part of the bulb base becomes exposed to the environment, and on cracks in the fresh outer scales and basal plate late in the season or shortly after lifting, if bulbs undergo a too rapid drying process. Thus, the periode during which infection is possible is rather restricted viz., between lifting and emergence of new roots in the next season. It is not very likely that infection takes place in the period that roots and foliage mature and die, as formation of abscission layers will protect the bulb against penetration. A similar explanation holds for infection of roots. Establishment of the pathogen in the cortex of a root is only possible when penetration precedes the suberization of the exodermis, which occurs very soon after emergence.

Symptom expression may take place shortly after infection, provided that the temperature exceeds 13 ^{o}C. A causal relationship does not seem to exist between root infection and basal plate infection. Nevertheless, presence of root rot in autumn always indicates that also infection of the basal plate has taken place earlier. Exceptionally, nematodes may injure roots some time after emergence so that then penetration of the roots would become possible, and theoretically, also invasion of the basal plate. Infection of bulbs through roots may also occur when immature bulbs are lifted very early at a time that formation of corked abscission layers has not yet been completed.

Although many infections may be due to incarefulness of the grower himself, no precautions can be taken to avoid natural wounding of the basal plate at the time of root emergence. Since disease control should aim at prevention of infection, measures should be taken which reduce inoculum at the infection site or which lead to unfavourable conditions for the pathogen at time of penetration. Besides avoiding mechanical damage, also sanitary measures, such as roguing during the season and discarding of diseased bulbs at lifting and in store, will contribute to reduction of the inoculum potential in a lot. An impression of the necessity to invest time and money in such measures can be obtained from inspection of bulbs sampled in December. Next, a cool (< 13 °C) and dry storage will also limit the total inoculum in a lot. When latent infections are expected to be present it is advisable to proceed with cooling until planting. In such cases it is also better to wait until the soil temperature at plant depth is lower than 13 °C.

If, in spite of all measures, bulbs become contaminated with inoculum of *F. oxysporum* f. sp. *narcissi*, treatment with fungicides is recommended in the last resort. When such bulbs are hot-water treated or soaked in another way, addition of a chemical with fungicidal properties will even be a prerequisite. It was found that treatment of bulbs with organic mercury compounds, thiram or pimaricin can result in shifts in the microflora of narcissus roots and bulbs in favour of *Penicillium* and *Trichoderma spp.*, while *F. oxysporum* ff. spp. and *Cylindrocarpon destructans* are reduced. Formaldehyde does not influence the composition of the microflora. Changes in colonization of bulbs play a significant role in disease control as some of the fungi have the capacity to inhibit growth of *F. oxysporum* ff. spp. *in vitro* and *in vivo*. Besides favouring some fungi in their competition with other micro-organisms, fungicides can also induce production and secretion of metabolites having fungitoxic properties. Moreover, some of these metabolites may act synergistically when combined with the fungicide which induced their production.

It is emphasized here that the above mentioned indirect effects of fungicides on the pathogen have to be taken into account when alternatives are sought for the chemicals on narcissus bulbs. In fact, we have in this way a combination of chemical and biological control. Indirect action via antagonists may not only enhance the effect on the pathogen, but may also stretch the control period. Even when the fungicides has disappeared, the effect of the antagonists on the pathogen may continue.

It is obvious, that the use of chemicals which eliminate for example the *Penicillium* and *Trichoderma* antagonists should be avoided.

A formulation of the antibiotic pimaricin, named Delvolan, got an official approval for application against basal rot in narcissus bulbs. Use of this product, however, remained rather limited since its introduction in practice. This product has been withdrawn from the market now for that reason.

SUMMARY

The pathogenesis of *Fusarium oxysporum* f. sp. *narcissi*

Basal plates and roots of narcissus were infected by *Fusarium oxysporum* Schlecht f. sp. *narcissi* (Cooke & Massee) Snyder & Hansen during the period of emergence of the roots in autumn only. This observation contrasts with the generally accepted view that infections mainly take place when roots die at senescence of the plant in early summer. It is concluded from histological observations that before root emergence the basal plate tissue is externally protected by an outer periderm. Shortly after the infection period during which roots rupture the periderm, bulbs and roots appear to possess a natural resistance against penetration and infection by fungi. New infection of the roots is prevented by suberization of the root exodermis. A periderm formed in the parenchymatous tissue of the basal plate surrounding the roots also acts as a barrier. The central disc itself becomes protected in early summer by formation of lignified abscission layers across the cortex and stele of senescent roots.

For infection of the bulb and roots a temperature of above 13 °C and a high humidity favouring fungal growth are prerequisites. Root and basal rot will develop within a few weeks from the time that roots emerge. Decay of roots in early summer follows natural abscission of the roots.

Latent and active infections may be discerned depending upon their position with respect to the inner periderm. The relationship between the site of infection and the development of symptoms is discussed.

The commonly applied hot-water treatment contributes significantly to the spread of the pathogen. Control of basal rot requires killing of bulb-borne inoculum before planting and protection against soil-borne inoculum during the first week of root emergence after planting, which both can be achieved by application of a fungicide.

The relation between autumnal infection of narcissus bulbs by *Fusarium oxysporum* f. sp. *narcissi* and disease development in the following season

Bulbs of narcissus contaminated with conidia of *Fusarium oxysporum* f. sp. *narcissi*, in absence or presence of fungicides during a hot-water treatment

(HWT, 2 h at 43.5 °C), were planted in September and October. When, after the HWT, bulbs were stored for 4 days at 17 °C under humid conditions (RH > 90%) and planted in soil at 13-15 °C, root rot was observed in December and basal rot in July. New basal rot developed during subsequent storage in August. However, when similarly treated bulbs, prior to planting at 8-12 °C, were stored during an additional period of 14 days at a RH of 70%, various types of bulb rot were present in December instead of root rot. Then sickle-, dwarf- and non-emerging plants were noticed in April, and very advanced basal rot in relatively high percentages of bulbs in July. For bulbs planted at 13-15 °C, a high positive correlation was found between the incidence of root rot in December and the presence of basal rot at the end of the season. For bulbs planted at 8-12 °C after 14 days of storage at RH of 70%, a correlation was found between the incidence of bulb rot in December and the subsequent development of symptoms in the foliage in April and the presence of basal rot in July.

The relationship between autumnal infections of roots and basal plates and the development of symptoms later in the season holds for most situations in practice. Early knowledge about the health condition of a bulb lot in the field will support the setting up of an effective programme of roguing in spring and will indicate the most appropriate cultural measures to be employed from the time of lifting.

The role of antagonists in the chemical control of *Fusarium oxysporum* f. sp. *narcissi*

Treatment of narcissus bulbs with methoxy ethyl mercury chloride, pimaricin or thiram provided control of basal rot and primary root rot, caused by *Fusarium oxysporum* f. sp. *narcissi* and secondary root rot and 'skin disease' caused by *F. oxysporum* ff. spp. A similar treatment with formalin was only effective against basal rot and primary root rot. Newly formed roots of bulbs treated with the mercurial, pimaricin or thiram became more heavily colonized by *Penicillium* and *Trichoderma* spp. than those of untreated or formalin-treated bulbs. Especially *P. janthinellum* and *T. viride* appreared to protect the roots against the pathogens. These fungi were found to be antagonistic to *F. oxysporum* f. sp. *narcissi* and *F. oxysproum* ff. spp., both in experiments *in vitro* and *in vivo*. A synergism between *P. janthinellum* and thiram was observed with respect to inhi-

bition of mycelial growth of *F. oxysporum* f. sp. *narcissi in vitro* and to control of root rot and basal rot *in vivo*. *In vitro*, similar effects were also found for pimaricin and organic mercury compounds. Pimaricin was found to stimulate the production of antibacterial metabolites by *Cylindrocarpon destructans in vitro*. In the field, a synergistic effect was observed between pimaricin and *C. destructans* with respect to control of basal rot.

The influence of incubation conditions after lifting on development of basal rot and skin disease and on effectiveness of chemical control measures

Fusarium oxysporum f. sp. *narcissi* and other formae speciales of *F. oxysporum* were more affected by heating during a hot-water treatment (HWT, 2 h at 43.5 °C) than bulb-borne *Penicillium* and *Trichoderma spp.* were. The latter fungi also survived a forced drying process after a HWT better. Such treatment of bulbs and bulb parts resulted in a changed distribution of micro-organisms on the bulbs in favour of these saprophytes. Addition of either a mercurial thiram or pimaricin accelerated these effects on the bulb microflora, whereas formalin did not. A period of cool (< 15 °C) storage enhanced both the growth rate of *Penicillium spp. in vitro* and their capacity to colonize bulb tissue in natural soil. Higher temperatures stimulated development of *Trichoderma viride*. *Cylindrocarpon destructans* and *F. oxysporum* ff. spp. Late planting at temperatures below 8 °C reduced disease development substantially.

Further it was found that *Penicillium corymbiferum*, *Penicillium janthinellum* and *Trichoderma viride* are able to contribute to control of root rot and skin disease caused by *F. oxysporum* f. sp. *narcissi* and/or *F. oxysporum* ff. spp.

The data obtained contribute to a better understanding how incubation conditions from lifting onwards can influence disease development in the following season. It was possible to elucidate why a low dose of a fungicide like pimaricin, applied during a HWT could give better control of basal rot and skin disease than higher dosages did.

SAMENVATTING

Sedert de jaren dertig hebben kwikhoudende fungiciden een belangrijke rol gespeeld bij de bestrijding van ziekten in land- en tuinbouwgewassen. Door hun hoge activiteit tegen schimmels, hun grote chemische stabiliteit en lage prijs werden ze vooral toegepast ter ontsmetting van zaaizaad, bloembollen en pootaardappelen. Een grotere mate van milieu-bewustheid leidde in het begin van de jaren zeventig tot een aantal, van overheidswege opgelegde, beperkende maatregelen met betrekking tot de toepassing van deze kwikmiddelen. Uit fytosanitair oogpunt waren die te rechtvaardigen omdat inmiddels nieuwe en minder giftige fungiciden geïntroduceerd waren voor toepassing in de consumptieteelten. Algehele vervanging vond evenwel niet plaats hetgeen voor het toenmalige Ministerie van Volksgezondheid en Milieuhygiëne reden was een werkgroep in te stellen met de opdracht de mogelijkheden te onderzoeken van vervanging in de vermeerderingsteelten. De auteur kreeg als taak dit onderzoek uit te voeren onder toezicht van een Begeleidingscommissie bestaande uit vertegenwoordigers van het Ministerie, diverse landbouwkundige instituten en instellingen alsmede een farmaceutisch bedrijf.

Omdat de vervanging van kwik met betrekking tot de bestrijding van het bolrot in narcissen het meest urgent was, werd dit het eerste onderwerp van studie. Uit een aantal oriënterende experimenten bleek dat een gedegen onderzoek naar de pathogenese van *Fusarium oxysporum* f. sp. *narcissi*, de veroorzaker van het bolrot van narcissen gewenst was, omdat de literatuur hieromtrent onduidelijk en ten dele tegenstrijdig was (hoofdstuk 2). Inzicht in de relatie tussen aanwezige bolinfecties in de herfst en ziekte-ontwikkeling in het daarop volgende seizoen bleek belangrijke informatie op te kunnen leveren voor de teler in verband met de planning en uitvoering van bestrijdingsmaatregelen naast die van een behandeling met fungiciden (hoofdstuk 3). Daar antagonistische schimmels, aanwezig op bollen en in de grond een rol bleken te spelen in de ziekte-ontwikkeling en bestrijding met fungiciden, werd besloten ook aan dit aspect aandacht te schenken (hoofdstuk 4). Tenslotte diende de invloed van milieu-omstandigheden onderzocht te worden, ten aanzien van zowel de ontwikkeling van bolrot in het veld en de bewaring als op de effectiviteit van chemische bestrijdingsmaatregelen (hoofdstuk 5). Presentatie en evaluatie van de belangrijkste conclusies vonden plaats in hoofdstuk 6.

De pathogenese van *Fusarium oxysporum* f. sp. *narcissi*

Infectie van de bolbodem en de wortels van narcis door *Fusarium oxysporum* Schlecht f. sp. *narcissi* (Cooke & Massee) Snyder & Hansen vindt alleen tijdens de periode van beworteling in de herfst plaats en niet, zoals algemeen aangenomen werd, ten tijde van het afsterven van de wortels bij veroudering van de plant in de voorzomer. Histologisch onderzoek toonde aan dat vóór het uitlopen van de wortels, het weefsel van de basale plaat beschermd wordt door een buiten-periderm. Reeds kort na de infectieperiode tijdens het doorbreken van dit periderm, blijken de wortels evenals de bol weerstand te bieden tegen infectie door schimmels. Verkurking van de wortel-exodermis voorkomt dan infectie van de nieuwe wortels. Een periderm, dat in het parenchymatische weefsel van de basale plaat gevormd wordt en de wortels omgeeft, fungeert eveneens als barrière. De centrale bolschijf zelf wordt in de voorzomer beschermd tegen binnendringen van schimmels vanuit de dan niet meer functionele wortels door vorming van verhoute abscissielagen dwars door de schors en de centrale cilinder van de wortels heen.

Voor binnendringing van de bol en de wortels zijn temperaturen boven 13 °C nodig en een hoge vochtigheid. Ontwikkeling van wortelrot en bolrot zal binnen enkele weken volgen mits de temperatuur vanaf het begin van de beworteling gedurende een periode van tenminste 2 weken hoger is dan 15 °C. Wegrotten van wortels in de voorzomer vindt meestal plaats na afstoting van de wortels als gevolg van de vorming van een abscissielaag.

Afhankelijk van hun positie ten opzichte van het binnen-periderm kan onderscheid gemaakt worden tussen latente en actieve infecties. Verder blijkt er een oorzakelijk verband te bestaan tussen het type infectie en de symptoomexpressie.

De algemeen toegepaste warm-waterbehandeling draagt bij tot de verspreiding van de ziekteverwekker. Chemische bestrijding vereist vóór het planten doding van het op en in de bol aanwezige inoculum en tijdens de eerste week van de beworteling na het planten, een beschermende werking van het fungicide tegen het in de grond aanwezige inoculum.

Het verband tussen herfst-infectie van narcisbollen door *Fusarium oxysporum* f. sp. *narcissi* en ziekte-ontwikkeling in het daarop volgende seizoen

Narcisbollen, die tijdens een wwb (2 uur bij 43,5 °C), met conidiën van

Fusarium oxysporum f. sp. *narcissi* besmet werden in aanwezigheid of afwezigheid van fungiciden en daarna geplant in september en oktober, vertonen een verscheidenheid van ziekteverschijnselen. De aard van de symptomen werd bepaald door de bewaaromstandigheden tussen de wwb en het planten, en de bodemtemperatuur gedurende de eerste weken na het planten. Bewaring gedurende 4 dagen bij 17 °C onder vochtige omstandigheden (RV > 90%) na de wwb en planten bij 13-15 °C resulteerde in de aanwezigheid van wortelrot in december en bolrot bij het rooien in juli. Nieuw bolrot ontwikkelde zich ook tijdens en de daarop volgende bewaring in augustus. Bollen met een zelfde voorbehandeling, echter met een daarop aansluitende extra bewaarperiode van 14 dagen bij 17 °C en een RV van 70% vóór het planten bij 8-12 °C gaven in december verschillende typen bodemrot te zien in plaats van wortelrot. Sikkel-, dwerg- en niet-opgekomen planten ('blinds') werden dan in april waargenomen en relatief hoge aantallen met bolrot in juli.

Voor bollen, die bij 13-15 °C geplant werden, werd een duidelijke positieve correlatie gevonden voor de mate van wortelrot in december en de aanwezigheid van bolrot aan het einde van het seizoen. Een overeenkomstige relatie werd voor bollen geplant bij 8-12 °C gevonden ten aanzien van het bolbodemrot in december en de daarop volgende ziekte-ontwikkeling in april en de aanwezigheid van bolrot in juli.

Het verband tussen in de herfst optredende infecties en de later in het seizoen gevonden symptoomontwikkeling gaat voor de meeste in de praktijk voorkomende gevallen op.

Vroegtijdige bekendheid met de gezondheidstoestand van een partij te velde zal in een belangrijke mate het nemen van een beslissing ondersteunen over te gaan op intensief "ziek-zoeken" in het voorjaar. Tevens kan op grond van die kennis vanaf het rooitijdstip een gericht programma van kultuurmaatregelen afgewerkt worden om hierdoor onnodige risico's in het volgende seizoen te voorkomen.

De rol van antagonisten in de chemische bestrijding van *Fusarium oxysporum* f. sp. *narcissi*

Behandeling van narcisbollen met methoxyethylkwikchloride, het antibioticum pimaricine of thiram geven een bestrijding van bolrot en primair wortelrot, veroorzaakt door *Fusarium oxysporum* ff. spp. Een dergelijke behandeling met formaline was alleen effectief tegen bolrot en primair wortelrot. Nieuw

gevormde wortels van bollen behandeld met het kwikmiddel, pimaricine of thiram werden in sterkere mate door *Penicillium* en *Trichoderma spp.* gekoloniseerd dan van onbehandelde of met formaline behandelde bollen. Vooral *P. janthinellum* en *T. viride* bleken de wortels tegen de ziekteverwekkers te beschermen. Deze schimmels bleken antagonisten van *F. oxysporum* f. sp. *narcissi* en *F. oxysporum* ff. spp. te zijn, zowel in experimenten uitgevoerd *in vitro* als *in vivo*. *In vitro* werd voor *P. janthinellum* en thiram een synergisme waargenomen ten aanzien van de remming van de myceliumgroei van *F. oxysporum* f. sp. *narcissi*, en *in vivo* ten aanzien van de bestrijding van wortelrot en bolrot. Dergelijke effecten werden *in vitro* ook voor pimaricine en organische kwikverbindingen gevonden. Pimaricine bleek tevens de produktie van bacterie-remmende metabolieten gevormd door *Cylindrocarpon destructans* te stimuleren. Voor laatstgenoemde fungicide-schimmel combinatie werd in het veld een synergistisch effect ten aanzien van de bestrijding van bolrot waargenomen.

De invloed van incubatie omstandigheden op de ontwikkeling van bolrot en huidziek en op de effectiviteit van chemische bestrijdingsmaatregelen

Fusarium oxysporum f. sp. *narcissi* en andere *formae speciales* van *F. oxysporum* werden sterker onderdrukt door verwarming tijdens een warm-water-behandeling (wwb, 2 uur bij 43,5 °C) dan met de bol overgaande *Penicillium* en *Trichoderma spp.* Deze laatste schimmels overleefden ook een snel droogproces beter. Dergelijke behandelingen van bollen en boldelen resulteerden in een gewijzigde bezetting van de bol met micro-organismen ten gunste van genoemde saprofieten. Toevoeging aan het dompelbad van hetzij een kwikmiddel, pimaricine of thiram versterkte deze effecten op de bol-microflora, dit in tegenstelling tot een toevoeging van formaline. Een koude bewaring (< 15 °C) versterkte zowel de groeisnelheid van *Penicillium spp. in vitro* als hun vermogen bolweefsels in natuurlijke grond te koloniseren. Hogere temperaturen stimuleerden de ontwikkeling van *Trichoderma viride*, van *Cylindrocarpon destructans* en van *Fusarium oxysporum* ff. spp. Laat planten bij temperaturen beneden 8 °C onderdrukte ziekte-ontwikkeling aanzienlijk. Verder werd gevonden dat *Penicillium corymbiferum*, *Penicillium janthinellum* en *Trichoderma viride* in staat zijn bij te dragen in de bestrijding van wortelrot en huidziek veroorzaakt door *F. oxysporum* f. sp. *narcissi* en *F. oxysporum* ff. spp.

De verkregen gegevens maken het eenvoudiger te begrijpen hoe de incubatie omstandigheden vanaf het rooien de ziekte-ontwikkeling in het volgende seizoen kunnen beïnvloeden. Het werd ook mogelijk een verklaring te vinden voor het feit dat een lage dosis van een fungicide als pimaricine, toegepast tijdens een wwb, een betere bolrot en huidziek bestrijding kon geven dan hogere doses.

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CURRICULUM VITAE

Cornelis Johannes Langerak werd op 22 december 1947 geboren te 's-Gravendeel. Na het behalen van het einddiploma HBS-B aan het Christelijk Lyceum te Dordrecht in 1965 begon hij in hetzelfde jaar zijn studie aan de Landbouwhogeschool te Wageningen. Het propaedeutisch examen werd met goed gevolg in juni 1966 afgelegd. Het kandidaatsexamen in de richting Plantenziektenkunde werd behaald in juni 1969 en het doctoraalexamen in dezelfde richting, met als hoofdvak Fytopathologie (verzwaard) en de bijvakken Biochemie en Plantenfysiologie, in september 1972.

Van 1 juli tot 1 oktober 1972 was hij werkzaam als wetenschappelijk assistent, en vanaf 1 oktober 1972 tot 1 januari 1978 als gastmedewerker, voor de door het Ministerie van Volksgezondheid en Milieuhygiëne ingestelde werkgroep "Vervanging van Kwikhoudende Fungiciden", aan het Laboratorium voor Fytopathologie van de Landbouwhogeschool te Wageningen.

Per 1 januari 1978 werd hij verbonden aan het Rijksproefstation voor Zaadonderzoek (RPvZ), Binnenhaven 1, Wageningen, alwaar hij tot heden is aangesteld als hoofd van de afdeling Gezondheid. Uit hoofde van die functie nam hij zitting in de begeleidingscommissie van voornoemde Werkgroep en trad daarnaast op als coördinator en adviseur van het team van medewerkers belast met de afronding van het project in december 1978. Verder vond in 1980, 1982 en 1984, steeds gedurende enige maanden, uitzending plaats als adviseur in zaaizaadprojecten in Kenia (N.S.Q.C.S.) en de Filippijnen (I.R.R.I.) voor het Directoraat Generaal Internationale Samenwerking van het Ministerie van Ontwikkelingssamenwerking, en werd hij naast zijn werkzaamheden op het RPvZ in staat gesteld het proefschrift te bewerken.