STUDIES ON BLACK ROOT ROT, A SEED-BORNE DISEASE OF CARROTS, CAUSED BY *ALTERNARIA RADICINA* MEIER, DRECHSLER & EDDY.

A Thesis submitted in partial fulfilment
of the requirements for the Degree

Master of Agricultural Science

at

Massey University

by

Donald John Scott

1970

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ACKNOWLEDGEMENTS

Grateful acknowledgement is made to Dr. H. T. Wenham for his guidance and helpful advice given willingly throughout this study.

Thanks are also due to the laboratory staff of the University Department of Microbiology and Genetics for laboratory assistance, to Miss D. Scott for photographic processing, to the Library staff for obtaining numerous publications and to Mrs. R. Couling for typing this thesis.

The author was assisted financially by the 1968 John Alexander Hurley Scholarship, and by the New Zealand Department of Agriculture.

Special thanks to my wife for her patience, encouragement and assistance.

CHAPTER I

INTRODUCTORY STUDIES ON THE DISEASE AND PATHOGEN.

THE DISEASE.

THE PATHOGEN.

INTRODUCTION

The production of table carrots (*Daucus carota* L.) for the domestic market has become a highly specialised field of horticulture in many growing districts of New Zealand. In the Manawatu and Ohakune areas alone some 850 acres of carrots are grown annually, and an evaluation of some disease factors which are, or could potentially limit production is undoubtedly worthwhile.

Almost all the seed sown by commercial growers in New Zealand is imported in bulk by the main seed companies from such countries as Australia, United States, France and Denmark. Several overseas workers have recorded the presence of potentially destructive fungal pathogens being associated with seed (Neergaard 1948, Grogan & Snyder 1952, de Tempe 1962, Hewett 1964, I.S.T.A. 1966). These include the following two pathogens:

Alternaria dauci (Kuhn) Groves & Skolko.

Alternaria radicina Meier, Drechsler & Eddy.

The possibility that either or both of these pathogens may be entering this country with seed prompted a preliminary investigation into the health status of such seed. Some imported seed lines available to commercial growers early in 1968 were screened in accordance with the current recommendations of the International Seed Testing Association (I.S.T.A.), and at that time only *A. radicina* was found to be present in some of the lines.

According to overseas work (Grogan & Snyder, 1952) both pathogens are capable of infecting seedlings and mature carrot plants.

Recently A. dauci has been found in New Zealand causing considerable foliage damage, especially in the Pukekohe area (Dingley, pers. comm.). However, it was not observed, even insipiently, in the Manawatu and Ohakune areas during 1968, but was apparently present prior to this (Wenham, pers. comm.).

Even though A. radicina has been recorded as a foliage pathogen, it is better known overseas as the incitant of black rot of carrot roots, a condition which may occur in the field, but more especially develops under moist storage conditions (Meier, Drechsler & Eddy, 1922; Lauritzen 1926). A preliminary survey of carrot growing properties in the Manawatu and Ohakune areas has revealed that A. radicina is present in some crops as the causal agent of an apparently insignificant root rot condition. However with the emphasis on blemish-free, washed carrots this disease may be contributing to the enormous quantities of carrots rejected after washing because of their poor consumer appeal.

Since only A. radicina was initially found on commercially available seed and in locally grown carrot crops, this thesis will consequently be confined to a study of this organism alone, although comparative references will be made to A. dauci when and where appropriate.

The first chapter describes preliminary studies which mainly involve establishing the identity and pathogenicity of the fungus. In addition certain aspects concerning the cultural characteristics of the pathogen are investigated.

Since it was first described, the pathogen has been referred to various related genera, including Alternaria, Thyrospora and Stemphylium. The bases for the delimitation of these and other related genera have

been described by many workers (Wiltshire 1938, Neergaard 1945, Subramamain 1961, Simmons 1968), but there still appears to be confusion regarding the generic affinity of the carrot black rot pathogen. In chapter two, therefore, the nomenclature of this pathogen is reviewed and an experimental investigation into its generic placement is undertaken.

The third chapter concerns many aspects relating to the seed transmission of A. radicina. With reference to the nature and significance of seed-borne inoculum, there appears to have been no thorough attempt in the literature to incorporate individual findings into an overall picture. An understanding, such as this, was considered to be essential if an effective approach to the problem of control was to be made. An investigation was therefore conducted to determine the nature and significance of seed-borne inoculum, and to determine a satisfactory method of control.

A. THE DISEASE

Alternaria radicina was first recorded by Meier, Drechsler & Eddy (1922) as causing a condition of mature carrot roots they called "black rot". According to these workers :

> "the trouble as it occurs on the root is characterised by progressive softening and blackening of the tissues. Infection seems to occur frequently at the crown, as a result of which the black decay extends down the core of the carrot. Many cases have also been observed where decay had originated at other points on the surface of the carrot."

Lauritzen (1926) provided a similar but more concise description of the disease on the roots :

> "Lesions as they develop on the sides of the roots are circular to irregular in outline, slightly depressed, usually shallow, penetrating from 1-3 mm. deep. When infection occurs at the crown the decay usually penetrates rather deeply, particularly into the core. As a rule the color of the decay is almost jet black, but is sometimes greenish-black. The black fungous growth may or may not occur on the surface of the lesions."

Lauritzen (1926) conducted extensive experiments to determine the conditions which best favoured the development of the disease. He found that relatively high temperatures, with an optimum of 28°C, and conditions of high humidity, favoured the development of severe infection on carrot roots under storage. He further stated that ;

> "the decay caused by *A. radicina* represents only part of the loss that may result from the presence of the disease, for the black rot lesions afford a favourable opportunity for the entrance of other fungi, such as *Sclerotinia* and *Botrytis*."

Grogan & Snyder (1952) are the only workers to have demonstrated that foliage infection can occur naturally in the field. On four occasions in commercial crops grown for roots they observed foliage blight, which was most severe on the outer, more mature leaves. Meier, Drechsler & Eddy (1922) and Lauritzen (1926) did not observe naturally occurring

Plate 1.

Black rot symptoms on mature carrot roots
(naturally infected).

foliage infection but demonstrated that it could be achieved artificially by inoculating foliage with conidial suspensions and holding under conditions suitable for penetration and infection.

During 1968 and 1969 visits were made to the Ohakune area where large acreages of carrots are grown for harvest in the winter and early spring. An inspection for the presence of black rot was made in the field and more especially in the "cull piles" of washed carrots rejected on account of being unsuitable for the market. Only rarely were infected carrots found and these made up an insignificant fraction of those carrots rejected. Of greater importance was rejection of carrots due to their being undersized, oversized, deformed, split, and infected with a number of different unidentified micro-organisms. In the Ohakune district there is an urgent need for a comprehensive study into the various physiological and pathological causes of the excessive rejection of harvested carrots.

Crops grown in the Manawatu were also periodically examined during 1968/69, although little black rot was found. However, during late spring and early summer of 1969, Manawatu grown pre-packaged washed carrots being sold in a Palmerston North supermarket were found to be infected with A. radicina. Initially infection of the attached petiole bases was observed and this was followed by the appearance of typical black rot lesions (Plate 1) on the sides of the carrot roots after holding the plastic packages for one or two weeks. These findings tend to support the belief that black rot is essentially a post-harvest, storage disease, and with the increasing use of plastic packaging may become even more important. The source of infecting inoculum will be discussed in detail in another section.

Apart from the petiole base infection, no natural infection of the foliage was observed in any of the crops examined.

Prior to this investigation the occurrence of A. radicina in New Zealand had not been recorded (Dingley 1969). It must be emphasised that during the current study only those crops in the Ohakune and Manawatu districts were examined for the presence of the pathogen. There is a distinct likelihood that the pathogen will also be found in carrot crops growing in other districts of New Zealand.

B. THE PATHOGEN

The essential mycological features of *Alternaria radicina* were first determined by Meier, Drechsler & Eddy (1922) and, although the accuracy of their description has not been disputed, the pathogen has since been classified in various related genera, namely *Alternaria*, *Thyrospora*, and *Stemphylium*. In the literature the fungus is currently referred to as *Stemphylium radicinum*, although (for reasons outlined later) the name *Alternaria radicina* is retained.

The host range of *A. radicina* was studied extensively by Neergaard (1945) who found infection to be confined essentially to carrot with an ability to produce weak symptoms on related plant species such as parsley and celery. The complete morphological description of *A. radicina* has been given by a number of workers (Meier, Drechsler & Eddy, 1922; Lauritzen, 1926; Neergaard, 1945) and the most conspicuous diagnostic features are as follows :

> The fungus produces dark, erect conidiophores, mainly unbranching, 10 - 200 µ long by 4 - 9 µ wide, and not swollen terminally. The dictyosporous conidia are most distinctive; they measure 19.6 - 69.5 µ long by 9.1 - 33.2 µ wide, are smooth, oval to ellipsoid, rounded at both ends and dark brown in colour. (Plate 2) Under a stereoscopic microscope the conidia are shiny and black in appearance (Plate 2).

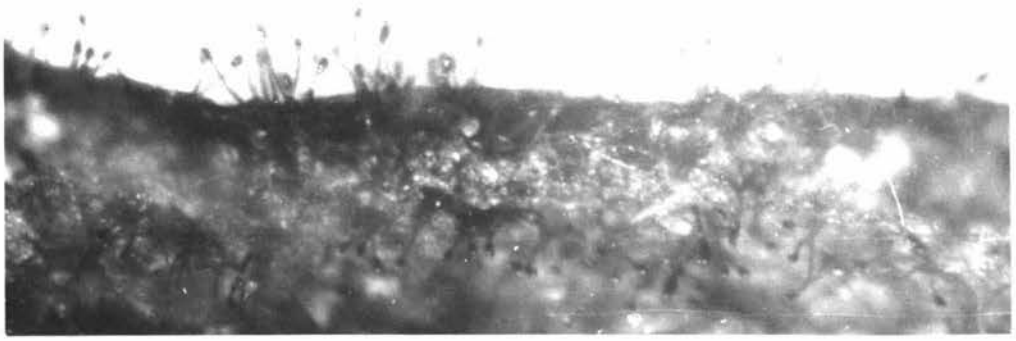
Further details of conidial dimensions are presented in Table 1. Although it was possible to identify the fungus by morphological characteristics and dimensions alone, its pathogenic association with carrot was an obvious aid to its identification.

An interesting observation was consistently made when infected tissue (from root lesions, infected petiole bases and infected seedlings) was subjected to high humidity. Profuse greenish-grey aerial mycelium

Plate 2.

(a)

(b)



Conidia of A. radicina

(a) Conidia from an infected seedling.

(b) Appearance of conidia under a stereoscopic microscope.

developed in most cases and it completely enclosed the conidia which were produced closer to the infected tissue surface. Such a "mycelial net" was found to be stable during quite vigorous air movement thereby acting as a screen and restricting the dissemination of conidia. This may account for the apparent lack of foliage infection, even when environmental conditions are favourable. The large numbers of conidia formed could only be liberated under a more severe mechanical influence, such as harvesting.

1. Establishment of Pathogenicity

Meier, Drechsler & Eddy (1922), Lauritzen (1926) and Neergaard (1945) demonstrated the pathogenicity of different isolates of A. radicina by artificial inoculation. In this study isolates of A. radicina were obtained from imported, infected seed, and also from infected plant material collected in the field. Their pathogenicity was confirmed by subjecting each isolate to the requirements of Koch's Postulates, and this required consideration of techniques of isolation, inoculation and reisolation.

(a) Materials and Methods

(i) Origin of working isolates

Strains from as many different sources were sought and this was achieved by isolating from infected seed imported from Australia and Holland, and also from infected plant material collected in Ohakune. Thirty-two isolates of A. radicina were cultured and of these several were chosen for further pathogenicity, nomenclature and cultural studies (Table 2).

Table 1. Conidial Dimensions of *A. radicina*

Origin of Information	Substrate	Temperature °C	Incubation period	Length (microns) mean	Length (microns) range	Width (microns) mean	Width (microns) range	Length & Width ratio	Number of Spores measured
Scaramella (1929) after Neergaard, 1945	*	*	*	45.0	*	22.0	*	2.05	*
Jorgensen (1934)	Potato agar	*	*	*	20.0-75.0	*	15.0-35.0	*	*
Neergaard (1945)	Malt agar	*	*	36.4	15.0-69.0	16.7	9.0-26.0	2.18	100
Neergaard (1945)	Host	*	*	31.1	19.5-45.0	16.2	7.5-25.5	1.89	100
Author	Carrot root	20-25°C	14 days	36.1	19.6-57.6	20.4	11.8-32.1	1.77	50
"	Carrot seedling	22°C	10 days	48.6	27.0-78.1	19.3	13.4-32.7	2.52	100
"	Carrot foliage	20-25°C	7 days	41.6	22.7-61.7	14.4	9.1-20.0	2.89	100
"	P.D.A.	24°C	7 days	39.7	23.0-69.5	23.1	16.0-33.2	1.72	100

* no details given

Table 2. Origin of culture isolates of A. radicina.

Isolate number	Origin: Isolated from	Origin: Source	Presence of yellow pigment (see Section 2.)
1	Seed line 3	Australia	-
4	" " 4	"	+
10	" " 4	"	-
11	" " 7	"	+
14	" " 19	"	+
22	" " 26	Holland	+
25	Carrot lesion	Ohakune	-
28	" "	"	+
32	" "	"	-

(ii) Isolation to agar

Only one method of isolation was routinely used to obtain pure cultures of A. radicina. The large dictyosporous conidia were readily transferred on a sterile needle tip from sporulating infected tissue to potato dextrose agar (P.D.A.) slopes. Those isolates free of contamination were held in a refrigerator until required and periodically subcultured without apparent degradation or saltation. Neergaard (1945) found that the pathogen maintained its sporulating capacity in culture for at least 25 years, and on that basis he considered it to be stable in culture.

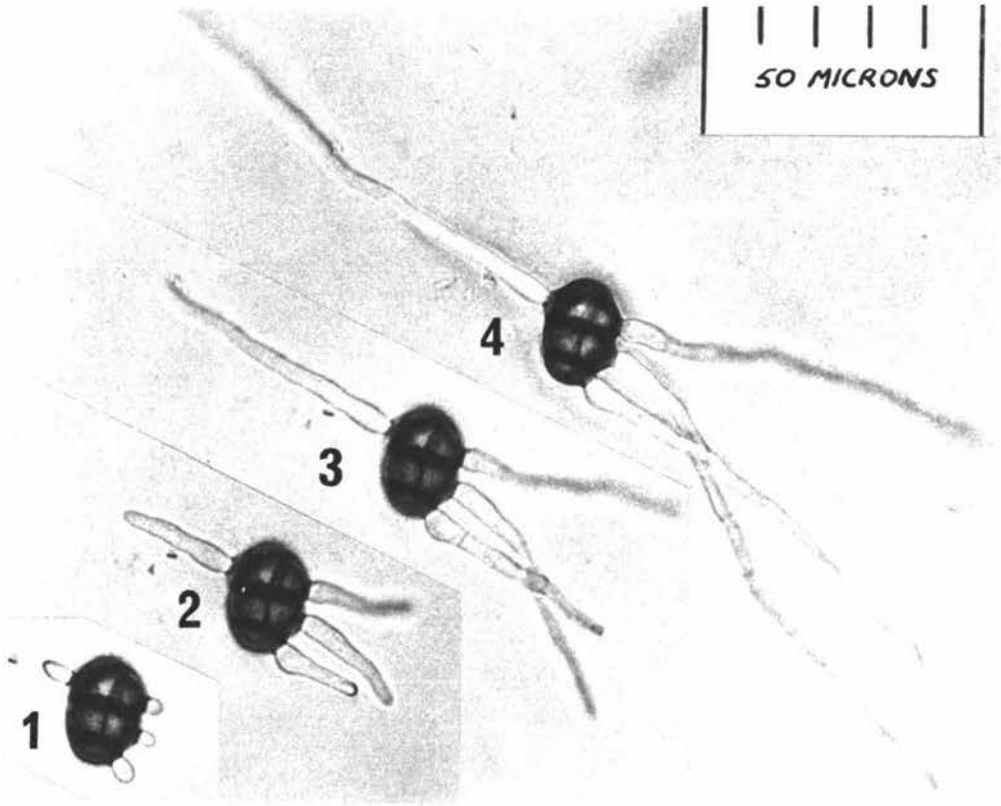
Cultures of A. radicina were also obtained by plating surface-treated, infected seeds to P.D.A., but were not used for pathogenicity or nomenclature studies.

(iii) Inoculation and Reisolation

A. radicina characteristically produces large numbers of conidia on P.D.A. and for this reason the pathogen readily lent itself to artificial inoculation. Accordingly there was no need to consider using any other types of media for the production of inoculum.

Inoculum was prepared by growing each culture on P.D.A. until the colony diameter reached 5 - 6 cms. (about 10 days growth), cutting the agar into strips using a sterile needle and placing into a 150ml. beaker of sterile water. This was shaken for about five minutes until the suspension became dense with conidia, and strained through sterile muslin to remove mycelial and agar fragments. The concentration was adjusted with sterile water to approximately 50,000 spores per ml.

Plate 3.



Spore germination at 24°C

1. After 2 hours incubation.
2. After 3½ hours incubation.
3. After 5 hours incubation.
4. After 6½ hours incubation.

The ability of conidial inoculum to germinate was demonstrated by spreading a drop of the suspension on a glass slide and incubating at room temperature in an atmosphere of high humidity. Plate 3 shows the growth of germ tubes at intervals between two and six and a half hours.

Conidial inoculum was prepared from six different isolates, namely, isolates number 4, 11, 14, 22, 28 and 32, and the pathogenicity of each was tested by inoculating carrot seedlings, foliage and roots.

(b) Results

(i) Seedlings

Six surface sterilised pathogen-free seeds were sown on a filter paper "platform" within each of a number of test tubes. When the hypercotyls were approximately one inch long each tube was inoculated with five drops of conidial inoculum, and held for one week to allow development of disease symptoms. Four tubes were inoculated with a spore suspension from each isolate, and in addition four tubes were inoculated with distilled water to act as controls. In all treatments, except the controls, the seedlings were killed by the pathogen, and from each of the infected seedlings A. radicina was reisolated to agar, thus fulfilling the requirements of Koch's Postulates.

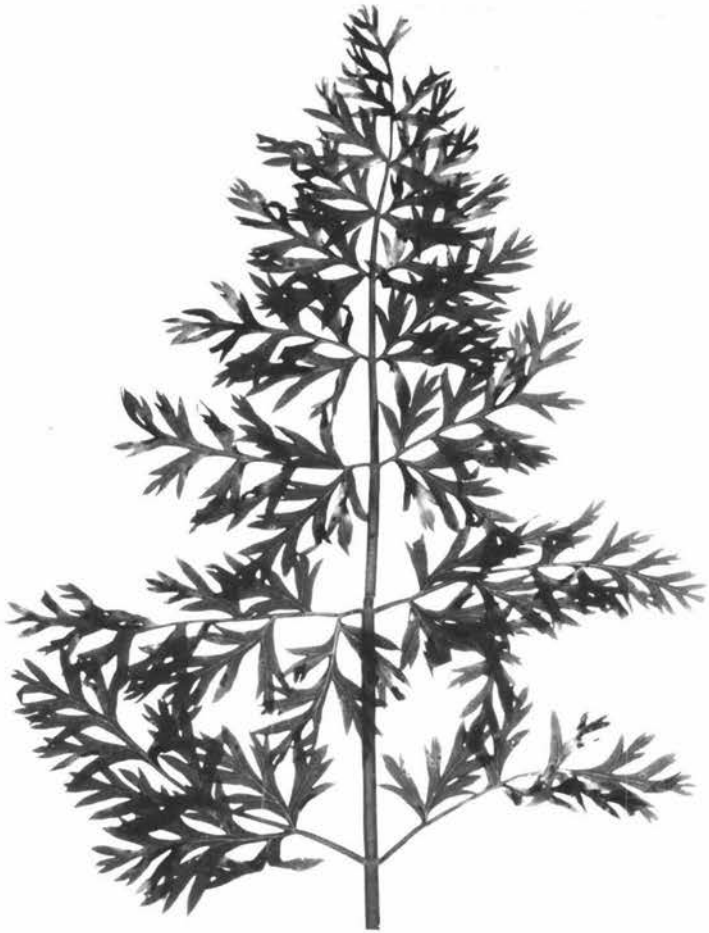
(ii) Foliage

For routine pathogenicity tests seven small carrot plants were placed in separate petri plates and each spray inoculated with a conidial suspension of A. radicina. All treatments, except a control, produced pathogenic symptoms on the inoculated plants after one week (Plate 4a), and reisolation of the fungus to agar confirmed the pathogenicity of all

Plate 4.

(a)

(b)

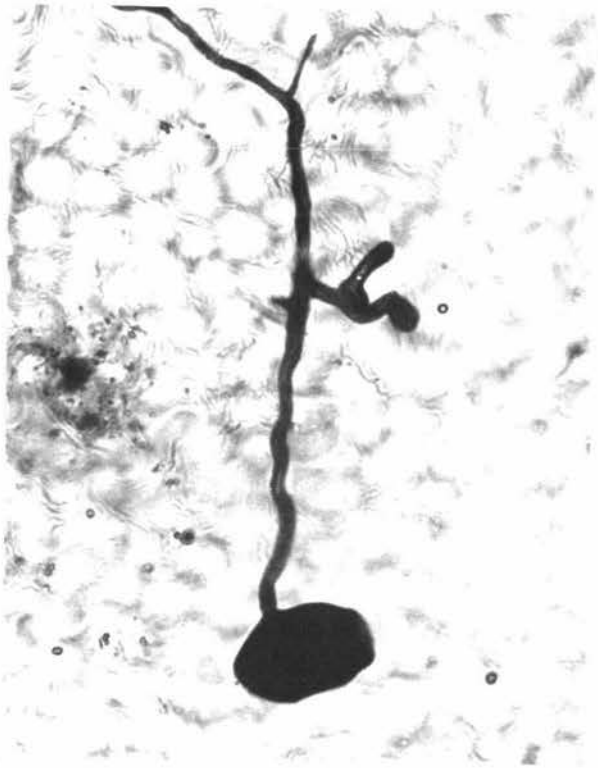


Pathogenic symptoms induced by artificial inoculation.

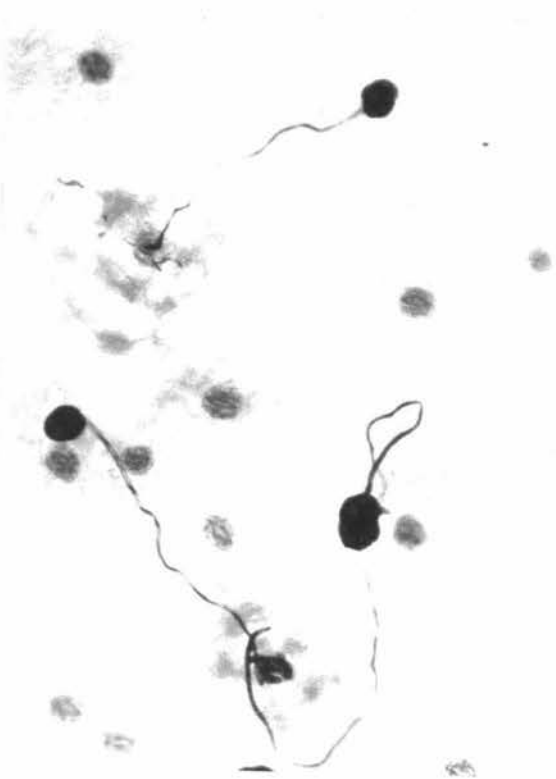
(a) On semi-mature carrot plants.

(b) Lesions on mature foliage.

Plate 5.



(a)



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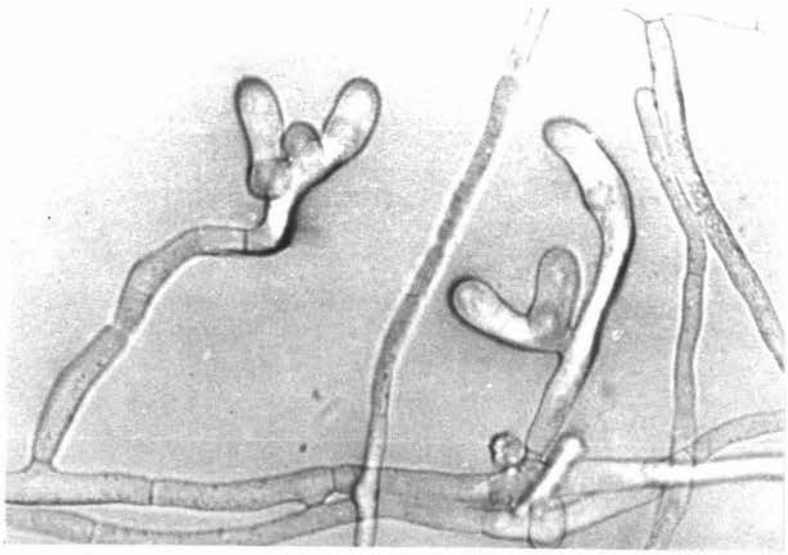


(c)

Penetration.

(a) Direct penetration of a carrot leaflet.

(b) Indirect penetration through stomata of leaflet.

(c) Formation of appressoria on glass surface.

isolates tested.

In addition to the above tests, carrot plants were grown in the glasshouse and when they had reached maturity the foliage was spray-inoculated with a conidial suspension of the pathogen. The inoculated plants were held in a high humidity cabinet for three to four days to allow infection to take place. Leaf-spot symptoms invariably developed, as illustrated in Plate 4b . By cleaning the leaflets in a warm 50% mixture of glacial acetic acid and ethyl alcohol (95%) and staining with lactophenal blue direct and indirect penetration was clearly observed (Plate 5). In both cases appressoria were produced on the leaf surface, together with distinct discoloration of tissue immediately surrounding the point of penetration. Appressoria were also shown to form on the glass surface within a humidified slide chamber (Plate 5).

(iii) Roots

Conidial inoculum of the six isolates was used to spray inoculate two mature carrot roots (one wounded, one non-wounded) from each of four varieties. The carrots were held in polythene bags for three weeks and in all cases typical black rot symptoms developed. By reisolating to agar, the pathogenicity of each isolate was once again confirmed.

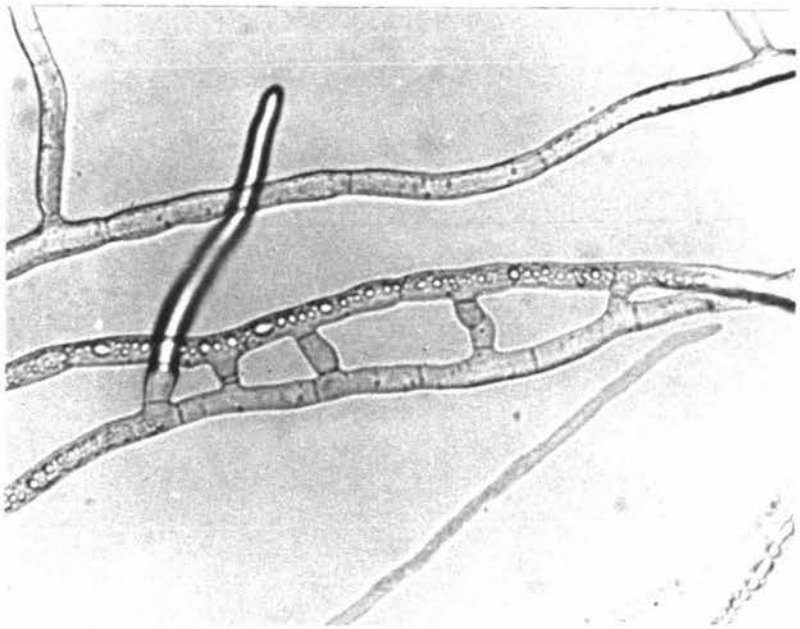
On the basis of the tests conducted on carrot seedlings, foliage and roots, the pathogenicity of all isolates of A. radicina used in this study was established beyond doubt.

2. Cultural Studies

A brief study of A. radicina in culture was conducted for a number

Plate 6.

(a)



(b)

Certain cultural characteristics of A. radicina.

(a) Hyphal anastomosis.

(b) Crystal formation.

of reasons. Firstly, the cultural characteristics of the pathogen were a further aid to its identification. Secondly, it was considered necessary to ascertain the growth rate and sporulation capacity of the pathogen in culture since subsequent investigations involved the use of artificially produced inoculum. Finally there was a possibility that certain hitherto unrecorded cultural phenomena might be observed.

(a) Gross colony characteristics on agar media.

On P.D.A. the pathogen characteristically produced a uniformly dense web of grey to dark green aerial mycelium. Within this mycelium vast numbers of conidia were produced on dark conidiophores arising as short branches of the aerial hyphae or directly from the agar surface. These characteristics are in accordance with those observed by Neergaard (1945). Meier, Drechsler & Eddy (1922) reported that hyphal anastomosis frequently occurred in culture; this was also observed in the current study (Plate 6a).

Neergaard (1945) commonly observed crystal formation in malt agar and other media, but of many hundred pure cultures grown on various media during this study, on only one occasion were crystals formed (Plate 6b). The fact that crystals were of such infrequent occurrence in this study indicated that they could not be regarded as a diagnostic feature of the fungus.

(b) Growth studies on agar media.

Brancato & Golding (1953) studied the use of colony diameter as a measure of growth rate and considered that it was sufficiently reliable for determining growth rates and for comparing the effect of environmental

factors on a fungus culture on the same medium. The method was therefore considered suitable for growth studies of A. radicina.

Inoculum for growth studies was prepared by growing the fungus on water agar and cutting into discs using a 6 mm. diameter cork borer. One disc was then transferred asceptically to the centre of each 10 cm. test plate, and corrected diameter measurements recorded after the appropriate period of incubation. All growth studies were conducted using Oxoid P.D.A. medium.

(i) Growth rate on agar media

Preliminary experiments indicated that the available isolates of A. radicina varied markedly in their growth rates on agar media, and for this reason a more detailed investigation was conducted.

Petri plates of P.D.A. were inoculated in the manner already described, and the colonies incubated at 24°C. The colony diameter of each isolate was calculated daily by taking the average measurement along a marked axis of four replicated test plates.

The results are presented in Figure 1, and confirm the work of Neergaard (1945), who found that different isolates of A. radicina grew at different rates on agar media. Some of the possible explanations for the differences have been studied by Curren (1968). He found that in culture media certain nutritional requirements of two isolates of A. radicina differed. For example, potassium nitrate (KNO_3) was a good source of nitrogen for isolate No. 2 but not for isolate No. 1. Similarly his two isolates differed in their vitamin requirements. Isolate No. 1 was deficient for thiamine while isolate No. 2 did not need added vitamin for

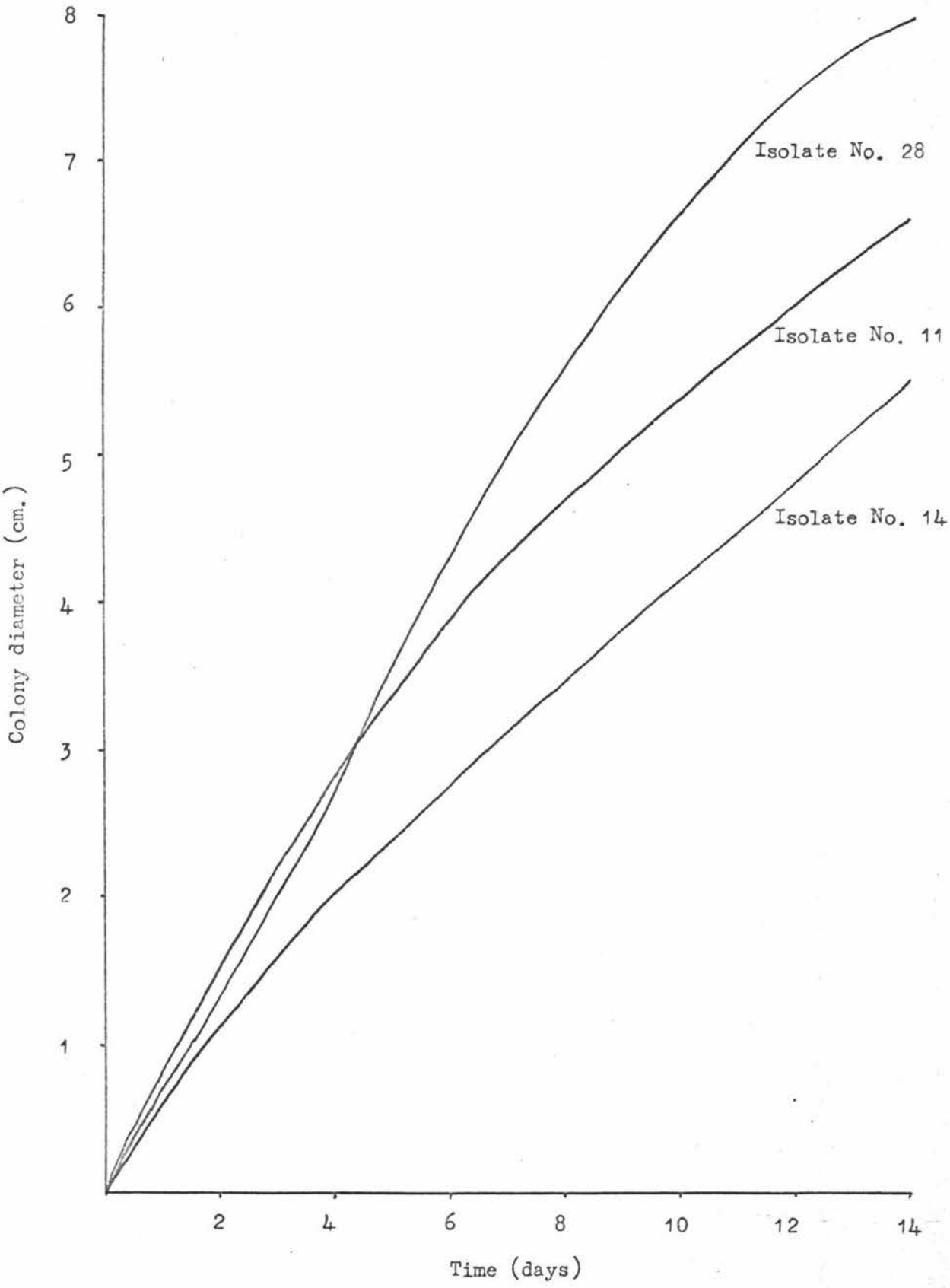


Fig. 1. Growth rate of three isolates of A. radicina on oxoid P.D.A. at 24°C.

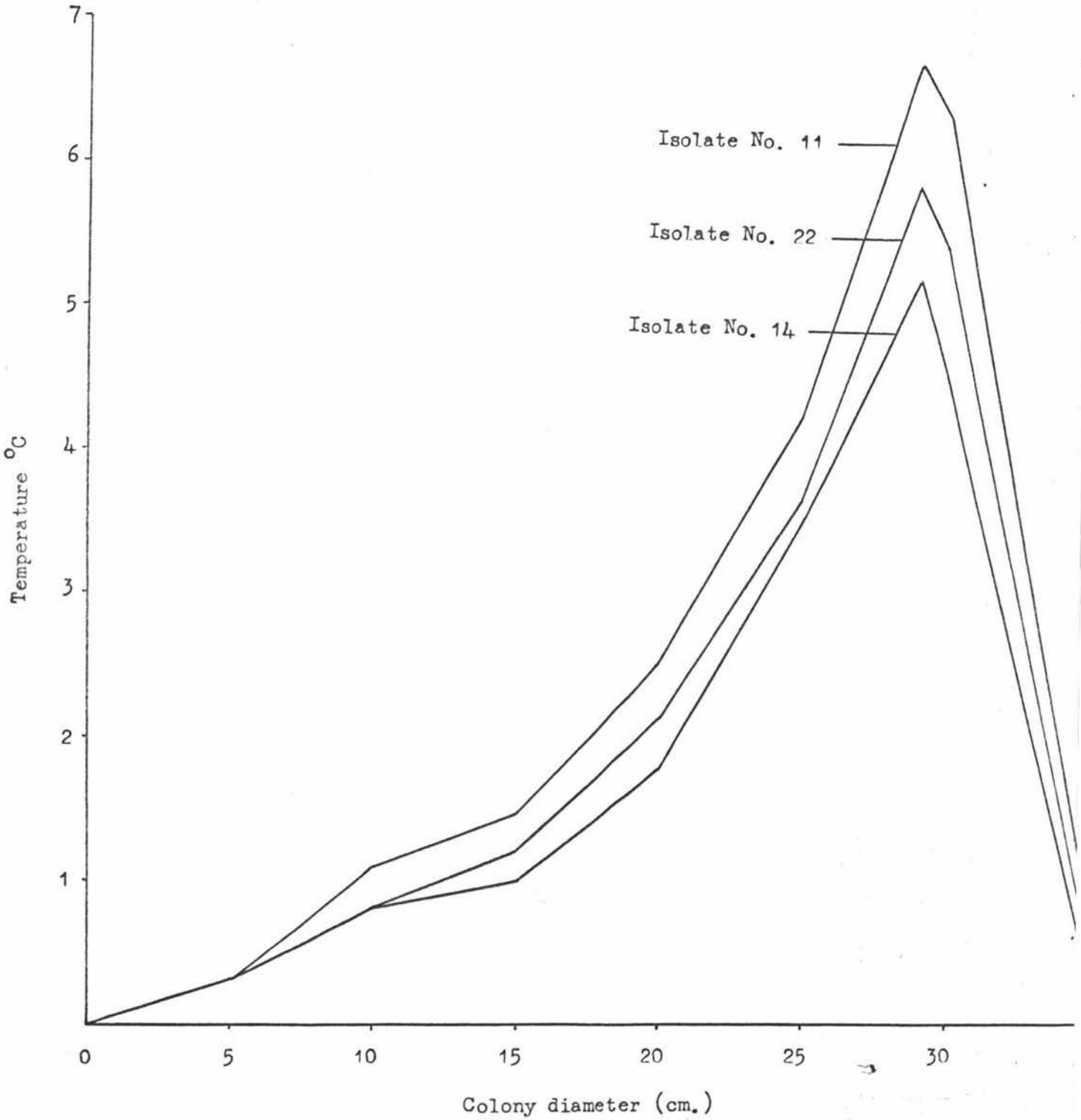


Fig. 2. Effect of incubation temperature on the growth of three isolates of A. radicina on P.D.A.

normal growth.

(ii) Influence of temperature on growth rate

Lauritzen (1926) and Neergaard (1945) demonstrated that the optimal temperature for the fungus was 28°C and this was confirmed in the current investigation. Average colony diameters of three isolates of A. radicina were measured after seven days over a range of temperatures from 5°C - 35°C. The results are presented in Figure 2.

(c) Pigmentation and Antibiosis in Culture

Isolates of A. radicina were routinely cultured on lab. P.D.A., and on this medium only, two distinct cultural types were identifiable.

1. Pigment-producing isolates. After incubation at 24°C for one week certain isolates (Table 2) produced a distinct yellow pigment which readily diffused into the surrounding medium. As the colour intensified the radial growth of the fungus slowed and stopped before reaching the edge of the petri plate.

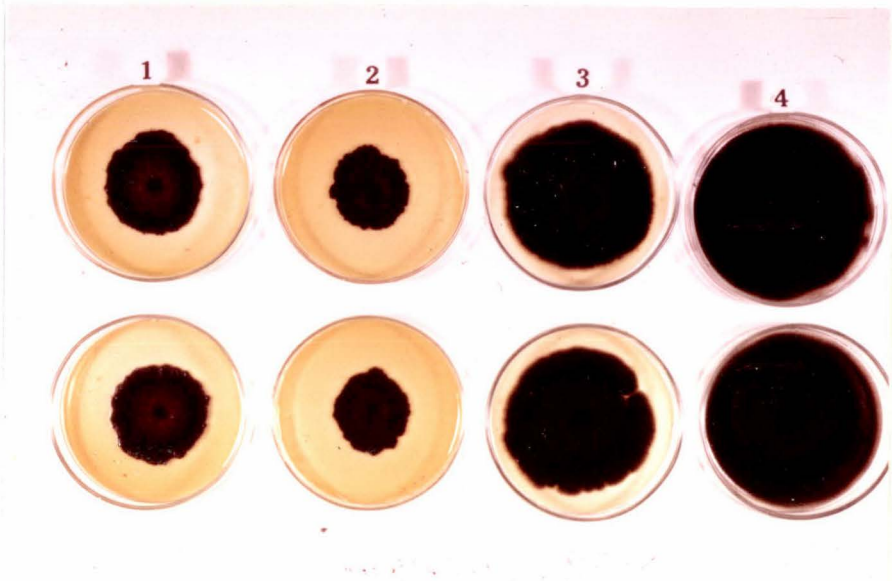
2. Non pigment-producing isolates. These isolates did not produce the yellow pigment and their growth rate was unaffected until they had grown to the edge of the plate.

The two types are illustrated in Plate 7 , and the differences in growth rate clearly demonstrated in Figure 3. The occurrences of this phenomenon has not previously been recorded, although Meier, Drechsler & Eddy (1922) observed a cessation of growth of A. radicina on potato agar medium after 10 to 15 day's incubation.

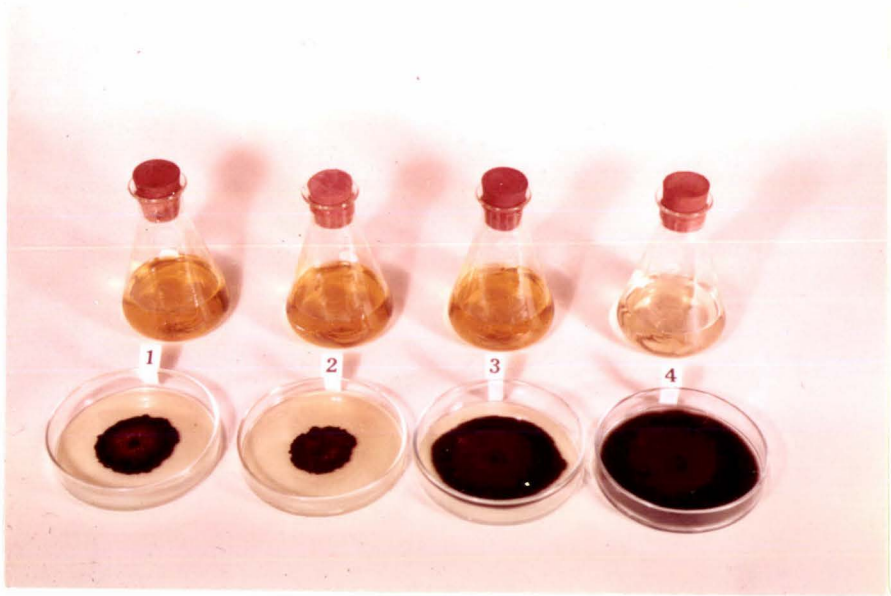
A further interesting observation was made of those plates

Plate 7.

(a)



(b)



Production of yellow pigment in Lab. Potato Dextrose medium (24°C).

(a) Cultural characteristics on agar (at 10 days) showing the pigment produced by isolates No. 4, 14 and 28. No pigment was produced by isolate No. 32.

(b) Pigmented and non pigmented liquid culture filtrates produced after four weeks incubation in relation to radial growth on agar media after 10 days incubation.

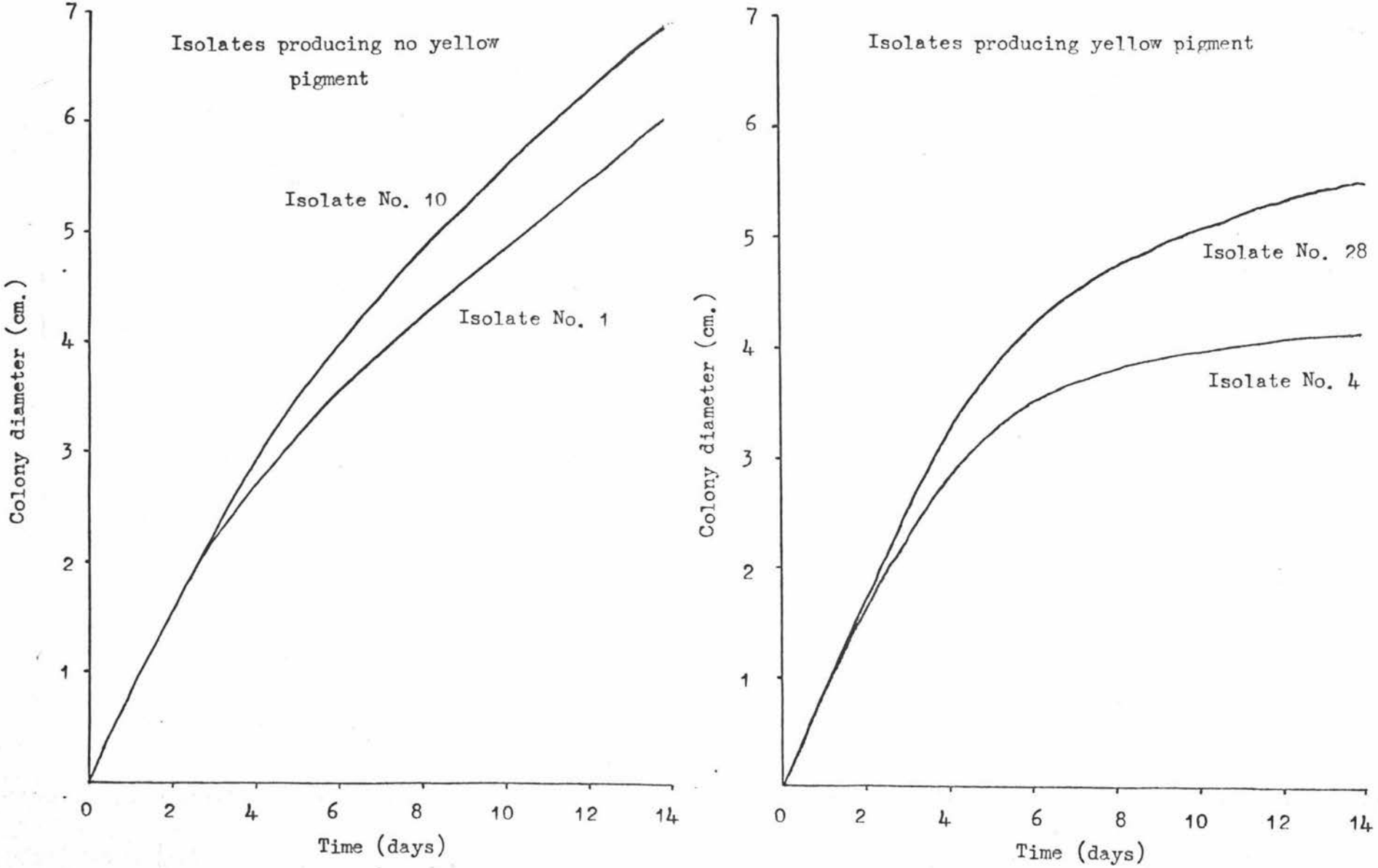


Fig. 3. Comparison of growth rate on lab P.D.A. between isolates of A. radicina that produced a yellow pigment and isolates that did not produce a yellow pigment (Incubation at 24°C).

containing the pigment-producing isolate. Although the circle of pigmented medium surrounding the fungal growth was frequently exposed to the air by removal of the plate lid, at no time did fungal contaminants develop as a result.

It appeared, therefore, that the pigment was itself, or was associated with a self inhibitory, antifungal substance. For this reason experiments were conducted to produce the pigment in liquid culture, and then test the crude filtrate for its effect on the growth of other fungi.

(i) Growth in liquid culture

A potato dextrose broth was prepared by simmering 200 gms. of peeled, sliced potatoes in one litre of water, adding 10gms. of dextrose and autoclaving in 100ml. lots in 200ml. Erylenmeyer flasks at 15 p.s.i. for 20 minutes. Four isolates were selected for this experiment, three of which produced the pigment on agar media (isolates Nos. 4, 14, and 28), and one of which did not (isolate No. 32). Three flasks per isolate were inoculated with several mycelial pieces, plugged with sterile cotton wool and incubated at 24°C for at least three weeks.

All isolates of the fungus grew rapidly in liquid culture, and after 10 days a yellow coloration began to develop in those flasks containing isolates Nos. 4, 14 and 28, although no such coloration was produced by isolate No. 32. After one month's incubation the yellow coloration appeared to reach its maximum intensity, and at this stage the bulk of the fungal material was removed by filtration through a No. 1 Whatman filter paper. The occurrence of the yellow pigment in agar media and in the liquid culture filtrate is illustrated in Plate 7 .

(ii) Bioassay of Culture filtrates

For bioassay purposes the culture filtrates were concentrated by evaporation of water using a "Rotovapor" evaporator operated at 35°C. The 80mls. of culture filtrate from each isolate were reduced to three mls. of crude concentrated extract.

The three fungi on which each extract was tested were selected from diverse taxonomic groups, and inoculum was prepared as follows:

Monilinia fructicola (Wint.) Honey., (Ascomycetes). Conidial inoculum of this fungus was prepared by "washing" the surface of a profusely sporulating colony with sterile distilled water.

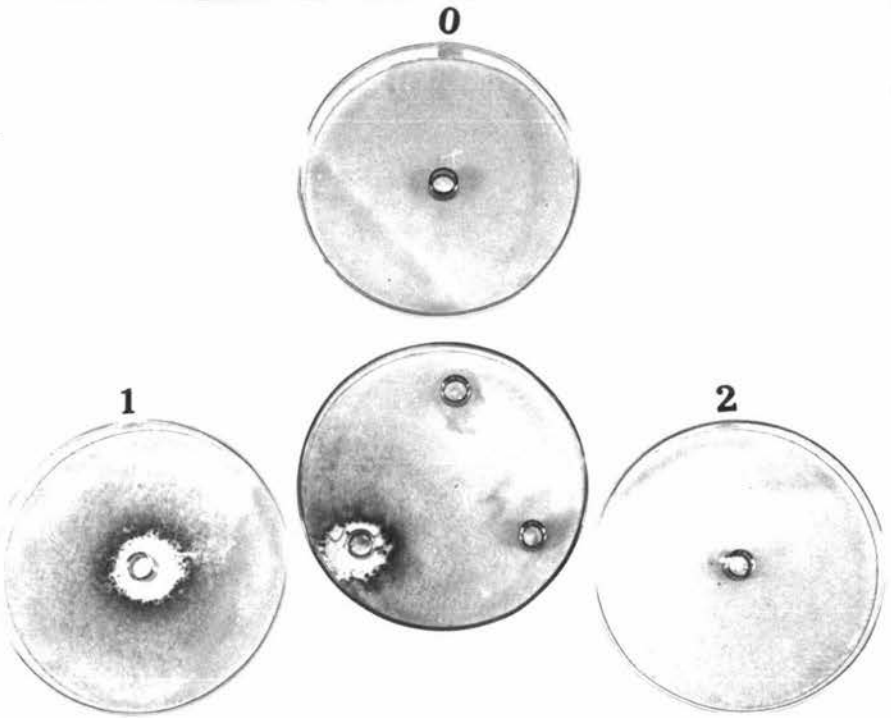
Stereum purpureum (Fr.) Fr., (Basidiomycetes). Mycelium from a fresh P.D.A. culture was fragmented by shaking vigorously in a McCartney bottle containing glass beads and sterile water.

Phytophthora cinnamomi Rands, (Oomycetes). Fragmented mycelial inoculum was prepared in the same way as for S. purpureum.

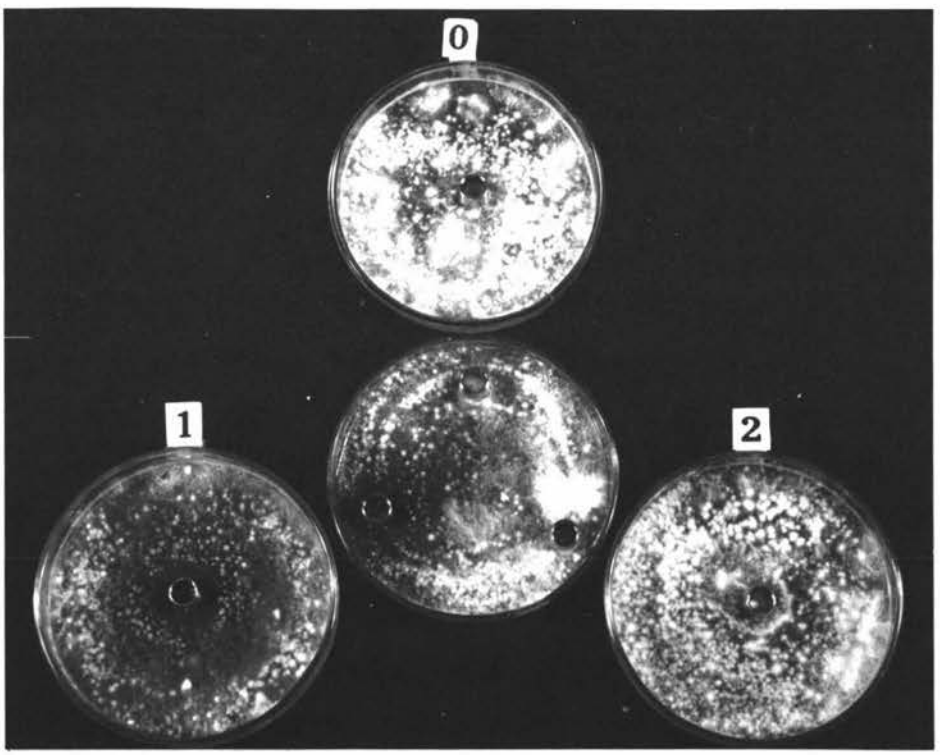
One ml. of inoculum was spread evenly over the surface of each oxoid P.D.A. test plate using a glass spreader. Hot stainless steel antibiotic assay rings were then partially embedded in the agar and allowed to cool. Five drops of concentrate from the appropriate isolate were placed within each antibiotic assay ring and the plates incubated at 24°C for four days. By this time the concentrate had diffused into the surrounding agar and the test fungi had grown sufficiently to reveal whether or not antibiosis had occurred. Drops of distilled water were used as a control.

Plate 8.

(a)



(b)



Effect of culture filtrates on growth of other fungi.

(a) Monilinia fructicola.

(b) Stereum purpureum.

The results of this experiment confirm that the yellow pigment produced by some isolates of A. radicina is associated with the inhibition of several unrelated fungi. This is recorded in Table 3. and clearly illustrated in Plate 8 .

Another important observation was made when the inoculum of P. cinnamomi was inadvertantly contaminated with bacteria. The extracts from both pigmented and non-pigmented isolates distinctly inhibited the growth of contaminant bacteria about the antibiotic assay rings. This phenomenon was also observed when isolates of the pathogen were grown on different media (malt agar, carrot dextrose agar) which had inadvertantly been contaminated with bacteria.

Discussion

As far as is known, the production of antifungal and antibacterial substances from Alternaria radicina has not previously been recorded. No attempt was made in the current study to determine the chemical identity of the compounds involved, although the antifungal pigment should readily lend itself to biochemical analysis since a direct comparison would be possible between the filtrates containing the pigment and those not containing it. The pigment apparently requires lab. potato dextrose medium to provide the specific chemical precursers for its formation. Furthermore there is no evidence to show that the pigment itself is the active antifungal agent; it may only be produced in close association with the active principle.

An antibiotic substance was shown by Bruehl, Millar & Cunfer (1969) to be significant in the saprophytic survival of Cephalosporium gramineum Nisikado & Itaka and although no evidence is presented, the

Table 3.

Effect of crude concentrated filtrates of

A. radicina on growth of other fungi

	Control	Pigmented			Non Pigmented
		Isolate 4	Isolate 14	Isolate 28	Isolate 32
Monilinia fructicola	-	+	+	+	-
Stereum purpureum	-	+	+	+	-
Phytophthora cinnamomi	-	+	+	+	-

\+ positive effect

\- no effect

possibility of a similar phenomenon occurring in the case of A. radicina cannot be overlooked.

On the basis of the findings in this study it is suggested that a more comprehensive investigation be conducted into the chemical composition and the etiological importance of the antibiotic substances produced by A. radicina. It is possible that the same substances are known to be produced by other micro-organisms, in which case their importance may already have been evaluated.

CHAPTER II

THE NOMENCLATURE OF THE PATHOGEN.

REVIEW OF LITERATURE

DEVELOPMENTAL MORPHOLOGY STUDIES

INTRODUCTION

The causal organism of black rot of carrots has undergone a number of changes in nomenclature since it was first described as *Alternaria radicina* by Meier, Drechsler & Eddy (1922). The pathogen has been reclassified in such genera as *Thyrospora* Tehon & Daniels (1925), *Stemphylium* Wallroth (1933), and *Pseudostemphylium* Subramanian (1961), and its obligate synonomy is as follows:

Alternaria radicina Meier, Drechsler & Eddy, 1922.
Thyrospora radicina (M.D. & E.) Neergaard, 1938.
Stemphylium radicinum (M.D. & E.) Neergaard, 1939.
sub-genus *Pseudostemphylium* sensu Wiltshire, 1938
Pseudostemphylium radicinum (M.D. & E.) Subramanian, 1961.

Such changes in its classification are attributed to the historical uncertainty surrounding the criteria by which these genera are differentiated. This section of the study, therefore, concerns the generic placement of the pathogen which involves a review of literature and an examination of its developmental morphology. Unless otherwise indicated the pathogen will hereafter be referred to as *A. radicina*.

A. REVIEW OF LITERATURE

Meier, Drechsler & Eddy (1922) assigned this pathogen to the genus Alternaria Nees 1817, sensu Elliot (1917) because they considered that it conformed to the original description of the genus. They specifically referred to certain morphological characteristics of the pathogen which apparently confirmed their classification, as follows:

> On weak substrate "the fructifications showed not infrequently a catenulate arrangement of the spores, usually regarded as the distinctive character of the genus Alternaria. Proliferation of secondary spores usually occurred at the tip of the primary spores, which were modified to form short, hyaline beaks. Such modification was not observed in spores that had not given rise to secondary spores."

The original classification remained undisputed by subsequent workers (Lauritzen 1926, Salmon & Ware 1934, Jorgensen 1934, Doyer 1938) until Neergaard (1938) proposed that the fungus be transferred to the genus Thyrospora Tehon & Daniels, on the basis of its spore form, as Thyrospora radicina (M.D. & E.) Neergaard. Tehon & Daniels (1925) had described a brown leaf spot of alfalfa caused by an echinulate-spored fungus they considered identical to Macrosporium sarcinaeforme Cav., a pathogen of red clover. Following Elliot's (1917) suggestion that M. sarcinaeforme was not a typical member of the genus Macrosporium, and assuming the forms on red clover and alfalfa to be identical, they erected the genus Thyrospora, with Thyrospora sarcinaeforme (Cav.) Tehon & Daniels, as the type species. Wiltshire (1938) and Smith (1940) demonstrated that Tehon & Daniels were incorrect in identifying their organism as M. sarcinaeforme, as the spores of the organism they described were echinulate whereas the spores of the red clover organism were smooth. Wiltshire (1938) showed furthermore, that the genus Thyrospora, erected

by Tehon & Daniels was identical with the genus Stemphylium and accordingly proposed the transference of members of the genus Thyrospora to Stemphylium. On the basis of Wiltshire's proposal Neergaard (1939) reclassified Thyrospora radicina as Stemphylium radicinum (M.D. & E.)

Wiltshire (1938) also studied the original and modern conceptions of the genus Stemphylium, a topic which was also concisely reviewed by Tate (1968). Wiltshire found that two morphologically distinct concepts had arisen as the type for the genus Stemphylium. He maintained that the original description of Stemphylium sensu Wallroth must be retained, and to this end proposed that all such stemphylia be placed in the sub-genus Eustemphylium. The later concept, Stemphylium sensu Harz, was based on a misidentification, and to avoid the necessity of transferring species of this type to another genus, Wiltshire proposed that both concepts be retained in the one genus Stemphylium, with the latter being placed in the sub-genus Pseudostemphylium.

Although Wiltshire demonstrated a clear distinction between his two sub-genera, there still remained an inadequacy in the distinction between the sub-genus Pseudostemphylium and the genus Alternaria. It appeared that delineation was based only on highly variable criteria such as conidial shape and ability to form chains (catenulation). This inadequacy is well illustrated in the classification of Alternaria radicina. Although both the following workers recognised Wiltshire's sub-generic treatment of Stemphylium, Neergaard (1939) transferred the pathogen from Thyrospora to Stemphylium, whereas Groves and Skolko (1944) chose to retain it in Alternaria for the following reasons:

"When the spores from near the growing margin of a colony were

> examined, a considerable number were observed that tapered towards the distal end as in Alternaria, but when mounts were made from older cultures few of the spores were found. The spores appeared to undergo secondary thickening in such a way that they became more ellipsoid than clavate, and rounded at both ends. They thus resembled Stemphylium spores in shape although they lacked the conspicuous constriction at the median septum that is apparently characteristic of Stemphylium..... On the basis of Drechsler's figure, which clearly illustrates short chains in which the distal spore is the younger, we are retaining the species in Alternaria."

Neergaard (1945), in an addition to his monograph on Danish species of Alternaria and Stemphylium, referred to the work of Groves & Skolko (1944) and stated that:

> "I must still maintain that the species should be referred to Stemphylium, namely to the sub-genus Pseudostemphylium Wiltshire (1938), as the spores very much resemble the spores of the type of this sub-genus S. lanuginosum Harz."

Grogan & Snyder (1952) agreed with Neergaard (1945), that the pathogen should be included in the sub-genus Pseudostemphylium, although they qualified this by the following statement:

> "considering Wiltshire's work (Wiltshire, 1929) on saltation from Pseudostemphylium to Alternaria and vice versa (Wiltshire, 1932), it is possible that further work on natural variability of members of this group might indicate that some or all of the Pseudostemphylium types should be included in Alternaria."

Subramanian (1961) subsequently elevated Pseudostemphylium and Eustemphylium to generic rank and although this acknowledged a clear morphological distinction between the new genera, it did not aleviate the difficulty in distinguishing between Pseudostemphylium and Alternaria for some species, especially Alternaria radicina.

A widely accepted approach to hyphomycete taxonomy was proposed by Hughes (1953), based on the developmental morphology of the conidium-

producing structures. He divided the Hyphomycetes into eight main sections according to the types of conidiophore and the manner of conidial production. This modern concept was adopted in principle by Tubaki (1958) who added a ninth section and divided some of Hughes' sections into sub-sections. Section IV of Hughes' classification grouped all those hyphomycete fungi producing their conidia (porospores) from pores on the conidiophore, which included the genera Alternaria, Ulocladium and Stemphylium. Simmons (1968) followed Hughes' approach and individually typified the genera Alternaria, Ulocladium and Stemphylium, basing their delimitation on differences in conidiophore shape and mode of proliferation, and on differences in juvenile conidium shape. The fundamental weaknesses in the criteria used to delimit species of Pseudostemphylium, Eustemphylium and Alternaria were recognised by Simmons. He concluded that the type species of the genus Pseudostemphylium, namely P. lanuginosum (Harz) Subramanian, closely resembled the type species Ulocladium botrytis Preuss. On that basis he proposed that P. lanuginosum be transferred to Ulocladium, which meant that the genus Eustemphylium Subramanian was restored to Wallroth's original concept of the genus Stemphylium. The criteria used by Simmons for the delimitation of the genera Alternaria, Ulocladium and Stemphylium are summarised as follows, (sensu Tate 1968);

Key to Stemphylium, Alternaria and Ulocladium

A Conidiophores terminally bulbous, with proliferation through the swollen apical surface ..... Stemphylium

A_1 Conidiophores not terminally bulbous, with proliferation lateral to and slightly below the apical surface.

B. Conidia fundamentally ovoid and distally

beaked ..... Alternaria

B_1 Conidia fundamentally obovoid and not distally beaked ..... Ulocladium

This classification scheme has already been accepted in principle by other workers (Tate 1968, Barron 1968) and is undoubtedly the best yet devised.

The scheme was used by Simmons (1968) to determine the generic affinity of Alternaria radicina as follows:

> "A. radicina M.D.&E. 1922, is a species of considerable importance which has been treated taxonomically with other pseudostem phylioid species.
>
> Conidia of A. radicina resemble those of some species of Ulocladium, only that they remain solitary or produce chains sparingly and never develop a narrowly tapered, true beak. The conidia are alternarioid in that they are ovoid at a very early age and to a great extent retain an ovoid or broadly ellipsoidal, apically broadly conical shape as they mature."

Although this argument appears to be wholly acceptable, Simmons did not provide illustrations to substantiate the placement. In this study, therefore, the objective was to demonstrate microphotographically that the pathogen belongs to the genus Alternaria.

B. DEVELOPMENTAL MORPHOLOGY STUDIES

Photomicrographic study of the developmental morphology (ontogeny) of hyphomycete conidia has been shown to provide valuable information regarding the generic affinity of certain fungi in accordance with modern taxonomic concepts (Kendrick, Cole & Bhatt 1968, Kendrick & Cole 1968, 1969, Cole & Kendrick 1968, 1969a, 1969b). The main objective of this section of the study was to record features of the conidium ontogeny of A. radicina in order to seek confirmation that the pathogen is correctly placed in the genus Alternaria. Direct comparisons were made between the conidium ontogeny characteristics of A. radicina and other fungi representing the genera Alternaria, Ulocladium and Stemphylium. More specifically, three basic determinations were made about each fungus, namely;

(i) The shape of the terminal end of the conidiophore.

(ii) The mode of proliferation of secondary conidia.

(iii) The shape of developing conidia.

1. Materials and Methods

Photomicrographic technique and methods of slide preparation were varied depending on the particular fungus under study and the information required about each.

(a) Photomicrographic equipment

A Leitz Wetzlar Ortholux compound microscope fitted with a Leitz Wetzlar 35 mm. camera was available and extensive use was made of this equipment for photomicrographic recording. Agfa F.P.3, ASA 125 film

was used in the camera and by maintaining the light reading and exposure time at predetermined values (F4 at 0.2 seconds) satisfactory results were consistently obtained for magnifications of 125, 250, and 400 times.

(b) Slide preparation

Two similar methods of slide preparation were adopted for observing and photomicrographing the details of conidium development; one a slide culture technique and the other a slide chamber technique, as follows:

(i) Slide culture technique

This technique was developed by Riddell (1950) for obtaining permanent mycological preparations. A 1.0cm. x 1.0cm. square of P.D.A. was placed between a sterile glass slide and a sterile cover slip, and each side of the agar inoculated with mycelium of the particular fungus under study. By incubating in a humidified petri plate at 24°C the fungus was induced to grow out and sporulate close to the surface of the cover slip and slide. When the desired stage of conidium development had been reached the agar piece was gently removed, leaving the intact conidiophores and conidia adhering to the slide and cover slip. A drop of mounting fluid was then applied to each and another slide or cover slip placed on top. After sealing the cover slip in place the preparation was examined under the compound microscope.

(ii) Slide chamber technique

Kendrick, Cole & Bhatt (1968) extensively used a humidified slide chamber technique for studying the conidium ontogeny of many different fungi. Harvey (1970, pers. comm.) developed a modification of

this technique which proved to be ideal for the purpose of this study. It consisted of a shallow "perspex" chamber which was fitted on to the microscope stage, and within which the growth of the fungus on P.D.A. was maintained by pumping humidified air through it. Although essentially based on the slide culture technique described above, this method enabled the sequence of conidium development to be observed microscopically and recorded photomicrographically without interruption.

(c) Fungi selected for comparison

The fungi used in this investigation were selected from a stock culture collection held in the laboratory. To ensure adequate sporulation the specific cultural and environmental requirements of these fungi were examined and where necessary adjustments were made to the slide culture and slide chamber techniques, as follows:

(i) Stemphylium botryosum Wallroth.

This fungus required special environmental conditions to induce fructification on agar media. Tate (1968) demonstrated that exposure to an ultra violet (U.V.) light source for 10 minutes each day for three days induced prolific sporulation on P.D.A. Slide cultures of S. botryosum were therefore subjected to 10 minutes U.V. irradiation daily for three days, and for the purposes of this investigation induced adequate sporulation. The slide chamber technique (sensu Harvey) was unsuitable for observing conidium ontogeny of this fungus because of the muriform nature of the conidia and the build-up of a moisture film inside the chamber.

(ii) Ulocladium consortiale

The ontogeny of conidia of this fungus was observed and photomicrographed using both the slide culture and slide chamber techniques. U. consortiale readily produced fructifications on P.D.A. media, thus no special modifications of technique were necessary.

(iii) Alternaria dauci (Kuhn) Groves & Skolko

This pathogen produced few conidia on P.D.A. unless special techniques were used to induce fructification. Zimmer & McKeen (1969) investigated the interaction of light and temperature on sporulation of A. dauci and although their recommendations were taken into consideration a wounding and washing method described by Ludwig, Richardson & Unwin (1969) for Alternaria solani (Ell. & Mart.) Sorauer, was found to be most satisfactory. A P.D.A. colony of A. dauci was scraped free of mycelium using a clean glass slide and thoroughly washed with running tap water for 24 hours. For the purposes of this experiment small strips of P.D.A. and fungus (0.3 cm. by 2 cm.) were then cut from the colony using a razor blade, and placed on their side on the underside of the coverslip of a humidified slide chamber. By this means the fungus produced its fructifications approximately at right angles to the line of vision through the microscope, thus allowing ready observation of conidium development.

(iv) Alternaria radicina Meier, Drechsler & Eddy.

The slide chamber was used exclusively to obtain photomicrographs of various features associated with the development of conidia and conidiophores of A. radicina. Although the pathogen sporulated readily within the chamber the conidia did not appear to develop fully when grown

on P.D.A. Snyder & Hansen (1947) have advocated the use of natural media to promote typical fructification of fungi in culture, and on this basis a modification of the chamber technique was devised. Instead of using the inoculated P.D.A. block, a small piece of inoculated host leaflet was attached in close contact with the underside of the glass cover slip by using another piece of cover slip fixed at both ends with paraffin wax. Spores produced from the edge of the leaflet remained in contact with the cover slip and their development was readily observed under the microscope.

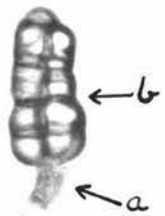
2. Results and Discussion

The results are presented in conjunction with a number of photomicrographs of the conidium ontogeny of S. botryosum, U. consortiale and A. dauci, each illustrating particular features which characterise the genera they represent in this study. These features were then used as the basis on which to determine the generic affinity of A. radicina.

(a) Stemphylium botryosum

According to Simmons (1968) there are a number of morphological characteristics by which species of Stemphylium are distinguished from species of Alternaria and Ulocladium. The main diagnostic features are the bulbous terminal end of the conidiophore (Plate 9), and proliferation through the swollen apical surface of the conidiophore. Tate (1968) clearly illustrated both of these features and although the distinctive mode of proliferation was observed in this investigation, no suitable photomicrographs were produced. Another diagnostic feature of this genus was also observed; namely the constricted median septum (illustrated in Plate 9).

Plate 9.



← 30μ →

Stemphylium botryosum. Conidium production.

(a) Bulbous terminal end of conidiophore.

(b) Median constriction.

(b) Ulocladium consortiale

The distinction between species of Ulocladium and Alternaria is based on the difference in shape of the developing conidium. In Ulocladium the conidium is fundamentally obovoid and not distally beaked as illustrated in Plate 10 .

(c) Alternaria dauci

The developing conidia of species of Alternaria are fundamentally ovoid in shape, often tapering distally to a true beak. These features are clearly illustrated in Plate 11 , which shows stages in the development of a single conidium of A. dauci.

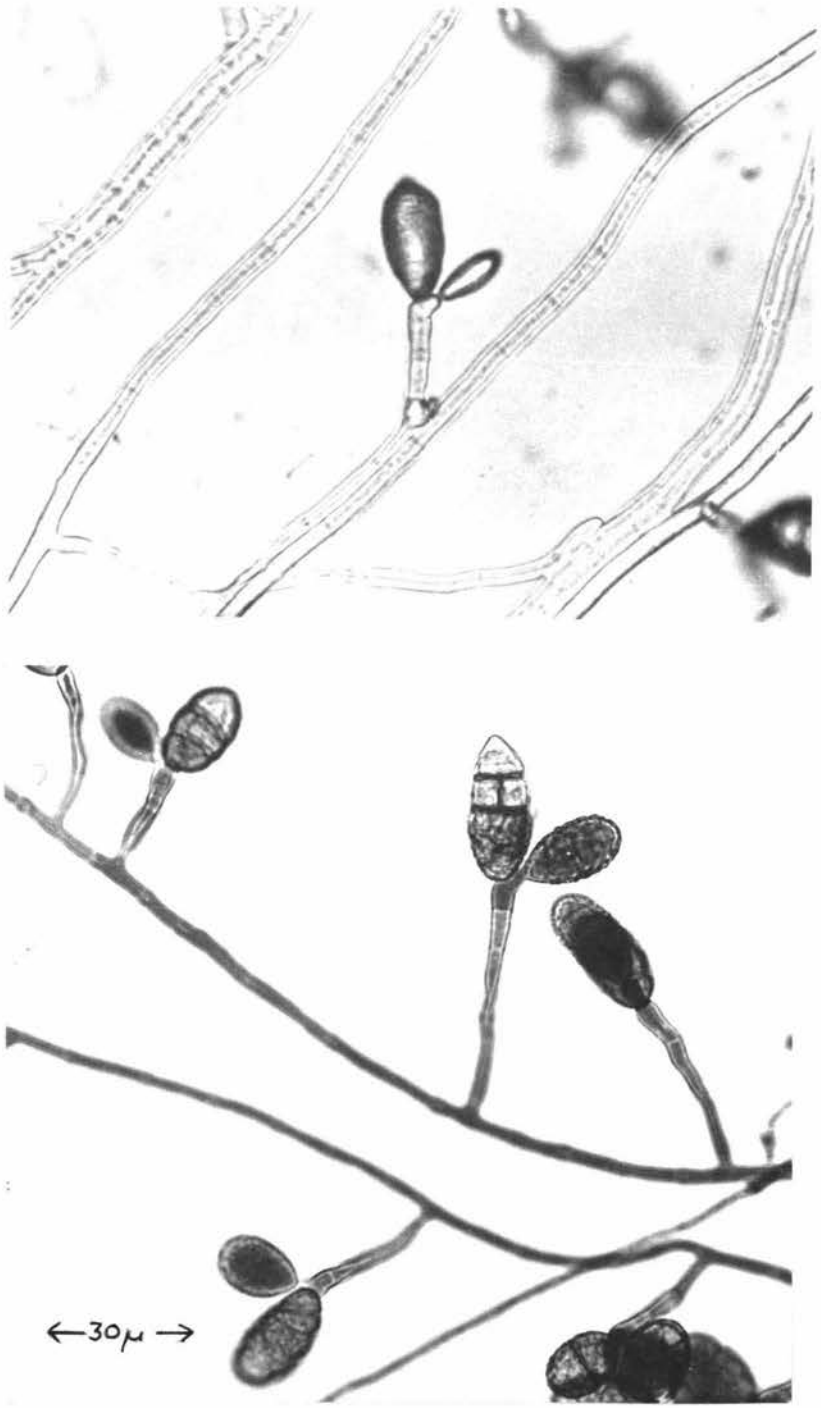
(d) Alternaria radicina

The diagnostic features of Stemphylium, Ulocladium and Alternaria, illustrated and described above, were used as a basis for determining the generic affinities of the pathogen.

The conidium ontogeny of A. radicina was carefully observed and photomicrographically recorded in the manner already described. The non-bulbous nature of the conidiophores and proliferation lateral to and slightly below the apical surface of the conidiophore are clearly illustrated in Plate 12 . These features indicate that the pathogen should be included in either Ulocladium or Alternaria.

In Plate 13 a typical developmental sequence of a single conidium of A. radicina is illustrated. The distinctly ovoid shape of the conidium is a further indication that the pathogen belongs to the genus Alternaria. This feature is also illustrated in Plate 12 , and

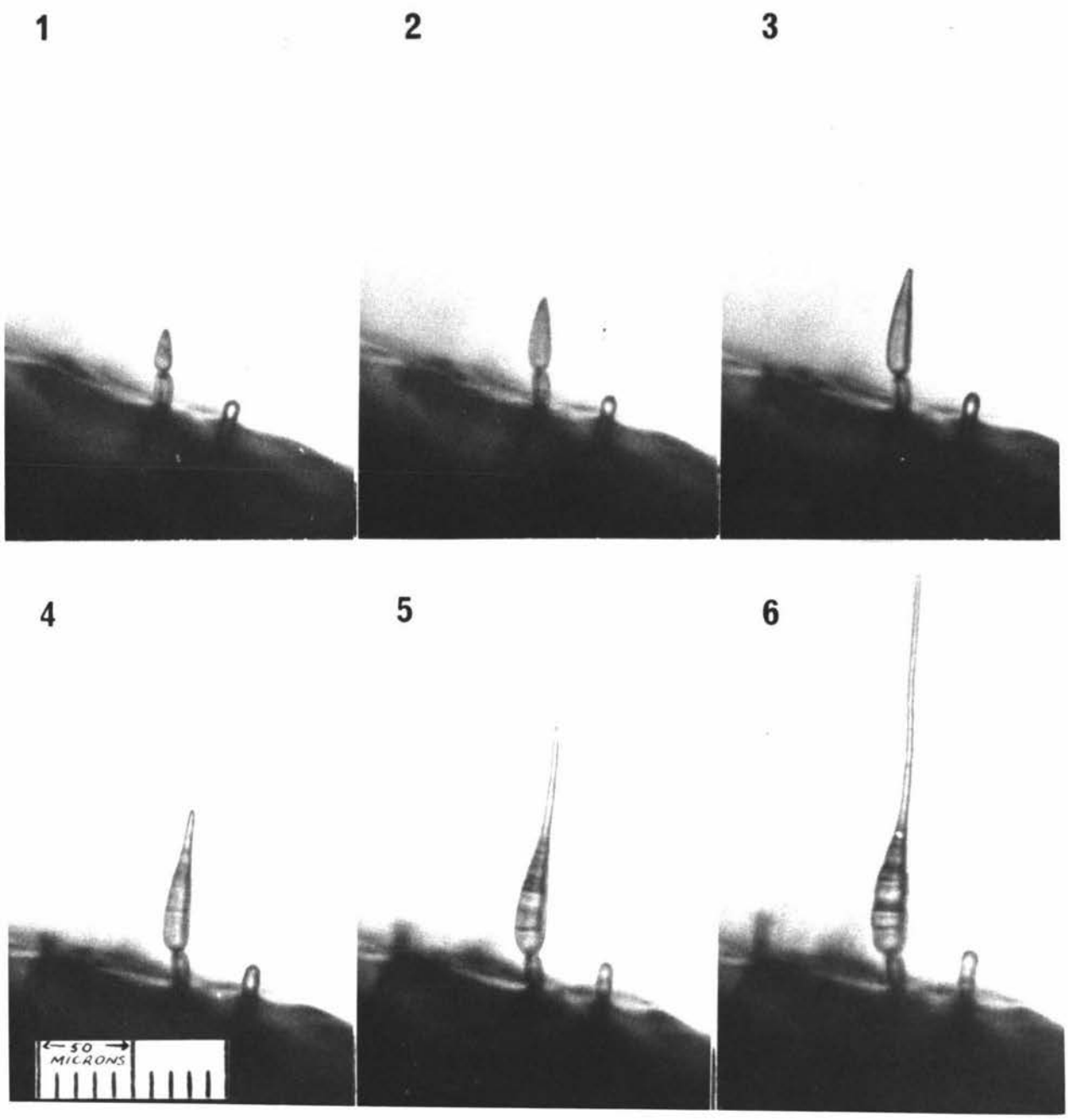
Plate 10.



Ulocladium consortiale. Conidium production.

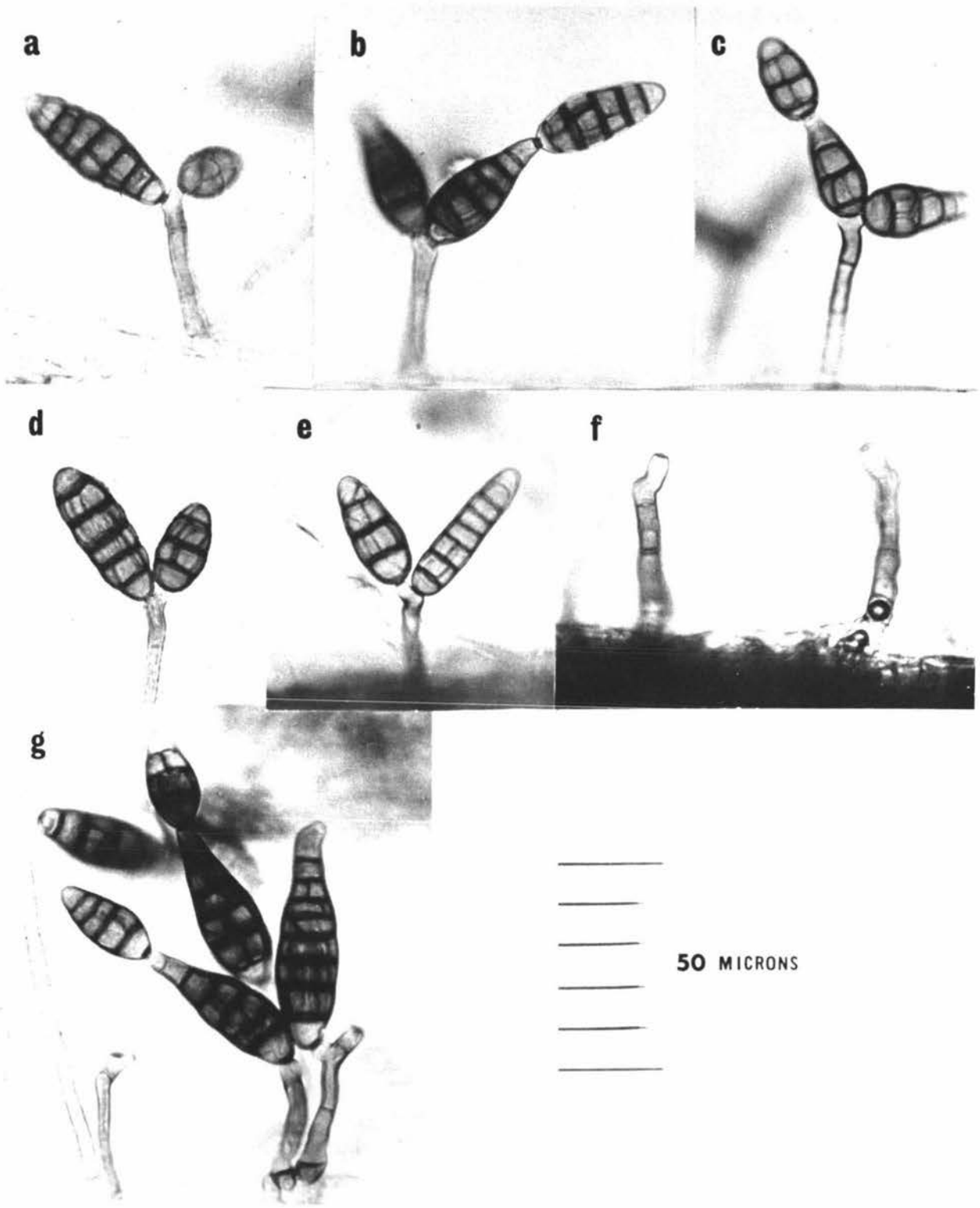
Illustrating the non-bulbous nature of the conidiophore and the obovoid shape of the juvenile conidium.

Plate 11.



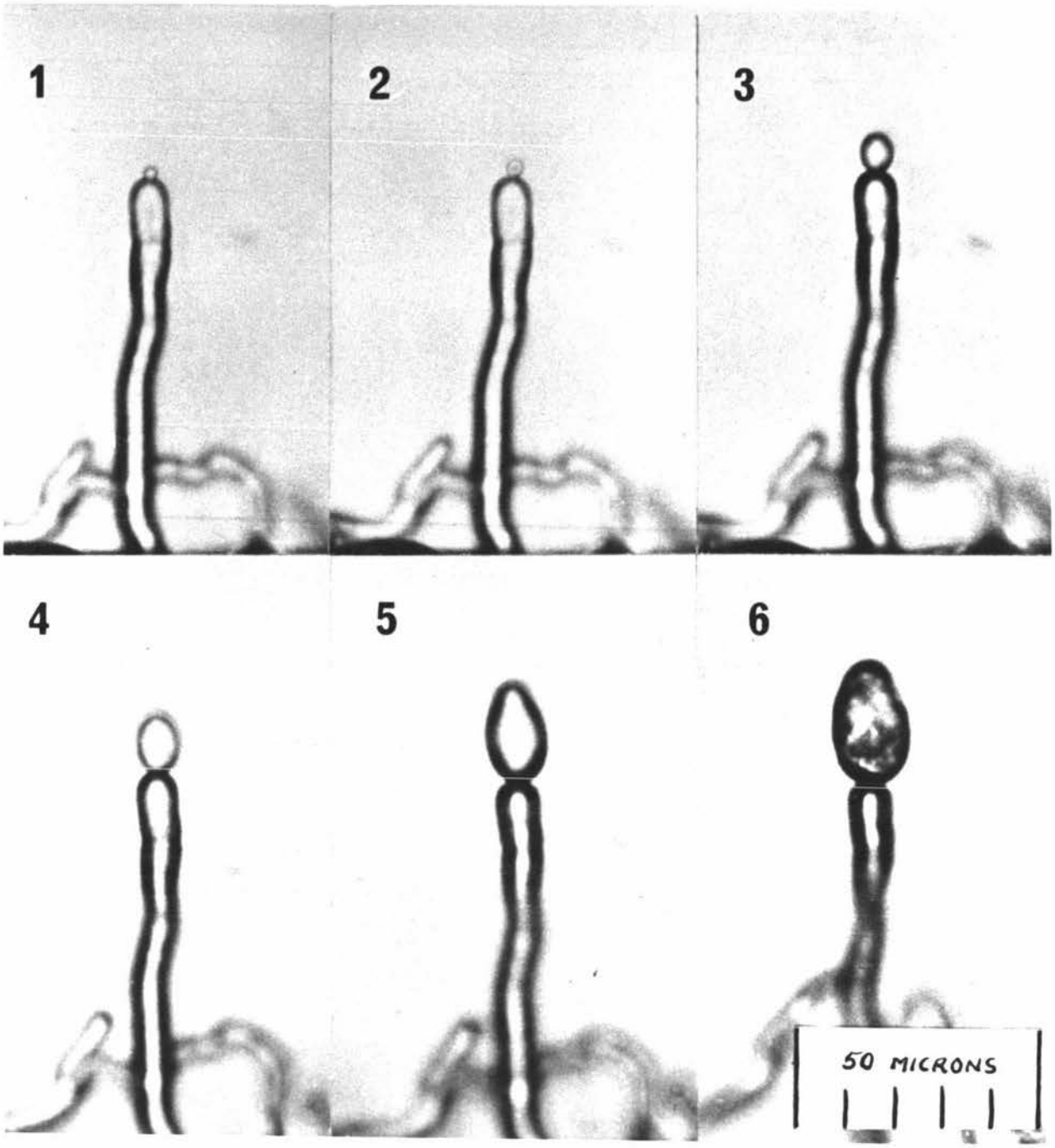
Alternaria dauci. Conidium ontogeny.

Plate 12.



Alternaria radicina. Production of conidia.

Plate 13.



Alternaria radicina. Conidium ontogeny.

is quite different from the obovoid shape of the juvenile conidia of U. consortiale (Plate 10).

The results of this investigation also confirm that the pathogen has the capacity to form short chains of conidia (Plate 12), and according to Simmons (1968) this is a phenomenon which does not occur in Stemphylium, and only rarely in Ulocladium. Furthermore the conidia do not possess a constricted median septum which is another characteristic of the genus Stemphylium.

Conclusion

Much of the past confusion in the classification of this pathogen is attibuted to the fact that highly variable criteria such as size and shape of the mature conidia were used almost exclusively to delimit these related genera. Simmon's modern approach to the typification of Alternaria, Ulocladium and Stemphylium does not rely on such variable criteria, but is based on more stable morphological features such as mode of conidium development (sensu Hughes, 1953). This system was accepted in the current study because it enabled clear distinction between the three genera. On this basis, therefore, it is the considered opinion of the author that the pathogen should be retained in the genus Alternaria, as Alternaria radicina M.D. & E.

CHAPTER III

SEED TRANSMISSION OF ALTERNARIA RADICINA

TRANSMISSION IN COMMERCIAL SEED LINES

NATURE OF SEED-BORNE INOCULUM

SIGNIFICANCE OF SEED-BORNE INOCULUM

CONTROL

A. TRANSMISSION IN COMMERCIAL SEED LINES

1. Evidence of Transmission

In their early studies on the disease and pathogen Meier, Drechsler & Eddy (1922) and Lauritzen (1926) made no reference to the possibility of A. radicina being seed-borne. However Jorgensen (1934) claimed that A. radicina was isolated from a sample of carrot seed in 1927 and that on inoculation to carrot roots it produced a deep, black rot. Only a brief abstract of Jorgensen's work is available; any further details have apparently never been published.

Doyer (1938) gave a concise description and illustration of how this pathogen affects young seedlings during routine germination assessments. This appears to be the first clear evidence that A. radicina is seed-borne in carrot. Mounce & Bosher (1943) described a seedling disease of carrots caused by A. radicina and showed that up to 40 percent of seed in some lots transmitted the pathogen. Groves & Skolko (1944) isolated A. radicina from seeds of carrot, parsley and vegetable marrow received from Nova Scotia, Manitoba, British Columbia, Connecticut, Minnesota, & California.

Neergaard (1945), in connection with his review of the genera Alternaria and Stemphylium, described results of his extensive investigation into the state of health of carrot seed, in part with reference to A. radicina. During the period 1935 to 1942, he screened 778 lots of commercial seed for the presence of A. radicina, and of these approximately 50 percent were found to transmit the pathogen at levels ranging from 0.1 - 30+ percent. Neergaard stated that - "undoubtedly the fungus is found in all European countries. It is markedly seed-borne, and on that

account an international spreading of the fungus is unavoidable".

Since then there has been a wealth of literature implicating A. radicina as a seed-borne pathogen of carrots (Khristova & Raikov 1948, Grogan & Snyder 1952, Rusakova & Raskin 1955, Roberts 1956, de Tempe 1962, Hewett 1964, Lang de la Camp 1966, de Tempe & Limonard 1966, Maude 1966).

2. Seed Screening Recommendations

Ideally a screening technique should attempt to reveal the presence of all pathogens transmitted by the seed and capable of subsequently infecting the host plant. If the conditions required to identify the various pathogens differ then separate seed health screening methods may need to be developed. In the case of carrot seed there are only two important pathogens known to be commonly seed-borne, namely A. radicina and A. dauci.

In most of the various screening recommendations simultaneous testing for both pathogens has been advocated since they require similar conditions for their identification. For this reason details of the development of carrot seed health testing techniques will, of necessity, refer to both A. dauci and A. radicina.

Until 1966 variations of the agar and blotter methods of testing carrot seed for the presence of A. radicina and A. dauci were advocated by several workers (Doyer 1938, Neergaard 1945, de Tempe 1962, Hewett 1964, Maude 1965, Noble 1966). The I.S.T.A. (1966) subsequently proposed a health screening procedure to be regarded as the official test for seed-borne A. radicina and A. dauci, as follows;

Working sample	:	400 seeds
Medium	:	moistened blotter in a closed container so that humidity will be maintained.
Spacing	:	at least 2 cm.
Incubation	:	10 days at 18 - 22°C.

Of particular importance in this recommendation was the adoption of the blotter as the testing substrate rather than agar medium. De Tempe (1963) had emphasised that the blotter test was a pathogenic one and as such automatically avoided confusing the identity of the pathogens on agar media with non-pathogenic strains and saprophytes.

In the current investigation, the I.S.T.A. testing recommendation was not strictly adhered to because more recent research had indicated the need for a re-evaluation and modification of certain procedures, and much of the equipment recommended was not available.

(a) Pre-treatment

Since the blotter test for A. radicina and A. dauci is a pathogenic one, the suppression of saprophytes by pre-treatment of seed did not help to distinguish between the pathogen and saprophytes. It was considered necessary to clarify this point since no mention of it was made in the I.S.T.A. recommendation.

(b) Light requirements

De Tempe (1962) found that the blotter health test in light more accurately indicated the field performance of the seed than the blotter test in darkness. U.V. lighting in 12 hour light/12 hour dark cycles was conductive to development and recognition of the two pathogens (de Tempe

1968). However, sophisticated U.V. lighting equipment was unavailable for use in this investigation so the germinators (with glass tops) were placed near the large laboratory windows just out of the direct sunlight. It was found that after 10 days very few of the decayed seedlings remained without the distinguishing conidia.

(c) Spacing

In experiments with A. circinans Neergaard (1945) found that - "on dense sowings (of cabbage seed) in the germinator neighbour infection appears to be of minor significance". With direct reference to carrot seed screening for A. radicina and A. dauci little attention has been given in the literature to the spacing requirement. Hewett (1964) allowed seeds "ample separation" and the I.S.T.A. indicated at least 2 cm. separation.

In the current investigation it was found convenient to use germination blotters which were already printed into half inch squares, one seed per square, 25 squares per blotter. This rather close spacing was found to be quite satisfactory because a careful examination of the few seedlings suspected of being cross-infected left no doubt as to the origin of the infection. Any inaccuracy introduced in this was considered to be of minor significance.

3. Seed Screening Procedure

The modified seed screening procedure adopted in the current investigation was as follows:

Approximately ten times the required amount of seed was taken from each sample packet, placed on a clean sheet of paper and then successively

divided in half until the amount of seed required for the test remained (200 seeds per sample).

After setting out the blotter pads in the Copenhagen germinators, plastic covers were placed over each and the glass germinator lid kept closed for the duration of the test to maintain an atmosphere of high-humidity around the germinating seedlings. The air temperature within the germinator was maintained at 22°C ± 2°C for the duration of the test, and the germinator placed near a laboratory window, out of direct sunlight. Plate 14 illustrates the use of the testing equipment.

After 10 days incubation the seed germination percentage was recorded and each sample was assessed for the presence of pathogens.

(a) Germination percentage

For the purposes of this study seed germination and disease level assessments were conducted concurrently. A seed was considered to have germinated when the emerging radical became visible, and although the official final germination assessment for carrot seed should have been made after 14 days, a 10 day test duration (at 22°C ± 2°C) was found to be more convenient, and from a comparative standpoint, sufficiently accurate.

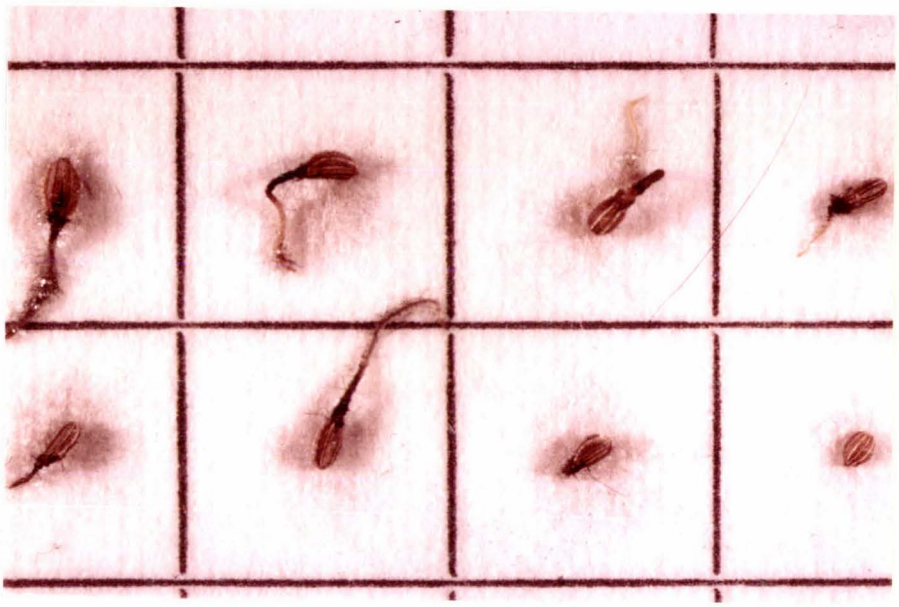
(b) Presence of pathogens

The first indication of either A. radicina or A. dauci was the presence of brown decay of seedlings. A close examination of each was then conducted using an "Olympus" stereoscopic zoom microscope, model SZ-3. At magnifications of between 30 and 120 times, the two pathogens were readily distinguished by their characteristically different conidia (Plates 2 & 16).

Plate 14.

Copenhagen germinator used for routine seed health testing.

Plate 15.



Seedling decay caused by seed-borne A. radicina.

Plate 16.

Conidia of Alternaria dauci (cf. Plate 2).

In the few instances where sporulation had not occurred on the decaying seedlings after 10 days they were held over and re-examined at 14 days. If by this time no evidence of sporulation was present then these seedlings were not considered to be infected by either of the pathogens.

4. Survey of Incidence of Seed Transmission of A. radicina

Since New Zealand seed merchants derive their carrot seed supply from overseas sources, the introduction of the pathogens A. radicina and A. dauci seemed inevitable in view of the absence to date of any mandatory carrot seed health screening requirements. To determine the validity of this hypothesis a survey was conducted to ascertain the state of health of commercially available imported carrot seed during the two years 1968 and 1969.

Carrot seed lines to be screened were obtained directly from several seed merchants throughout New Zealand in 1968 and 1969. Samples of as many different, recently imported seed lines were requested together with the following details about each sample:

(i) variety,

(ii) country of origin,

(iii) approximate date of harvest.

Results

During 1968, 55 seed lines were screened, and a further 31 samples likewise examined in 1969. Over the two years a total of 19 samples (22 percent) were found to be infected with A. radicina at levels ranging between one and thirty percent; and seven samples (eight percent) were infected with A. dauci at levels ranging between one and twenty-seven percent.

Table 4 Seed transmission of *A. radicina* and *A. dauci* in commercial carrot seed lines.

Sample Code No.	Variety	Origin	Germination	Percentage transmission *A. radicina*	*A. dauci*
1968					
3	Dividend	Australia	75	1	-
4	Egmont Gold	"	83	30	-
5	Selection 198	"	93	3	-
7	Topweight	"	90	5	-
9	*	"	91	3	-
12	*	"	88	1	-
15	*	Holland	91	-	1
18	*	Australia	91	19	-
19	Chantenay Long	"	92	1	-
21	Egmont Gold	"	82	17	-
26	Topweight	Holland	83	1	-
34	Holmes	Australia	87	24	-
51	Egmont Gold	"	85	14	-
53	Royal Red	Holland	73	3	-
1969					
101	Spring Maxicrop	Europe	88	-	22
102	Spring Market Improved	"	87	1	24
103	Manchester Table	U.S.A.	88	-	5
106	Red King	Europe	80	-	5
107	Topweight Improved	Australia	80	3	-
108	Amsterdam Forcing	Denmark	69	2	-
109	Topweight	Holland	85	-	13

Table 4 continued

Sample Code No.	Variety	Origin	Germination	Percentage transmission A. radicina	A. dauci
1969					
110	Royal Red	Holland	89	-	27
111	Nantes Early	France	84	1	-
118	Holmes Improved	Australia	80	11	-
121	Topweight	"	84	5	-

* Varietal name not indicated

Table 5

Extent of transmission of A. radicina and A. dauci in seed lines screened during 1968 and 1969

Year	Number of lines	A. radicina		A. dauci	
		Number of lines	Percentage of lines	Number of lines	Percentage of lines
1968	55	13	23.8	1	1.9
1969	31	6	18.0	6	18.0
Total	86	19	22.0	7	8.0

Details concerning the infected seed samples are presented in Table 4.

The information obtained enabled a detailed examination of several aspects of carrot seed health, as follows:

(a) The Extent of Seed Transmission

The term "extent of seed transmission" refers to the number of seed lines screened (expressed as a percentage) which had either or both pathogens present. Table 5 therefore shows the total number, and percentage of seed lines found transmitting each pathogen for each year of screening. The predominance of A. radicina in the 1968 results could have led to the conclusion that A. dauci was relatively unimportant. However, in 1969 as many lines were found to be infected with A. dauci as with A. radicina, thus illustrating the necessity of conducting such screenings over a number of seasons. (The fact that so little A. dauci infection was encountered during 1968 contributed to the emphasis being placed on A. radicina in this study.)

(b) The Level of Seed Transmission

The term "level of seed transmission" refers to the number of seeds within a line (expressed as a percentage) which had either or both pathogens present. In Tables 6 and 7 an analysis of levels of seed transmission found for both A.radicina and A. dauci is presented revealing a range from one to thirty percent. The importance of transmission level as it relates to stand establishment and in providing primary infection foci is considered in Section C. of this chapter.

Table 6 Range in level of transmission of A. radicina in seed lines screened during 1968 and 1969

Range	1968 (55 lines) Number of lines	1968 (55 lines) Percentage of lines	1969 (31 lines) Number of lines	1969 (31 lines) Percentage of lines
0-5%	8	14.3	5	15.0
6-10%	0	0.0	0	0.0
11-15%	1	1.9	1	3.0
16-20%	2	3.8	0	0.0
21+%	2	3.8	0	0.0

Table 7 Range in level of transmission of A. dauci in seed lines screened during 1968 and 1969

Range	1968 (55 lines) Number of lines	1968 (55 lines) Percentage of lines	1969 (31 lines) Number of lines	1969 (31 lines) Percentage of lines
0-5%	1	1.9	2	6.0
6-10%	0	0.0	0	0.0
11-15%	0	0.0	1	3.0
16-20%	0	0.0	0	0.0
21+%	0	0.0	3	9.0

(c) Source Country and Seed Transmission

The findings are presented in Table 8 and can be summarised as follows:

(i) There was almost complete absence of transmission of either A. radicina or A. dauci in seed originating from the U.S.A. This may possibly be attributed to unfavourable environmental conditions for the development of A. radicina and A. dauci in areas where carrot seed is grown in the U.S.A.

(ii) Some of the Australian produced carrot seed lines transmitted a high level of A. radicina. Eighty-seven percent of such lines screened transmitted the pathogen, at levels between one and thirty percent. Although Australian produced seed was undoubtedly most important in this respect, a number of European samples also transmitted the pathogen, but the individual levels were relatively low.

(iii) Seed from European countries almost wholly accounted for the presence of A. dauci in the screenings conducted in 1969.

(d) Varieties of Carrot Transmitting the Pathogens

In 1969 high levels of A. radicina were found to be associated with several lines of the variety Egmont Gold obtained from different seed companies. However, the transmission levels and germination percentages were comparable, which suggested that the lines may have originated from the same Australian grower. Since other varieties were also found to transmit A. radicina as seed-borne inoculum (Table 4), there was no justification for assuming that Egmont Gold was more susceptible to the pathogen.

(e) Seed Germination

In a strict germination test decaying seedlings are classified as "abnormal", and therefore not included in the germination figure. In this study, however, all seeds exhibiting any evidence of germination, regardless of infection, were included in the germination percentage.

Figure 4 shows the distribution of 10 day germination percentages observed for all the carrot seed samples screened during 1968 and 1969. Although this information did not take account of the effect of seed-borne inoculum on germination and emergence, it served as a basis on which to gauge its significance. The significance of seed-borne A. radicina inoculum was examined in a separate section of this investigation.

Table 8

Summary of the countries of origin of seed transmitting

A. radicina and A. dauci in 1968 and 1969

		Transmission of A. radicina			Transmission of A. dauci		
	Number of lines screened	Number of lines	As percentage of all lines	As percentage of lines from same origin	Number of lines	As percentage of all lines	As percentage of lines from same origin
Denmark	2	1	1	50	0	0	0
Holland	6	2	2	33	3	3.5	50
France	11	1	1	10	0	0	0
Europe (Unkn)	12	1	1	12	3	3.5	25
Total Europe	31	5	6	16	6	7	20
U.S.A.	37	0	0	0	1	1	3
Australia	18	14	16	87	0	0	0

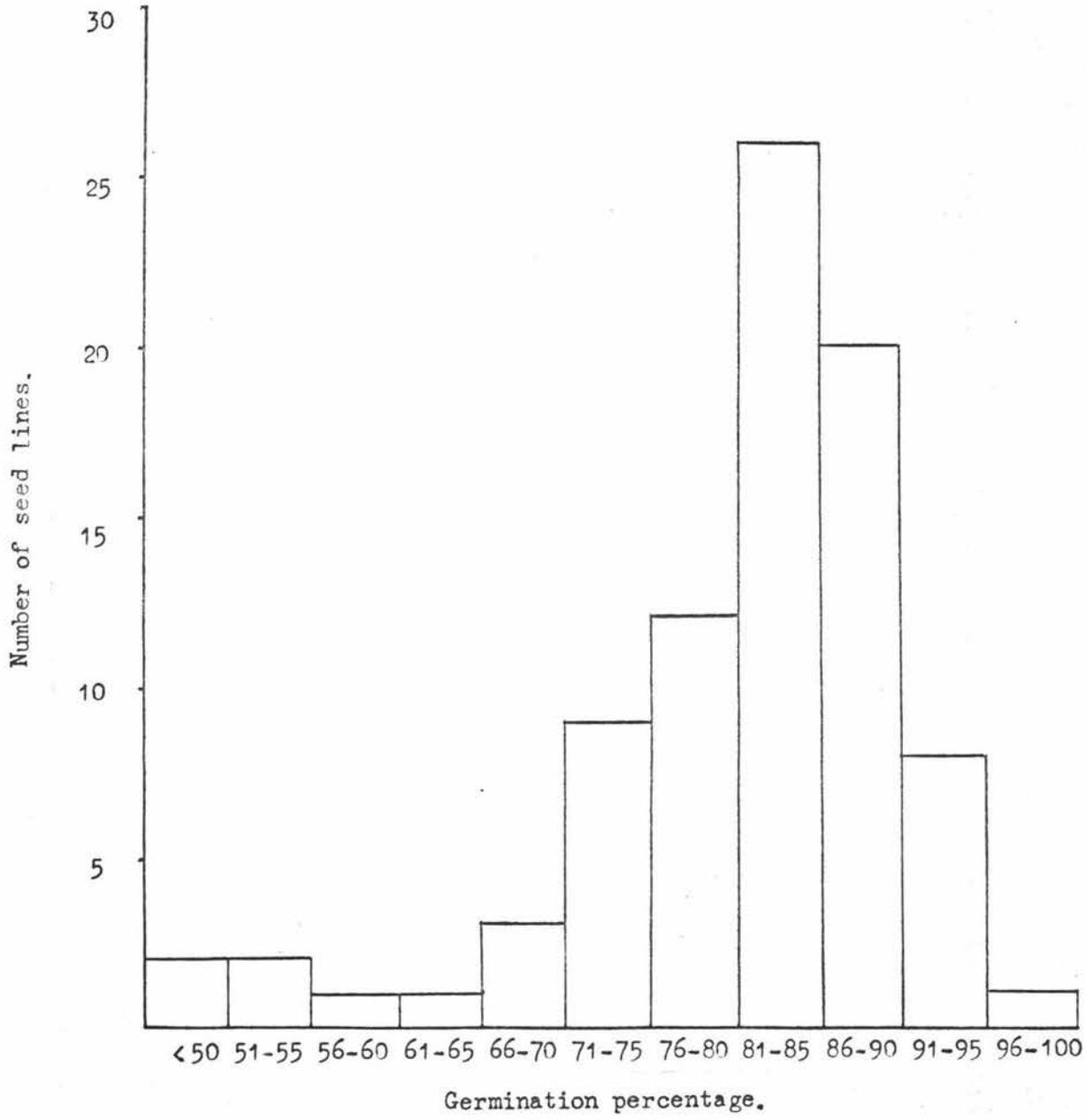


Fig. 4. Distribution of germination percentages at 10 days of seed lines screened during 1968 and 1969.

B. NATURE OF SEED-BORNE INOCULUM

A seed-borne disease may be defined as one which is potentially capable of becoming established in a crop as a result of sowing seed carrying the causal organism of that disease.

The nature of the association between pathogen and host seed can be divided into two broad categories, namely:

(i) Contamination; arises as a consequence of lesioned plant parts being included with the seed heads during harvesting. Conidia and mycelial fragments may become mixed with the seed and, so long as such externally borne propagules remain viable between harvest and sowing, they constitute an effective means of pathogen spread.

(ii) Infection; usually occurs as a result of seed-bearing structures of the host becoming infected during development, and the fungus then growing through into the testa or beyond into the endosperm or embryo.

1. Seed Contamination

Walker (1952) indicated that conidia of A. radicina can externally contaminate carrot seeds, although he provided no evidence to substantiate this. Grogan & Snyder (1952) have shown that contamination can occur naturally on seed from bolted plants of a root crop being grown during the rainy season. "Seed produced on such blighted umbels was invariably contaminated or infested by S. radicinum but was of course out of season for seed production". De Tempe (1962) in making a comparison of various methods of seed health testing for A. radicina mentioned the use of the Bolley technique, a method applicable to the identification of surface

borne pathogenic elements. This implied that seed contamination was possible, although no positive evidence of its nature or occurrence was presented.

Because of the lack of substantiated evidence of seed contamination in commercial seed lines, an attempt was made to determine the occurrence of seed contamination with A. radicina on imported carrot seed.

(a) Detection

The method developed by Bolley (1902) for examining wheat seed samples for the presence of adhering bunt spores (Tilletia caries (DC) Tul.) was considered to best suit the requirements of this experiment. Twelve lines of seed found by the seed screening survey to transmit A. radicina were routinely examined by the following method:

One gram of carrot seed was placed in 10 ml. of water in a 250 ml. Erylenmeyer flask. This was held for 10 minutes, agitated for five minutes, and the suspension centrifuged for five minutes at approximately 6,000 r.p.m. The centrifugate was then examined for the presence of dictyosporous conidia of A. radicina using a compound microscope.

In no instance were spores of A. radicina recovered from any of the seed lines examined by the above method. The level of seed-borne inoculum in some lines was so high (25 - 30 percent), that if conidial contamination was present, then it would have undoubtedly have shown up in the Bolley test.

The lines of seed examined were almost completely free of debris, although minute particles containing the pathogen could have adhered to the seed surface. These small pieces of debris, which were contained in

the centrifugate and subjected to high humidity, were routinely examined for germ tubes or mycelium, with negative results.

(b) Artificial Seed Contamination

Although the above results were negative, the absence of contaminating inoculum may have, by chance, been due to a biased selection of lines used in this study. The possibility of contamination occurring still remained, and for this reason further studies required the use of artificially contaminated seed.

Because of the interval between harvest of the seed overseas and sowing in New Zealand, it was necessary to demonstrate that contaminating inoculum is viable with time. However, mere demonstration of the presence and viability of inoculum does not prove that it is responsible for subsequent crop infection. Additional experiments are necessary to establish this.

(i) Contamination of Seed

A conidial suspension of A. radicina was prepared using 10 day old cultures growing on P.D.A. The concentration of this suspension was determined using a "Bright-line" haemocytometer, and then adjusted arbitrarily to produce the following three concentration series:

120,000 spores / ml.
60,000 spores / ml.
30,000 spores / ml.

Eighty mls. of each of these spore suspensions was immediately used to artificially contaminate samples of a disease-free seed line, as

follows. Twenty gram samples of disease-free seed were loosely enclosed in small muslin bags and placed in the appropriate spore suspension for about 10 minutes until thoroughly wetted. After removal from the suspension the seeds were allowed to dry at room temperature and then held in sealed envelopes until required.

The resultant contaminant spore loads were determined by subjecting seed to a routine washing method (sensu Bolley 1902) and determining the spore concentration of the resultant suspension using a haemocytometer. The spore load per gram of seed, and per seed was then calculated; the results are presented in Table 9.

Table 9 The relationship between inoculum concentration and spore load of artificially inoculated carrot seed.

Initial Conc.	Potential spore load *	Actual spore load achieved **	
spores/ml.	spores/gm/ of seed	spores per gm. of seed	spores per seed
30,000	120,000	16,000	18
60,000	240,000	31,000	35
120,000	480,000	58,000	66

* The potential spore load was calculated on the basis of 4ml. of inoculum per gram of seed.

** The spore load per seed was calculated on the basis of 870 seeds per gram.

The artificial spore loads were intended to be in excess of those considered possible in a natural situation to ensure that the inoculum level was such that the pathogen would produce its maximum potential effect.

(ii) Viability of Contaminating Inoculum

The importance of seed contaminating inoculum is dependent on its ability to remain viable, particularly in New Zealand, where it may take several months or even years to receive vegetable seed from overseas growers. Kramer & Pady (1968) studied the viability of air-borne spores and concluded that "Alternaria spores are remarkably resistant and apparently retain their viability for long periods of time". Ruckshenaite-Beretskere (1968) found that conidia of A. radicina maintained their viability in herbarium storage for up to 2½ years. These findings support the contention that the dictyosporous conidia produced by species of such genera as Alternaria and Stemphylium are quite capable of remaining alive as seed contaminants for the normal duration between harvest and sowing.

For these reasons the viability of conidia of A. radicina borne on carrot seed was considered to be worthy of examination. Since no naturally occurring seed-borne contaminating inoculum was found in this study, conidia which had been artificially applied to carrot seed were examined for their germination ability at intervals of up to six months.

De-contamination of seed was carried out using the washing method already described (sensu Bolley). The resultant spore suspension was centrifuged for five minutes at 6,000 r.p.m. and a drop of the centrifugate containing the conidia under test was spread over a clean glass slide. This slide was then placed in a high-humidity chamber for six

hours to allow the conidia to germinate. Two hundred such conidia were randomly examined for the presence of germ tubes, and the percentage of germinated conidia recorded. This determination was carried out concurrently on two lines of artificially contaminated seed; those lines containing an average of 18 and 66 spores per seed.

The results obtained substantiate the work of Kramer & Pady (1968) and Ruckshenaite-Beretskene (1968) in relation to the viability of conidia of A. radicina. Little reduction in the capacity of the conidia to germinate was observed until after 12 weeks; and after this period only a slow decline was observed (Table 10).

Table 10 Effect of storage duration on the viability of seed contaminating inoculum.

Storage duration	Percentage Spore Germination	
	18 spores/seed	66 spores/seed
1 day	96	92
4 days	90	90
1 week	87	89
2 weeks	87	85
3 weeks	88	90
4 weeks	90	89
8 weeks	85	87
12 weeks	82	80
16 weeks	68	66
24 weeks	42	38

(iii) Seedling Infection from Artificially Contaminated Seed

Having shown that A. radicina can remain viable as contaminant inoculum on carrot seed, it was necessary to demonstrate that such inoculum was capable of producing a pathogenic effect. Seed which had been artificially contaminated with conidia was therefore used to determine the nature and extent of pathogenic attack when sown in the greenhouse.

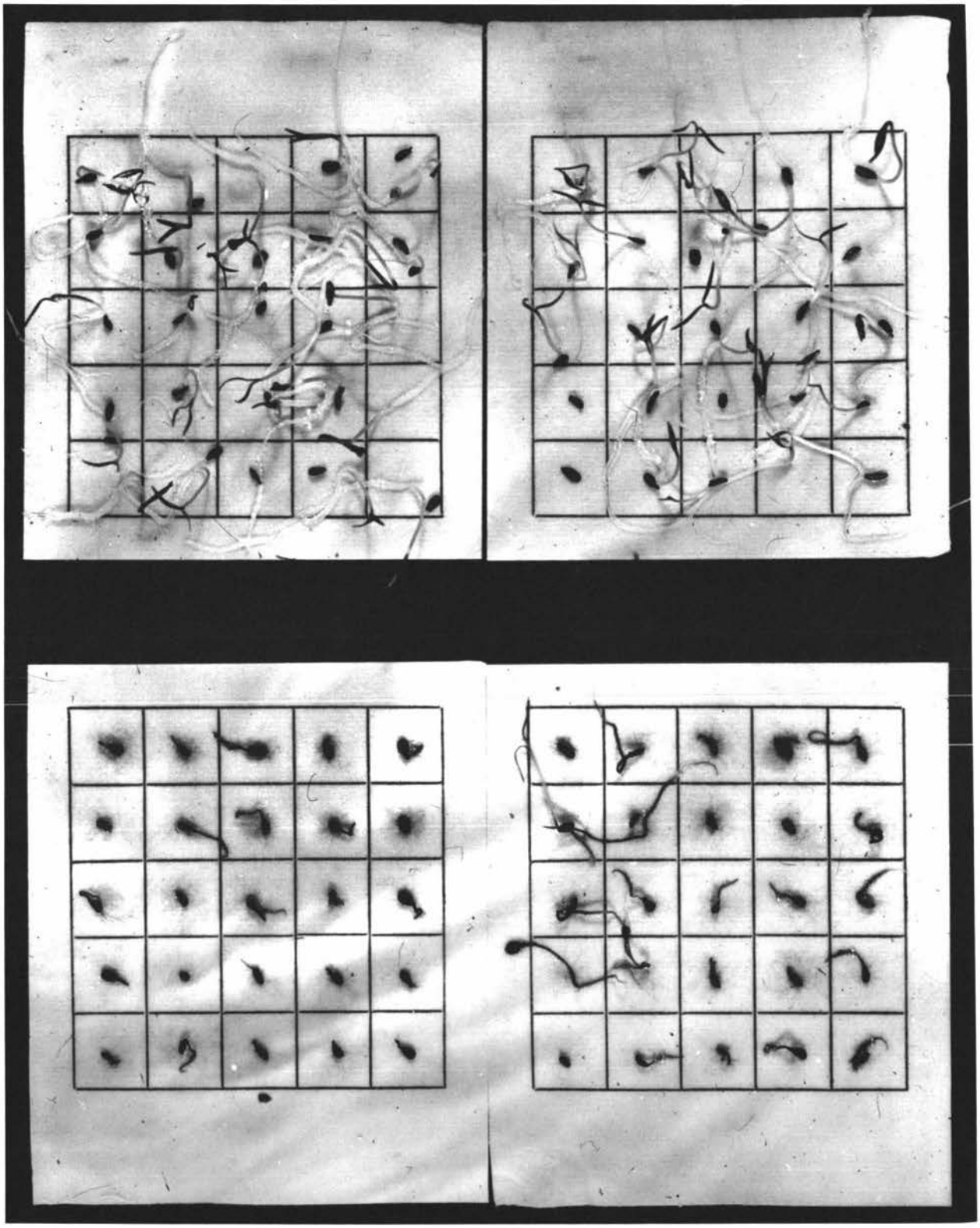
There were two treatments; disease-free seed (control) and artificially contaminated seed. Two replicates of 100 seeds were sown for each treatment. The seeds were individually sown one inch apart at a depth of approximately $\frac{1}{2}$ an inch, and the trays were kept well watered for the duration of the experiment. Assessments of seedling emergence and seedling infection were made three weeks after the first seedling emerged; that is, when most seedlings had produced their first pair of true leaflets.

The experiment was conducted immediately after artificial contamination and again following storage for 12 weeks. The line of seed with the maximum contaminant load was used in both cases to ensure that any potential pathogenicity was expressed.

The results (Table 11) confirm that surface-borne conidia of A. radicina are capable of causing a pre-emergence damping-off of seedlings, even after 12 weeks of storage of seed.

This was further established by subjecting the contaminated seed lines to a standard blotter health test at monthly intervals over a period of six months. One hundred percent infection of developing seedlings occurred in all the tests conducted (Plate 17).

Plate 17.



Seedling decay on blotters caused by artificial seed contamination with conidia of A. radicina.

Table 11 Effect of storage duration on the pathogenicity of contaminating inoculum

Treatment	Emergence 23/4/69 Percentage		Emergence 16/7/69 Percentage	
	Rep. 1.	Rep. 2.	Rep. 1.	Rep. 2.
Disease free seed control	76	72	75	70
Artificially contaminated seed	0	2	1	0

2. Seed Infection

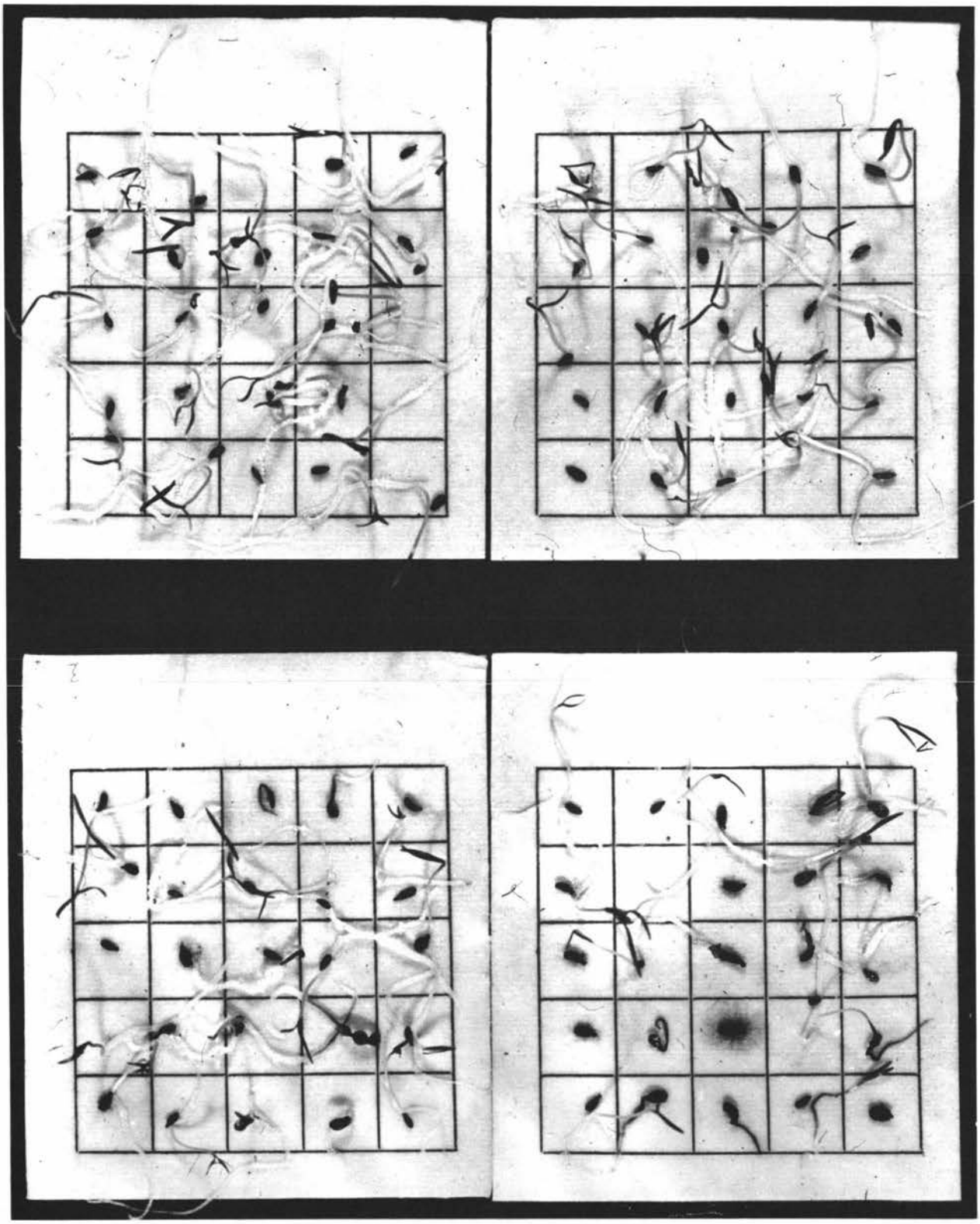
Doyer (1938), Neergaard (1945), Grogan & Snyder (1952), Noble & Richardson (1966) have suggested that A. radicina may be transmitted within carrot seed tissues, but they did not present clear evidence to support this.

The occurrence of A. radicina as contaminant inoculum on available seed has already been investigated, with negative results. It was inferred therefore, that in those lines known from the screening tests to transmit the pathogen the inoculum was present within the tissues of the seed (seed infection).

(a) Demonstration of Existence of Seed Infecting Inoculum

Studies aimed at demonstrating the existence of seed infecting inoculum involve firstly the elimination of contaminating inoculum without

Plate 18.



Seedling decay on blotters caused by natural seed infection with A. radicina.

Upper - non infected.

Lower - infected.

inactivating established infection within the seed, then plating the seed to agar.

(i) Removal of Contaminating Inoculum

Mercuric chloride (0.1%) was the chemical used as a surface sterilant in this study. A preliminary experiment was aimed at determining the minimum duration of surface sterilization which would eliminate all contaminant propagules of A. radicina. This was achieved by subjecting an infection free, artificially contaminated seed sample to a range of immersion times, and assessing the effectiveness of each treatment by plating the seed to P.D.A.

Approximately two grams of seed per treatment were loosely tied in muslin bags, the whole immersed momentarily in ethyl alcohol (50%), and then transferred to the mercuric chloride solution. At the end of the appropriate treatment time, each sample was successively washed three times in sterile distilled water to remove any traces of mercuric chloride, then placed between two pieces of sterile blotting paper to absorb excess moisture.

The results, presented in Figure 5 and illustrated in Plate 19a, indicate a rapid decline in viability of contaminating inoculum within the first three minutes of treatment. The remaining traces of viable inoculum were completely eliminated after five minutes immersion in the mercuric chloride solution. Before five minutes treatment could be regarded as a reliable means of distinguishing between contaminating and infecting inoculum, its effect on the level of infecting inoculum had to be determined.

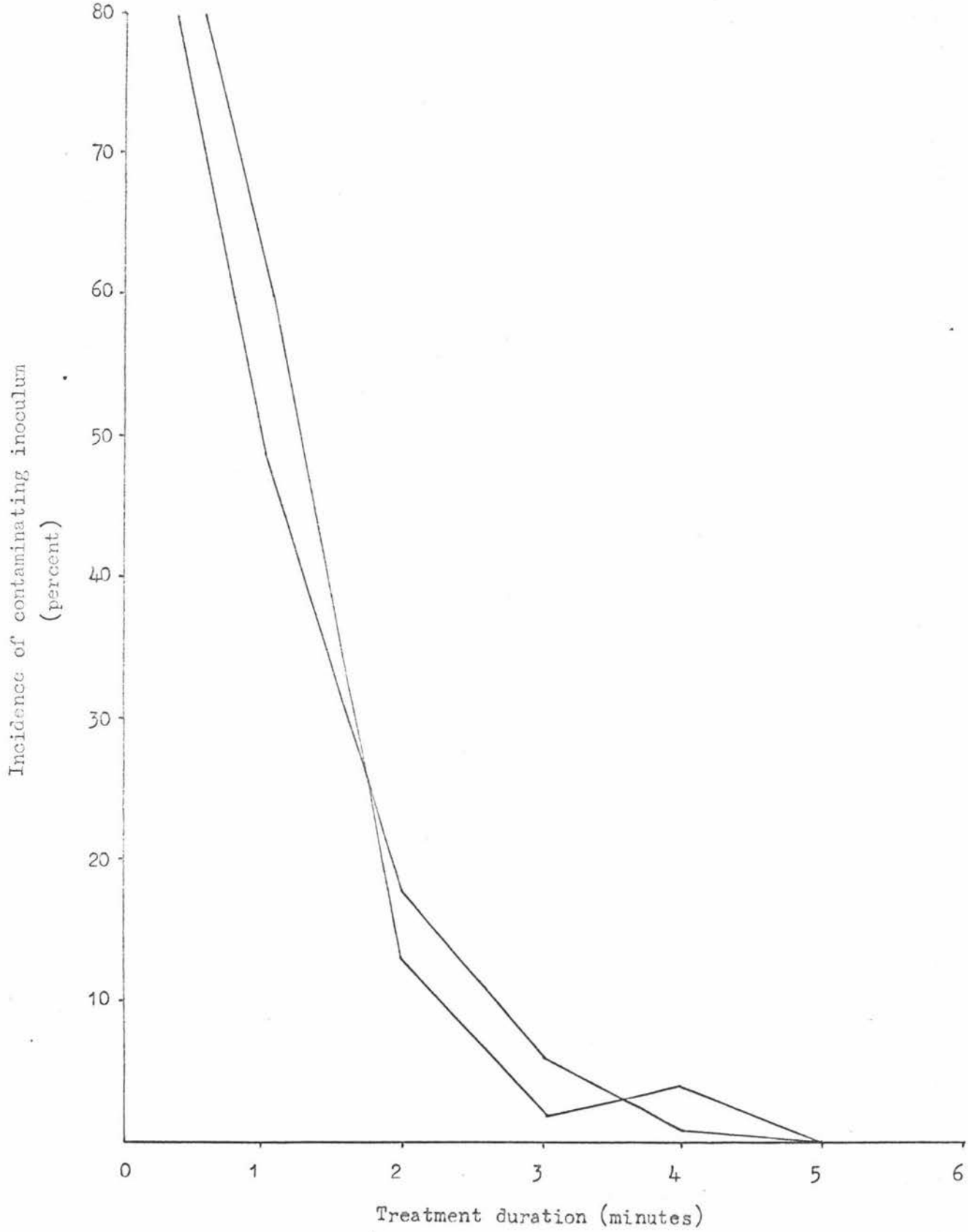


Fig. 5. Effect of surface sterilisation on viability of seed contaminating inoculum on P.D.A. (two replicates)

(ii) Application of the Method

Seed of a line which in a blotter test had revealed a 25 percent level of transmission of A. radicina, was subjected to mercuric chloride surface sterilization treatments of five, six and eight minutes, then plated to P.D.A.

As a result, four percent of seeds in each of the three treatments produced colonies of A. radicina indicating that some of the inoculum at least, was borne within the seed. This could be interpreted to mean that the remaining 21 percent of the inoculum was present as contaminating spores. However, the experiment on seed contamination had ruled out this possibility. A more plausable explanation was that the chemical treatment had eradicated mycelial inoculum established within the testa.

(b) Location of Seed Infecting Inoculum

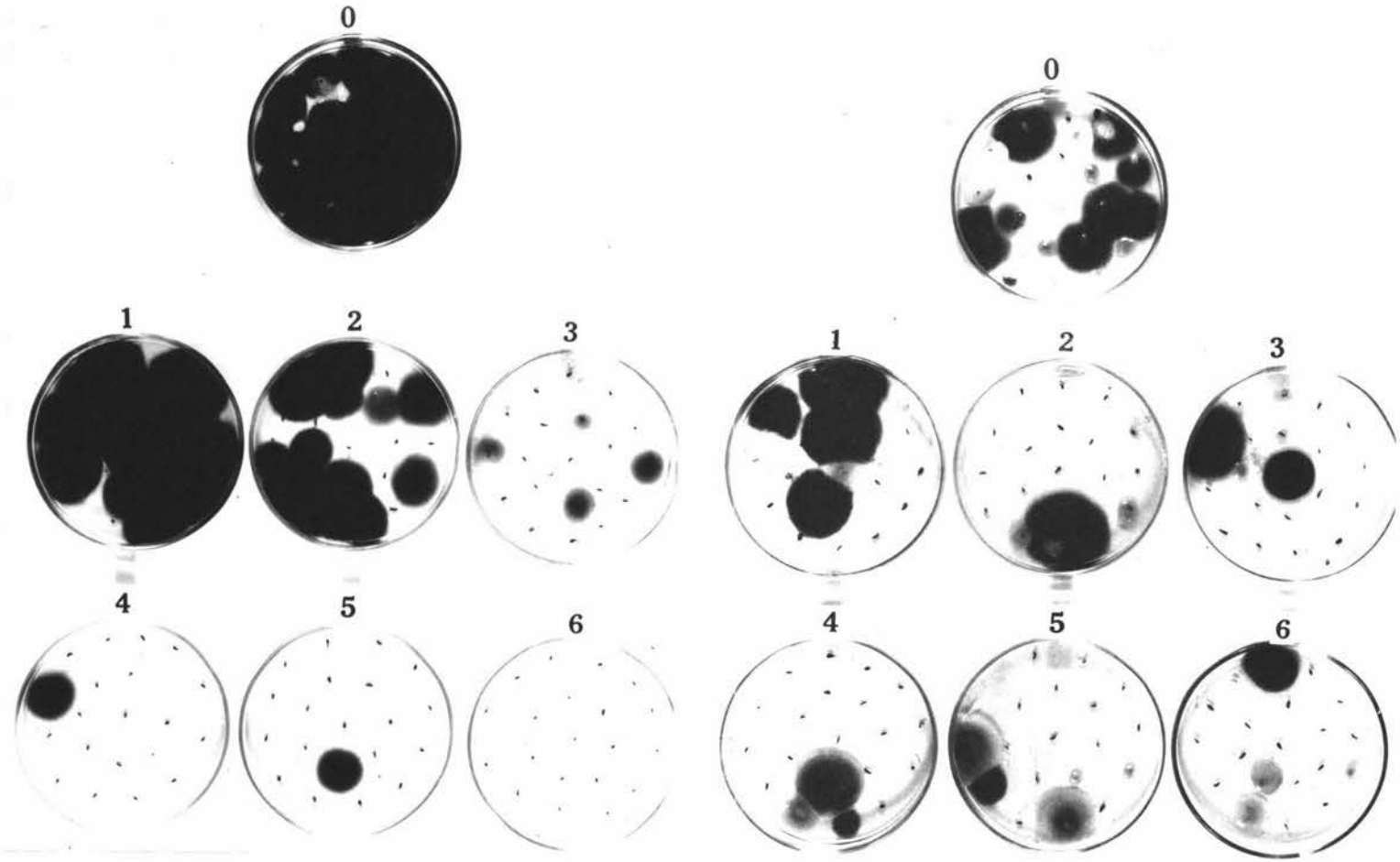
There are two possible broad locations of inoculum established within the seed tissues:

(i) Superficial infection of the seed coat

(ii) More deep-seated infection of endosperm and embryo

Netzer & Kenneth (1969) conducted experiments to determine the location and nature of carrot seed-borne A. dauci infection, using a microtome technique. By differentially staining the sections they found viable, thick-walled hyphae of A. dauci in the inner pericarp layer of seeds from infected seed lines. The general similarity between other seed pathology aspects of A. dauci and A. radicina , suggested that A. radicina could infect carrot seed in the same way. Since equipment for carrying out this type of experiment was unavailable, a more indirect

Plate 19.



(a) Contaminated seed. (b) Infected seed.

Effect of duration of surface sterilisation on level of seed-borne inoculum.

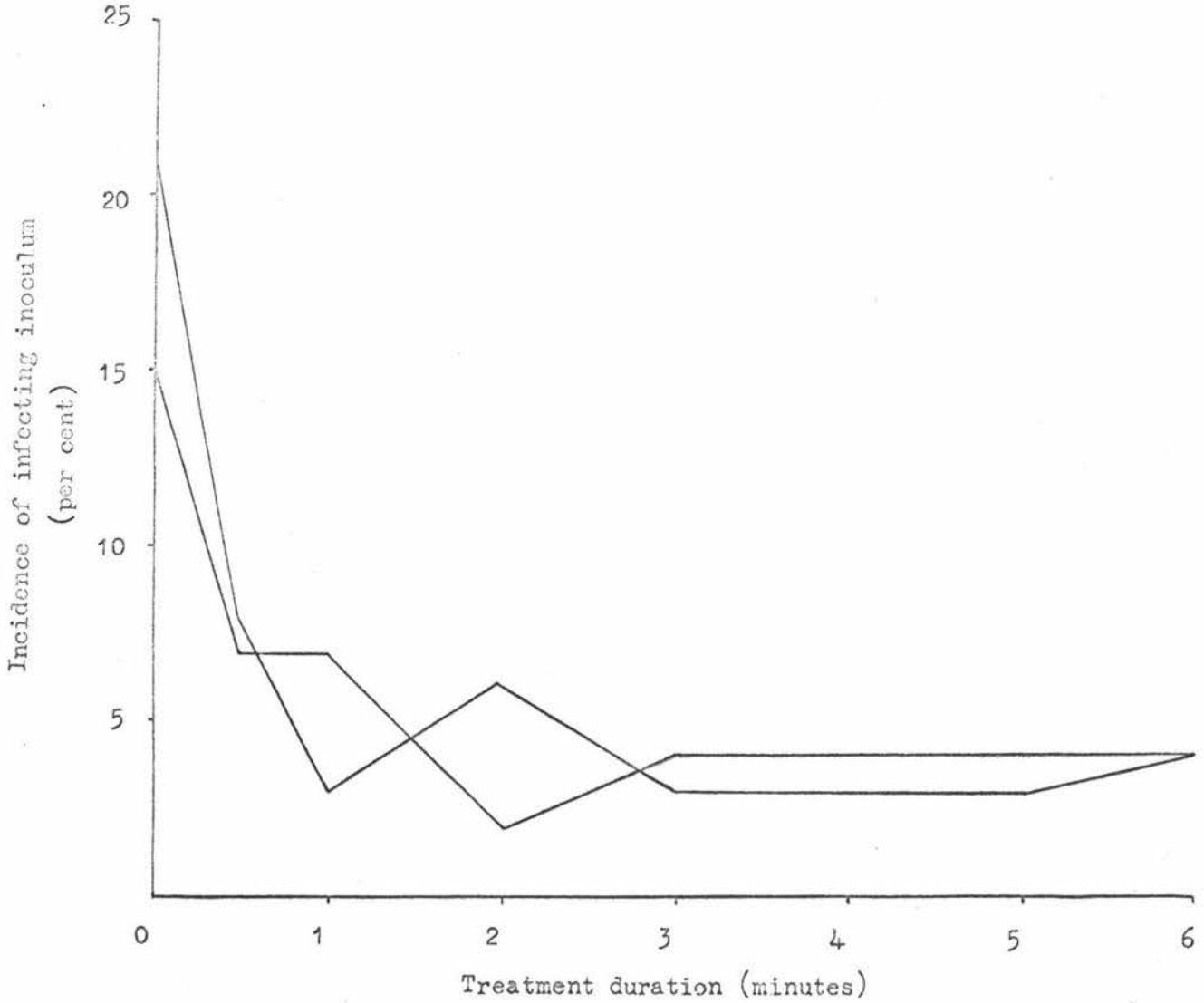


Fig. 6. Effect of surface sterilisation on viability of seed infecting inoculum on P.D.A. (two replicates)

method was adopted for determining the general location of seed infecting inoculum.

The ability of surface sterilisation treatments to eliminate seed-borne infection has already been demonstrated. It was argued that increasing durations of treatment would successively eradicate the infecting inoculum, according to its depth within the host seed tissues, and thus give an indication of its location. An experiment was conducted to determine the validity of his hypothesis, as follows:

Two gram samples of seed (line no. 4) were prepared for a series of treatments aimed at determining the effect of immersion time in mercuric chloride (0.1%) on the viability of infecting inoculum. The same immersion procedure was adopted as described in the previous section, and the seven time treatments used ranged between 30 seconds and six minutes. After immersion the samples were plated to P.D.A. at the rate of 20 seeds per plate, five plates per treatment (100 seeds per treatment). Colonies of A. radicina were readily identified after four days incubation at 24°C.

Results and Discussion

The results presented in Figure 6 and Plate 19b demonstrate that most of the inoculum was eliminated after relatively short surface treatments. This indicated that such inoculum was located relatively superficially within the seed coat tissues, although some still remained effectively more deep-seated, unaffected by the surface treatment.

These findings tend to support the findings of other workers. Doyer (1938) suggested that infection of carrot seeds by A. radicina remained superficial although Neergaard (1945) by the following state-

ment implied that quite deep seated infection occurred:- "there is a qualitative lowering of seed partly because the fungus kills a portion of the seeds and then lowers the germinating capacity of the lot (and) partly because many of the seedlings attacked in the form of damping-off". Noble, de Tempe and Neergaard (1959) referred to A. radicina as a "partially deep-seated" pathogen but did not substantiate this statement with any evidence. Various workers provided circumstantial evidence that infection remains relatively superficial. For example, Neergaard (1936), Mounce & Bosher (1943), Kristov & Raikov (1948), and Maude (1968), using soaks or surface treatment with various mercury based and organic fungicides, claimed complete or almost complete control of seed-borne A. radicina.

C. SIGNIFICANCE OF SEED BORNE-INOCULUM

With any seed-borne disease, inoculum associated with seed may be of significance in causing poor stand establishment and/or in providing primary infection foci for secondary spread.

A. radicina is most commonly known as the incitant of black rot of carrots, a disease of the mature carrot roots. Several workers have attributed the appearance of various pathological effects to the presence of seed-borne inoculum. Neergaard (1945) observed pre-emergence damping-off and various other workers (Jorgensen 1934, Doyer 1938, Mounce & Bosher 1943, Grogan & Snyder 1952, Maude 1968) observed seedling blight, both eventuating as a result of seed infection. In the literature there has been a tendency to study only a particular pathological effect of the pathogen without relating it to other pathological effects. There appeared to have been no thorough attempt to produce an account of the possible pathways of infection resulting from the use of infected seed.

Studies were therefore initiated to confirm experimentally that infected seed was potentially capable of producing different pathological effects on a carrot crop. More specifically this investigation was concerned with the potential for seed-borne inoculum to cause a reduction in stand establishment and cause seedling infection. On the basis of this investigation and the literature accounts, it was considered that enough information would be available to propose a scheme outlining the pathological effects of the fungus, and to suggest the pathways of infection existing between these effects.

1. Stand Establishment

The importance of seed-borne A. radicina infection causing a reduction in stand establishment can be considered under two main headings, depending on the stage of growth at which the pathogen caused host death, namely, pre-emergence damping-off and post-emergence damping-off. Preliminary studies, however, indicated that the pathogen was only important in causing pre-emergence damping-off under certain environmental circumstances.

Neergaard (1945) was the first to attribute poor carrot seed germination and seedling emergence to the presence of seed-borne inoculum. In the germinator damped-off seedlings were classified as "abnormal germs" and were not included in the germination figure. His experiments showed that the emergence of infected seed sown in autoclaved soil was lower than for uninfected seed. Grogan & Snyder (1952) also attributed germination failure to the presence of seed-borne A. radicina. They stated that "With the present trend toward precision planting, more attention must be given to seed-borne and soil organisms which cause seed failure or damping-off, such as A. radicina."

Pre-emergence damping-off can be divided into two sub-sections. The first, seed death before germination, is typified by seed infection of radish by A. raphani as described by McLean (1947). In that case the embryo was killed by the pathogen during maturation of the seed and the germination percentage could not be improved by seed treatment. Preliminary evidence indicated, however, that this situation did not apply to A. radicina infection of carrot seed, since certain seed-treatments were found to restore seed germination and emergence.

Furthermore there was no evidence in the literature to suggest that seed death before germination was a factor in pre-emergence damping-off of carrot seed.

Seed and seedling death at and after germination was considered to be the most likely alternative. In this case the pathogen, associated with seed in a dormant state, resumes activity under the favourable soil conditions, and may attack and kill the host seedling at any stage before 't emerges from the soil.

In view of these considerations an investigation was conducted to determine the potential effect of seed-borne A. radicina on germination and emergence of seedling carrots. More specifically the experiment aimed at determining if a relationship existed between the disease-free germination percentage and the percentage emergence in sterilised soil under glasshouse conditions favouring pathogen development.

(a) Materials and Methods

Three replicates of 200 seeds were sown in the glasshouse for each of an untreated, naturally infected sample, and a hot water treated sample of seed line No. 4. The seeds were individually sown in a sterilised peat-sand mix, one inch apart at a depth of half an inch, and the trays were kept well watered for the duration of the experiment. The final assessment of seedling emergence and seedling infection was made two weeks after the first seedlings emerged. Concurrently routine germination and disease level assessments were conducted on all seed samples. A soil temperature of 18 - 22°C was maintained throughout the experiment.

(b) Results and Discussion

Without conducting a test of significance it was obvious that seed infection with A. radicina caused a decrease in seedling emergence under conditions of high soil moisture content. (Tables 12 & 13)

Table 12 Blotter assessment of germination percentage and disease level.

	Infected Seed				Treated Seed			
	Rep 1	Rep 2	Rep 3	Average	Rep 1	Rep 2	Rep 3	Average
Infection Level %	86	85	85	85	86	87	86	86
Potential germination % *	28	21	22	24	1	0	2	1
Disease-free germination % *	58	64	63	61	85	87	84	85

* The potential germination percentage was determined by observing the number of seeds showing any signs of germination regardless of the presence of the pathogen. The disease-free germination percentage was calculated by subtracting the infection level from the potential germination percentage.

With reference to Table 13, it was concluded that a high level of seed infection (as assessed by the blotter method) was reflected in a correspondingly lower seedling emergence under the specified conditions of the experiment.

Table 13 Glasshouse assessment of seedling emergence

	Infected seed				Treated seed			
	Rep 1	Rep 2	Rep 3	Average	Rep 1	Rep 2	Rep 3	Average
Total Emergence %	64	67	69	67	84	83	84	84
Emerged and infected %	4	1	5	3	0	0	0	0
Emerged and disease free % **	60	66	64	64	84	83	84	84

** The emerged and disease-free percentage was calculated by subtracting the emerged and infected percentage from the total emergence figure.

An almost parallel situation was illustrated by Wallen & Cuddy (1960), and Wallen & Seaman (1963) investigating the influence of seed infection with Diaporthe phaseolorum (Cke. & Ell.) Sacc., on soybean seedling development. They found that the pathogen frequently killed its host during the germination and pre-emergence phases of host development. A certain amount of post-emergence damping-off and seedling blight was also found, indicating that under certain conditions the pathogen was less able to completely overtake and kill the emerging seedling.

In the current study little post-emergence damping-off was observed, because under the conditions of high soil moisture content in the glasshouse the pathogen killed the seedling before emergence. However, in Table 13 it was demonstrated that even under such conditions some infected seeds gave rise to infected, emerged seedlings. Casual observation revealed that such seedlings were occasionally overtaken and killed by the pathogen, but only under post-emergence conditions of

prolonged high humidity. In the field many emerged seedlings were infected by the pathogen, although only rarely were the seedlings completely killed by it. It appeared that unless the seedling was killed by the pathogen when it was very young (less than three weeks after emergence), then it survived to maturity virtually unaffected, but still showing signs of infection.

Conclusion

The potential for seed-borne A. radicina to cause stand reduction has been demonstrated and this occurs mainly during the post-germination, pre-emergence stage of development, when soil environmental conditions favour pathogen development. Depending on the level of seed infection and the soil conditions during germination, the amount of stand reduction may be considerable, although of little consequence when the seed is sown at higher seeding rates (sensu Wallen & Seaman 1963). However, with the adoption of precision sowing methods to obtain carrots of a particular size, the reduction in emergence caused by A. radicina could well affect the eventual yield and quality of marketable carrot roots. Control measures aimed at reducing the amount of seed-borne infection would therefore assist in making the results of precision sowing more predictable and reliable.

2 Seedling Infection

The occurrence of "seedling blight", caused by A. radicina, has been directly attributed to the presence of seed-borne inoculum (Maude 1966). With seedling infection it is presupposed that the pathogen persisted as a non-lethal infection on the developing seedling, whereas post-emergence damping-off is associated with the death of the seedling.

Although the existence of seedling infection has been demonstrated, its significance has received very little attention in the literature. Experiments were therefore conducted to confirm that seed-borne inoculum could give rise to infected seedlings, and if so, to determine the possible significance of such infection to the carrot crop. More specifically the investigation aimed at resolving the following points in a field situation;

The nature of seedling infection and its effect on host vigour,
The relationship between level of seed infection and level of seedling infection,
The secondary spread of infection from uninfected seedlings.

(a) Materials and Methods

Methods of ground preparation, layout and sowing are presented in Appendix III. For the purposes of this experiment four lines of carrot seed were used, as follows;

(i) An infected sample.
Seed-line No. 4 - It was the only line tested that contained a sufficiently high level of infection. i.e. 20 - 25 percent.

(ii) A hot water treated sample.
Seed-line No. 4 - Although complete pathogen elimination was not obtained using H.W.T. the infection level was reduced sufficiently for comparative purposes i.e. 1 - 2 percent.

(iii) A sample containing half the level of infection as in (i)

Seed-line No. 4 - This sample was prepared by mixing half infected seed and half H.W.T. seed. When tested on blotters it was found to be 10 - 13 percent infected.

(iv) An infection-free sample.

Seed-line No. 25 - Although of a different variety, this sample provided the necessary control as a check against the possibility of soil-borne inoculum, and to gauge the amount of disease spread between rows.

The occurrence and development of pathogenic symptoms were closely observed during the growth of the seedlings. Two assessments of infection percentage from each treatment were made by harvesting approximately 100 plants from a given length of row (two feet), and examining them for symptoms of infection. In the initial assessment, confirmation that those seedlings showing suspect symptoms were infected by A.radicina was made by subjecting a small lesioned tissue piece from each to 24 hours high humidity, and examining under a stereoscopic microscope for typical conidia. Further confirmation was made by isolating to agar.

All treatments were sown on 27/3/69, at a time when soil moisture content was insufficient for seed germination. Two days after sowing, however, heavy rainfall occurred, and although sufficient to stimulate germination, for another month the soil remained only just moist enough to support continued seedling growth. This was followed by an unusually dry winter and spring, although occasional periods of relatively wet weather produced conditions conducive to disease build-up and spread.

(b) Results

In spite of the dry conditions a good strike was obtained, resulting in a high density stand in all treatments. In contrast to the findings in the glasshouse trial, in the field there were no apparent differences in seedling emergence between treatments. This was attributed to the marked difference in seedbed moisture status between the two experiments, and its effect on the rate of development of the seed-borne pathogen.

(i) The nature of seedling infection

Symptoms typical of those described and illustrated by Maude (1966) were observed in some of the treatment plots about three months after sowing.

Plates 20&21 clearly illustrate the nature of the seedling infection most commonly found in the field. The cotyledons were first to show infection, followed by a gradual blackening of the older, outer petiole bases. A distinct circle of blackened petiole bases around the crown of the carrot resulted, but the infection remained relatively superficial. (Plate 20)

Only rarely were the younger, vigorous leaves infected by the pathogen; however the outer, moribund leaves, which had partially withered and fallen close to the infected petioles, frequently became infected. Except for a few small infected seedlings, suffering already from the effects of competition the pathogen did not appear to affect seedling vigour.

Plate 20.

(a)

(b)

Petiole base infection symptoms induced by A. radicina.

(a) An uninfected and an infected plant.

(b) Longitudinal sections of an uninfected and an infected plant.

Plate 21.

Petiole base infection symptoms.

Left - infected plants.

Right - uninfected plants.

(ii) The relationship between level of seed infection and level of seedling infection under field conditions.

The first assessment of disease incidence was made soon after disease symptoms became apparent in the early spring; that is, four months after sowing. It was considered that at this early stage, the incidence of field infection would have been more closely a reflection of the amount of seed-borne inoculum than later, after possible secondary spread of infection.

A correlation coefficient was calculated between the seed infection level and the average field infection level values from Table 14. A high positive correlation ($r = 0.99$) indicated a strong relationship between the two sets of observations.

(iii) The secondary spread of infection from infected seedlings

A second disease assessment was conducted two months after the first, after a period of relatively wet weather during which the pathogen had adequate stimulus and opportunity to disseminate, had it the capacity to do so.

Secondary spread of infection was detected in two ways:-

(a) as a build-up in disease incidence within rows: (within row spread).

(b) as a build-up in disease incidence in previously uninfected rows: (between row spread).

A comparison between figures in Table 15 and in Table 14, reveals a considerable build up of infection in all treatments during the two

months between assessments. The dense stands undoubtedly contributed to the amount of build-up in those treatments originating from infected seed, but the presence of infection in rows originally free from infection demonstrated the ability of the pathogen to spread further than to adjacent plants. Had the season been a normal wet one, the build-up of infection would probably have been even greater.

An examination of infected petiole bases and debris after periods of wet weather revealed the presence of abundant conidia of A. radicina. Rainsplash, wind and insects could each have contributed toward the dissemination of such inoculum both within and between rows.

Maude (1966) conducted similar experiments using infected seed, and likewise observed the occurrence and spread of petiole base infection in Autumn sown crops.

Table 14

The relationship between level of seed infection and level of seedling infection. First field assessment (19/8/69).

Treatment	Seed infection level percentage	Field infection level percentage			
		Rep. 1	Rep. 2	Rep. 3	Average
Infection-free seed (Control)	0	0	0	0	0
Hot-water treated seed	2	0	6	3	3
50/50% mixture H.W.T. & Dis. seed	13	15	19	15	16
Infected seed	24	33	27	30	30

Table 15

The relationship between level of seed infection and level of seedling infection. Second field assessment (13/10/69).

Treatment	Seed infection level percentage	Field infection level percentage Rep. 1	Rep. 2	Rep. 3	Average
Infection-free seed (Control)	0	3	8	5	5
Hot-water treated seed	2	10	11	9	10
50/50% mixture H.W.T. & Dis. seed	13	50	68	38	52
Infected seed	24	81	62	75	73

Discussion

The presence of petiole base infection alone appeared to be of little economic significance, although the blackened appearance of the infected crown could conceivably affect the consumer appeal of spring carrots which are sold with petiole bases still attached. This type of infection was noticed on carrots for sale in Palmerston North during October/November 1969.

Of greater potential importance concerns the observation that infected petiole bases constitute a substantial inoculum reserve for the infection of more mature plant parts. Lauritzen (1926) indicated that post-harvest infection on the sides of the roots originated as contamination from one or more of the following sources.

(i) from the washing of spores from the foliage to the soil and roots,

(ii) from contact with foliage at harvest,

(iii) from soil before and at the time of digging.

Soil-borne inoculum has been discounted as an important source of root infecting inoculum (Lang de la Camp, 1966). She demonstrated that drip inoculation of the crown with a conidial suspension was more successful than infection from the soil. Maude (1966) also found that "There was no infection of a ware crop grown on ground receiving an incorporation of infected carrot leaves and it seems unlikely that this source of disease is of commercial importance". From the literature accounts, therefore, it was assumed that inoculum originates mainly from above ground infections, although foliage infection is so uncommon that it cannot be considered capable of producing sufficient inoculum.

The findings of this investigation strongly implicate infected petiole bases as a potential inoculum source for infection of harvested carrots. It was frequently observed that infected petiole bases eventually fell to the soil surface causing infection of associated leaf debris. Under favourable environmental conditions large numbers of conidia were produced from such infected material, and it was thus postulated that the presence of such an inoculum reserve would unavoidably lead to the contamination of carrot roots during harvesting operations.

The occurrence of umbel infection has been observed by Grogan & Snyder (1952) and they considered it responsible for subsequent seed infection. The origin of the inoculum has not been fully investigated in the literature, although the findings of this study suggested that

petiole base and debris infection could theoretically account for eventual umbel and seed infection. The "bolting" umbel stem passes through a circle of petiole bases and would undoubtedly be capable of picking up a certain amount of infection as it did so. As well, progressive spread of infection from the base of the petiole stem to the umbel itself could take place during favourable environmental conditions.

Conclusion

In so far as petiole base infection occurs as a result of seed infection, it follows that black rot and umbel infection could possibly develop indirectly as a result of seed infection. Figure 7 is a scheme outlining the types of infection caused by *A. radicina* and the possible pathways of infection connecting them.

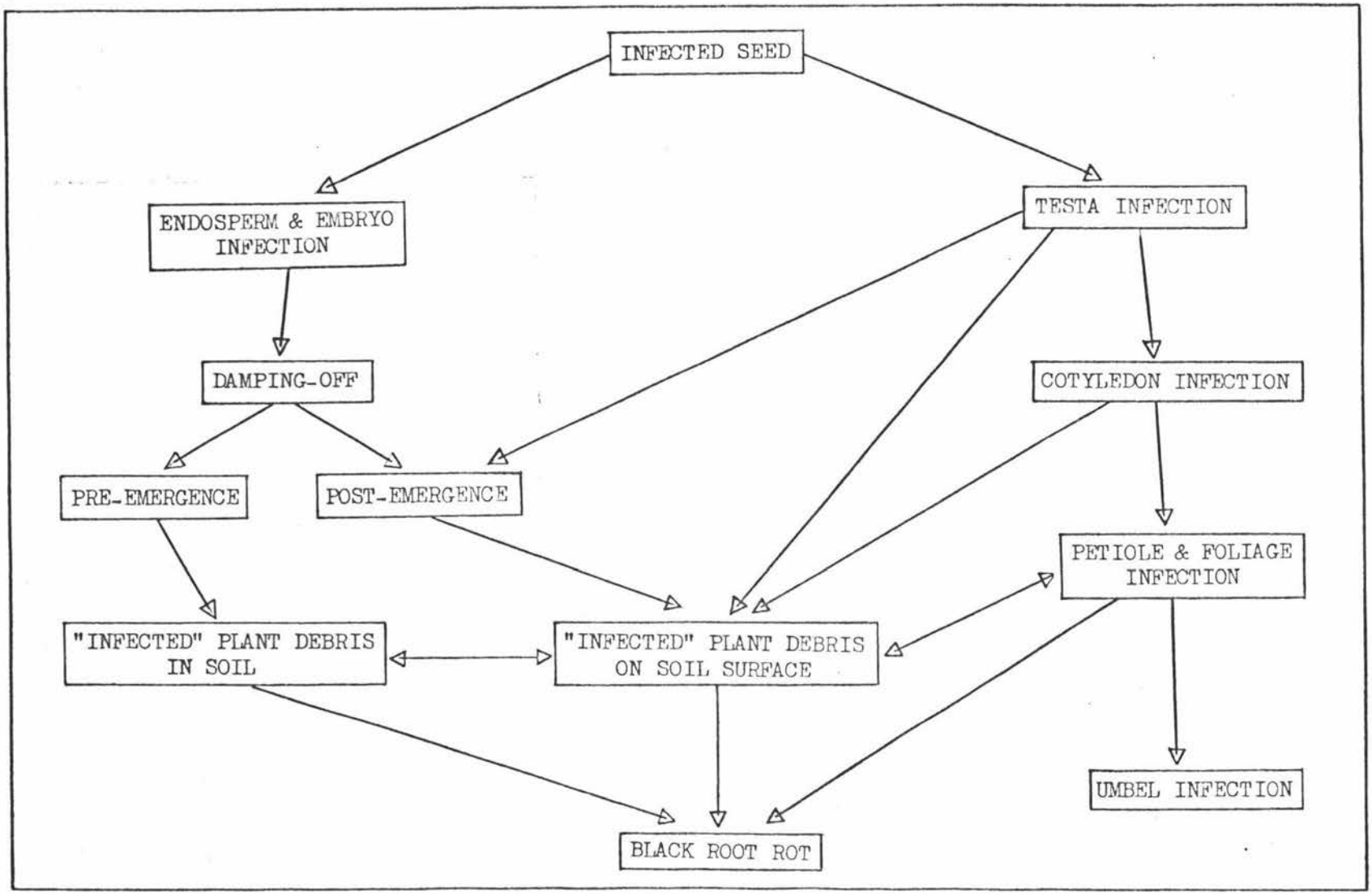


Fig. 7. Pathways by which seed, infected with A. radicina, could give rise to various pathological effects of carrot.

D. CONTROL

1. Introduction

The main emphasis in the literature has been placed on the use of various chemical seed treatments for the control of A.radicina infection of carrot seed. Doyer (1938) first suggested that since A. radicina infection remained superficial it might be controlled by chemical seed treatment. Subsequently a number of workers (Mounce & Bosher, 1943; Neergaard, 1945; Khristova & Raikov, 1948; Weber et al, 1954; Rusakova & Raskin, 1955; Grogan & Snyder, 1952; and Maude, 1966) using short soaks in or surface treatment with various therapeutants claimed complete or almost complete control of seed-borne A. radicina, without causing depression in germination or emergence. Roberts (1956) and Beaumont (1959) also achieved control of the pathogen using hot water treatment.

The control of seed-borne diseases centres around two basic approaches, namely; the prevention of the pathogen becoming seed-borne in the seed crop, or if that is not economically possible, the eradication of the seed-borne inoculum from the seed.

(a) Prevention of the pathogen becoming seed-borne

Ideally, if New Zealand seed importers could reliably obtain uninfected seed from overseas, the problem of controlling A. radicina would be solved. The production of disease-free seed overseas would involve growing the seed in arid areas where the environment does not favour above-ground infection, or spraying seed crops with fungicide to prevent them becoming infected.

In the U.S.A. effective control measures have apparently been

adopted where carrot seed is grown, because seed lines screened from this source were found to be almost completely free from both A. radicina and A. dauci. (Table 8)

(b) Eradication of the seed-borne pathogen

Since New Zealand growers have no direct control over the production of carrot seed the only method of ensuring disease-free crops is by eradicating the seed-borne pathogen when it occurs. Conceivably, this may be achieved by ageing the seed or subjecting it to some form of treatment.

(i) Ageing the seed

It is possible that during a period of storage infected seed could retain its germination, whereas the pathogen would die out, resulting in a measure of control. For example, Wallen & Seaman (1963) demonstrated that there was a marked decrease in the viability of Diaporthe phaseolorum during two years of storage of infected soybean seed. Similarly Krout (1921) showed that conidia and mycelium of Septoria apiicola lost their viability in three years whereas celery seed retained its germination capacity during the same period. Since no work of this nature had been reported for A. radicina, an experiment was conducted over a period of time to determine the viability of the seed-borne pathogen in relation to seed germination capacity.

Two lines of seed which were found in the initial seed screening tests to be infected with A. radicina were examined for their viable pathogen content and germination over a period of 18 months. Two hundred seed from each of the two lines were concurrently subjected to

health and germination tests, at each interval, using the previously described methods.

Table 16 Effect of duration of storage on percentage infection and seed germination

		March '68	June '68	Sept '68	Dec '68	March '69	June '69	Sept '69
Seed line								
4 -	Germination	83	87	84	89	85	86	89
	Infection	25	21	27	27	23	26	25
Seed line								
5 -	Germination	93	90	94	92	89	88	90
	Infection	3	3	6	5	4	3	6

Results are presented in Table 16 and indicate that no significant decline in either infection level or germination had taken place in the 18 months during which the seed lines were being tested. According to the details accompanying the seed lines, they were apparently harvested within one year before the first assessment was carried out, therefore making them up to two and a half years old when the final assessments were made.

On the basis of the results obtained the possibility of using seed ageing as a method of disease control was discounted.

(ii) Seed treatment

The effectiveness of various seed treatment techniques (such as hot water treatment, therapeutant soaks and dusting treatments) was examined in detail since they appeared to offer the best prospects for

the control of A. radicina and other carrot seed-borne pathogens. For this reason seed treatment will be dealt with separately in the following section.

2. Seed Treatment

Seed treatment refers generally to the use of certain physical or chemical agencies to eliminate seed-borne pathogens without affecting germination capacity of the seed.

From studies described in Section B of this chapter, inoculum of A. radicina was shown, indirectly, to be seed-borne as a predominantly superficial, testa infection. Depending on the prevailing environmental conditions, infection was found to be of significance in causing pre-emergence damping-off, and also in providing primary infection foci for relatively limited secondary spread. On the basis of these findings it was decided firstly, to examine the recorded methods of seed treatment for A. radicina and secondly, to make a comparison of the effectiveness of the methods in greenhouse and field sowings.

(a) Examination and Modification of Recorded Methods of Seed Treatment

There is a considerable amount of literature relating to various methods of treatment of carrot for seed-borne pathogens and in some cases the accounts given are short, with incomplete detail of how the treatments were experimentally selected and tested for effectiveness. For this reason a critical examination was made of various recommended treatments; these included hot water treatment, therapeutant treatments, and water soak treatment.

(i) Hot Water Treatment

Baker (1962) presented some ground rules and generalisations concerning thermotherapy of planting material, in which he outlined the possible causes of pathogen death from high temperature treatment, and the type of host injury sustained. With his work as a guideline the whole question of hot water treatment of carrot seed for A. radicina control was investigated.

Bant et al (1950, 1952) developed a hot water treatment for the control of fungal pathogens in broccoli and celery seed, and this technique was considered by Roberts (1956) as a possible method of eliminating A. radicina infection from carrot seed. Roberts investigated the effect of two hot water treatments on the infection level on one carrot seed line and its effect on the germination percentage of the same line and another uninfected one.

His results are summarised in Table 17. The germination percentage of both seedlines was reduced as a result of hot water treatment, although the more severe treatment caused the greater reduction. Both hot water treatments reduced the level of seed infection, but only the less severe one appeared to eliminate infection. The reason for this is difficult to account for, although it is suggested that insufficient replicates were used in the investigation.

Beaumont (1959) claimed that A. radicina was controlled by a hot water treatment of 50°C for 25 minutes, but he provided no evidence to support this.

Table 17 Effect of hot water treatment on level of infection and seed germination percentage (Roberts 1956)

Treatment	Infected Seed Line		Seed Line Free from Infection
	Germination percentage	Infection percentage	Germination percentage
25 minutes at 122°F (50°C)	11	0	23
30 minutes at 125°F	10	1	11
Untreated Control	50	15	41

Because of the lack of substantiated evidence the effect of different temperature / time combinations on pathogen and host viability was examined. The experiment, as a whole, was divided into two sections, the first trial investigated the effect of hot water on the viability of seed-borne inoculum: the second dealt with its effect on seed germination.

Trial 1. The effect of selected hot water treatments on viability of seed infecting inoculum.

One line of seed (number 4), found in the preliminary screening tests to be relatively heavily infected with A. radicina (25%) was used to determine the effect of hot water treatment on the viability of seed infecting inoculum. Thirty, one gram samples of this line were enclosed in small muslin bags ready for immersion in the various hot water treatments. The thirty combinations of the following temperatures and times were used as treatments:

temperature	44°, 46°, 48°, 50°, 52°, 54°C
time	15, 20, 25, 30, 35 minutes

After immersion in the hot water bath, each sample was cooled by dipping briefly in cold water, then dried at room temperature between two sheets of blotting paper. One hundred seeds per sample were laid out on standard germination blotters, one seed per square, and then incubated in a Copenhagen germinator at 20°C for ten days. After this time the level of infection in each sample was recorded.

The results are presented in Figure 8 and can be summarised as follows:

- : All treatments caused a reduction in the amount of viable seed-borne inoculum.
- : The treatment recommended by Roberts (1956) (50°C for 25 minutes) substantially reduced, but did not eliminate, infection.
- : A temperature of 50°C appeared to be most critical for the pathogen. Treatment at this temperature for 30 minutes and longer eliminated infection.
- : Treatments of 52°C and 54°C for 20 minutes and longer effectively eliminated infection.

Trial 2 The effect of selected hot water treatments on seed germination.

Baker (1962) suggested that certain heat treatments may delay or reduce germination, thus affecting the planting value of a seed line. He listed such host factors as variety, age, size, vigour, moisture content of seed that may affect susceptibility of a particular seed line to hot water

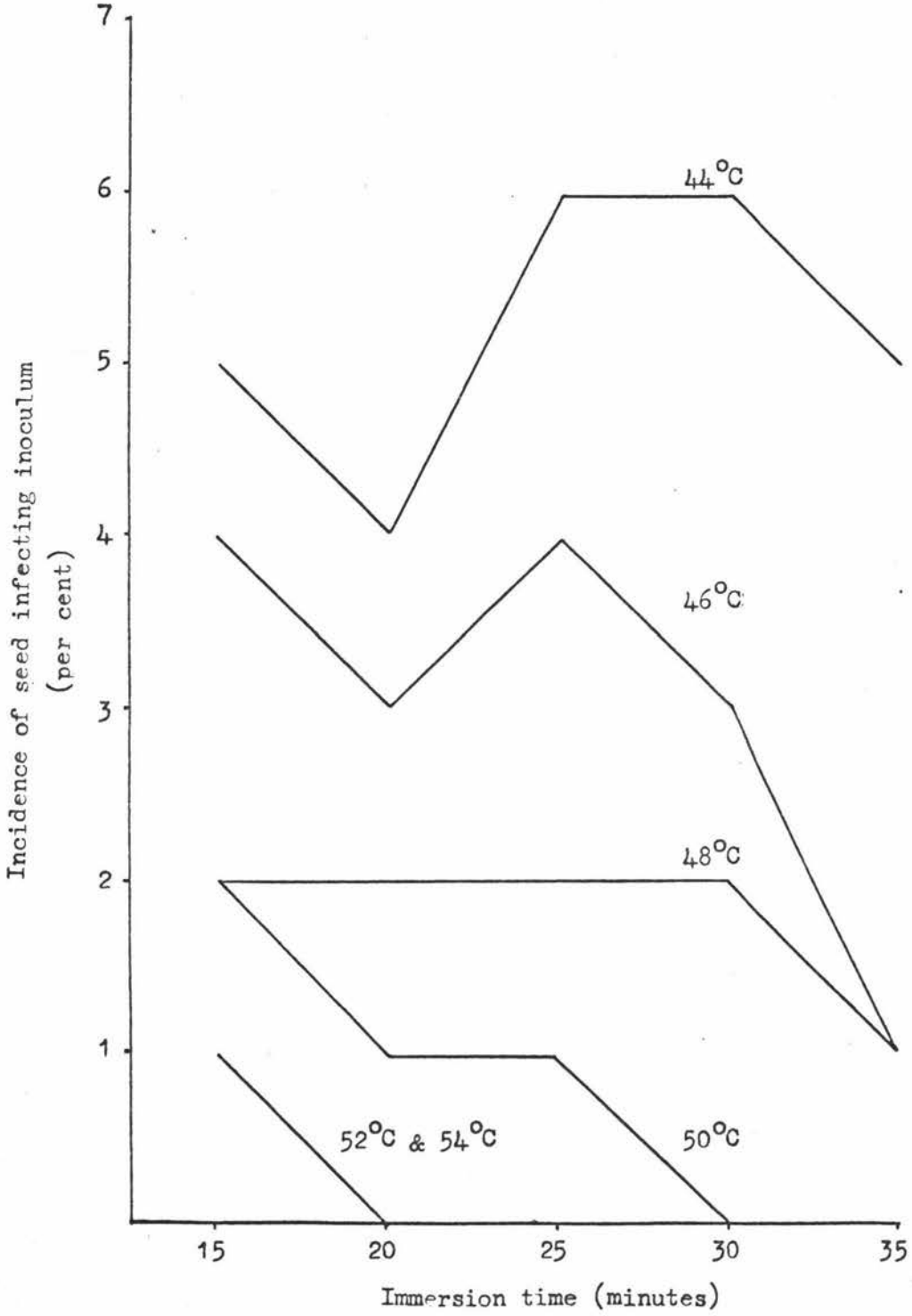


Fig. 8. Effect of selected hot water treatments on viability of seed infecting inoculum.

therapy. For this reason it was necessary to use three lines of carrot seed representing different varieties and different germination percentages, as follows:

line 4	(infected line) (as in Trial 1)	germination	85%
line 13	(uninfected line)	"	80%
line 44	(uninfected line)	"	87%

Exactly the same procedure as in trial 1. was adopted for treating and subsequent laying out of the samples, although in the case of the uninfected lines the seeds were placed four per testing square. (With regard to line 4, trials 1 and 2 were conducted concurrently.)

Germination assessments were made after four and seven day's incubation to detect any effect on rate of germination as a result of hot water treatment. Seeds exhibiting any evidence of germination were included in the germination percentage.

The results for the three lines are presented respectively in three tables (Appendix IV) and are summarised in Figures 9, 10 & 11. Assessments for 20 and 30 minutes immersion time have been omitted from the figures because it was considered that the 15, 25 and 35 minute observations alone satisfactorily showed the trend of treatment effects.

Except for line 44, temperatures up to 48°C did not markedly retard or reduce germination. From 50°C to 54°C germination was retarded when in combination with the 15 minute immersion treatment, and was progressively reduced as the duration of treatment increased to 35 minutes.

Ideally a treatment should completely eliminate the seed-borne inoculum without affecting germination of the seed. Certain treatments

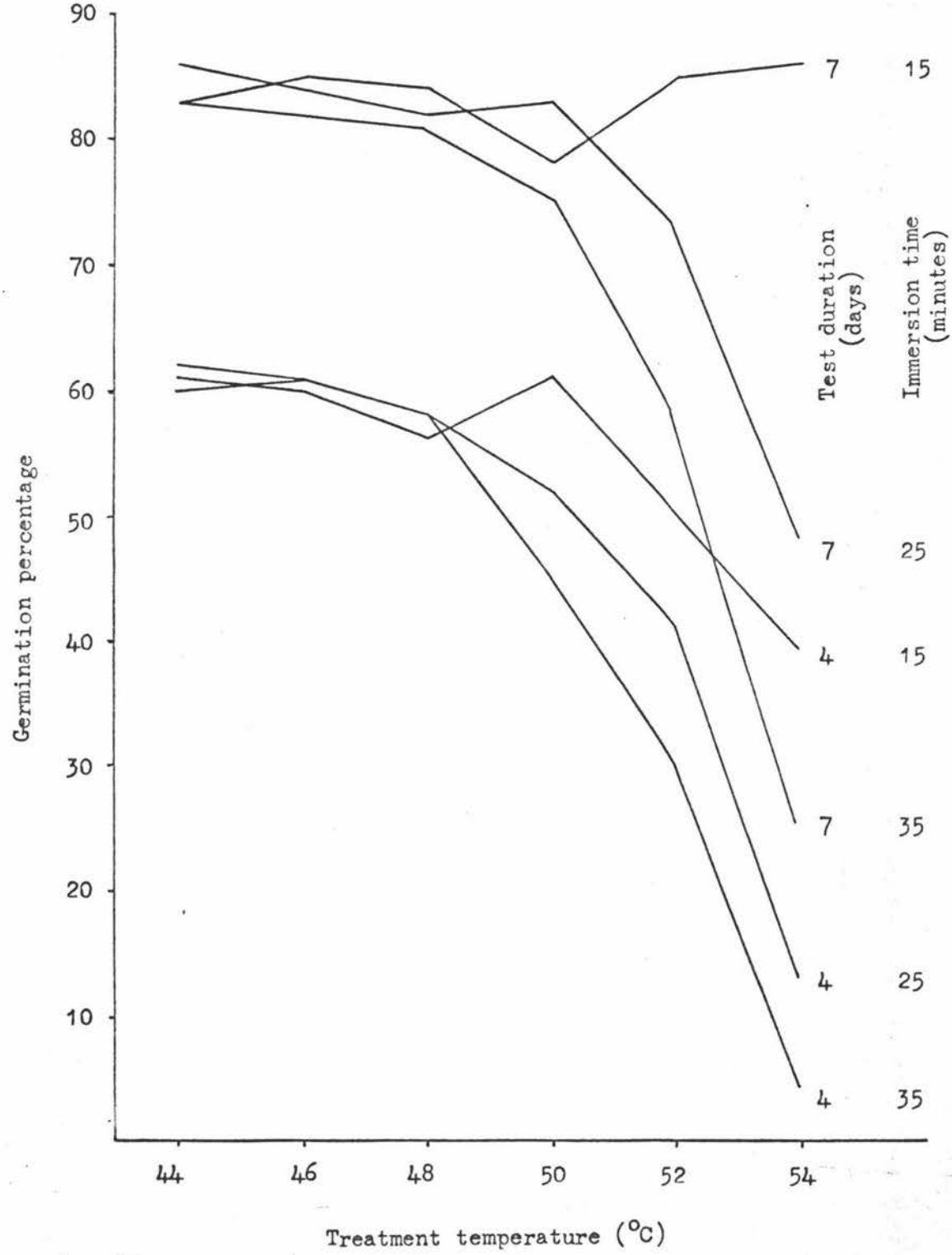


Fig. 9. Effect of selected hot water treatments on germination of carrot seed (seed-line No. 4).

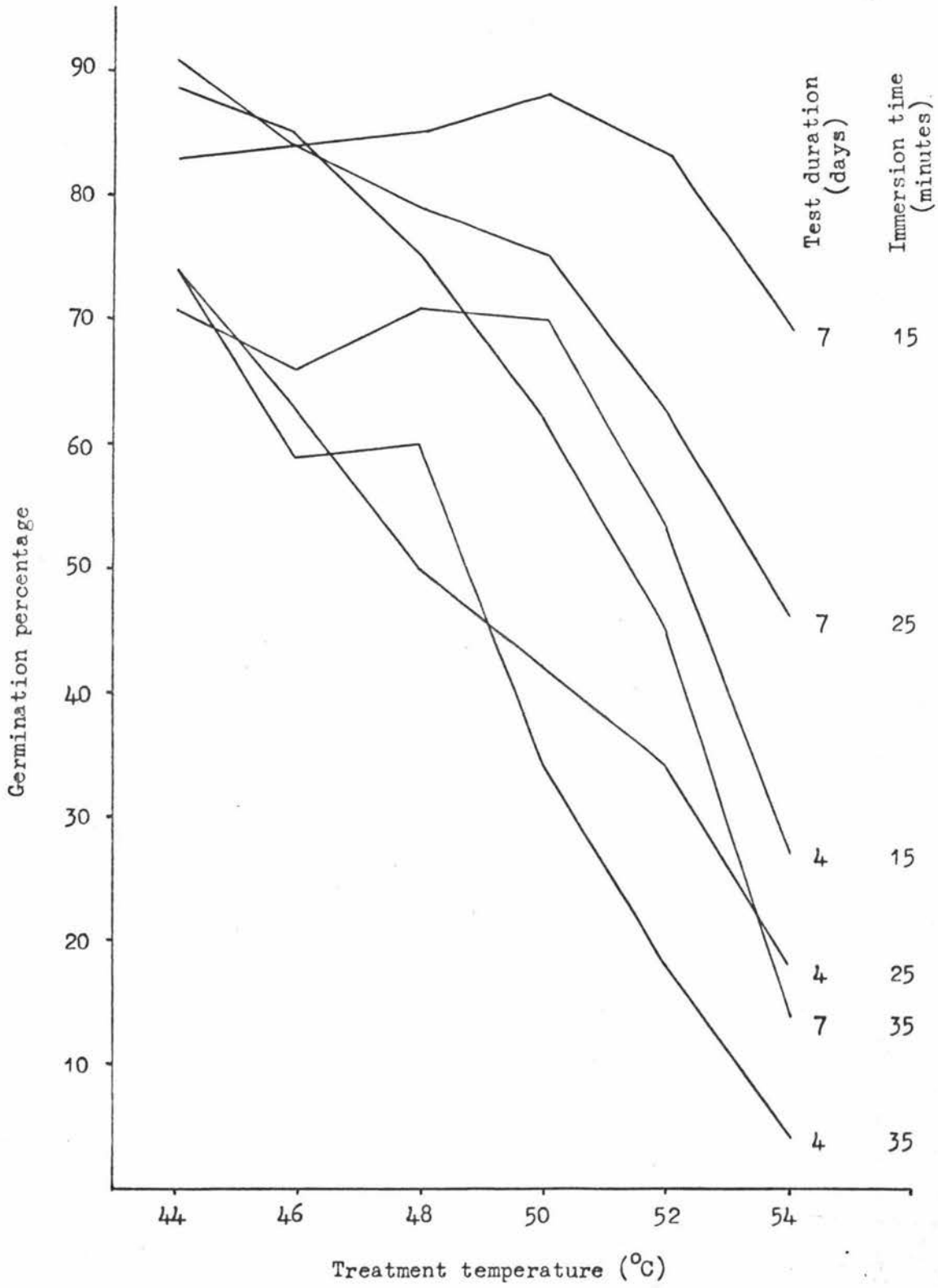


Fig. 10. Effect of selected hot water treatments on the germination of carrot seed (seed-line No. 13).

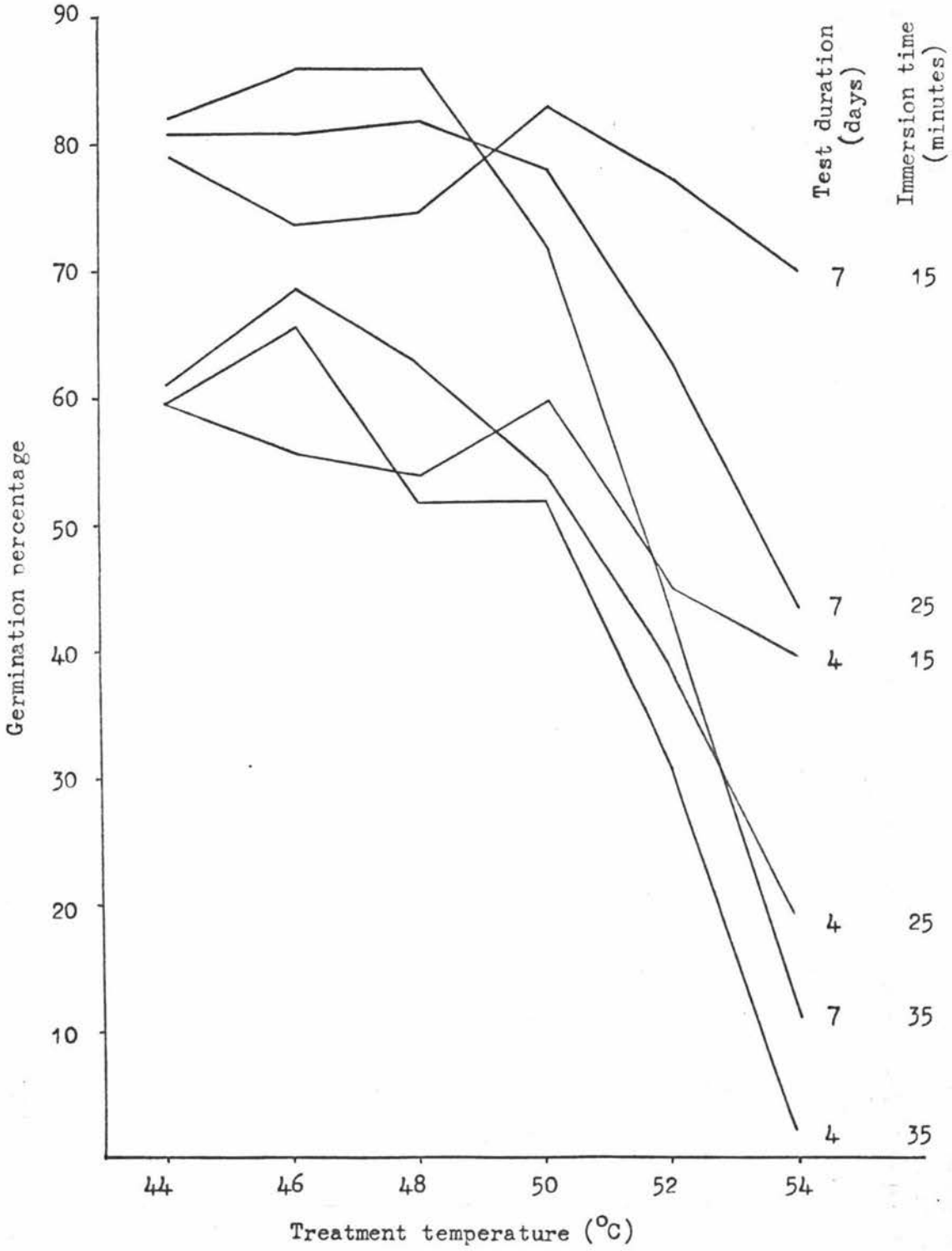


Fig. 11. Effect of selected hot water treatments on the germination of carrot seed (seed-line No.44).

which completely eliminated the pathogen from seed line four (selected from Figure 8) were thus assessed in relation to the delay or reduction in germination they produced in the three seed lines examined.

Table 18

Effect of selected hot water treatments on seed germination

Treatment	Percentage Reduction in Germination *				Percentage Delay in Germination **
	Line 4	Line 13	Line 55	Average	Average
50°C for 30 minutes	3	0	16	6	7
for 35 minutes	8	2	25	12	10
52°C for 20 minutes	15	0	21	12	11
for 25 minutes	17	3	25	15	12
54°C for 20 minutes	30	14	42	29	15
for 25 minutes	38	23	41	34	13

* The reduction in germination capacity of the seed, attributable to seed treatment (that is, the amount of seed actually killed by the treatment), was calculated by subtracting the seven day germination figure in the treated line from the seven day germination figure in the untreated control.

** The difference between the reduction in germination at four days and the reduction in germination at seven days was regarded as a measure of the delay in germination caused by the treatment.

Table 18 showed that although certain hot water treatments eliminated the pathogen they also caused a delay or reduction in

germination. As was expected, the magnitude of such effects was dependent on the severity of treatment and the lines of seed used. With this taken into consideration, a hot water treatment of 50°C for 30 minutes was selected as being most suitable because of those treatments giving complete elimination of seed-borne A. radicina, it least affected germination.

(ii) Therapeutant treatments

Therapeutants are commonly used for the control of seed-borne pathogens and their effectiveness depends largely on the methods of application and the nature of the seed-borne inoculum. The methods of application can be divided into two broad categories, as follows:

Seed dressing: The seed is either "dusted" with a wettable preparation of the therapeutant or dipped in a slurry containing the preparation and both aim at eliminating surface-borne (contaminant) inoculum. For example, covered smut of wheat (caused by Tilletia caries (DC) Tul.) has been effectively controlled in this way.

Therapeutant soaks: The seed is immersed in a solution or suspension of the therapeutant so that it may "penetrate" the seed and to some extent and eradicate infecting inoculum confined to the outer layers of the seed. (e.g. Septoria apiicola Speg.)

The use of various therapeutants applied as dressings or as soaks for the control of seed-borne A. radicina and A. dauci has been advocated by some workers. Khristova & Raikov (1948) claimed complete control of A. radicina "on heavily infected carrot seed" using granosan and ceresan dusts. Maude (1966) conducted a comprehensive glasshouse trial in which mercury based seed-dressings and short soaks in ethyl

mercury phosphate (E.M.P.) were compared mainly with a thiram (Tetramethylthiuramdisulphide) seed dressing (50% active ingredient) and with soaks in thiram suspensions (0.2%) for 24 hours at 30°C. He found that all treatments significantly reduced seedling infection, although the E.M.P. soak severely reduced germination. Only the 24 hour soak at 30°C in 0.2% thiram suspension appeared to give complete control without a depression of emergence.

In this study it was decided to check the effectiveness of Maude's "thiram soak" technique for eliminating seed-borne A. radicina using seed-line number four, known to transmit a high level of infection (25%). Samples of infected seed were loosely lied in muslin bags and immersed for 24 hours in a suspension of thiram (0.2%) held at 30°C. The treated seed was subsequently dried and subjected to a germination and infection assessment.

Table 19 Effect of "thiram soak" treatment (sensu Maude) on level of seed infection (seed line 4)

	Infection level (percent)	
Replicate No.	Treated seed	Untreated seed
1	1	24
2	2	20
3	2	21
4	1	23
5	3	27

This experiment was replicated five times but in no instance was complete control achieved (Table 19). The level of infection was reduced in each instance to between one and three percent, and the germination remained unaffected.

(iii) Water soak treatments

Certain overseas results have pointed to the possibility of controlling seed-borne A. radicina by soaking infected seed in water. Firstly, Arny & Leben (1956) demonstrated that water soaking oats and barley seed for 56 hours at 24 - 26°C markedly reduced Helminthosporium seedling blights in the subsequent crops. By plating treated and untreated seed to agar they found that species of Alternaria, present as contaminants on the seed, were also markedly reduced by the water-soaking. Maude (1966) included in his investigation into methods of control of A. radicina a water-soak treatment of 30°C for 24 hours to compare with his thiram soak method. He found that water soaking alone caused a slight reduction in infection. Thirdly, Limonard (1968) showed that seed-borne A. radicina was susceptible to the wet blotter effect, a situation where an increase in the water content of the testing substrate reduces the apparent level of infection.

On the basis of the above considerations an experiment was conducted to determine whether or not the duration of immersion in water would affect the viability of seed-borne A. radicina.

One gram samples of infected seed (seed line four) were loosely tied in muslin bags ready for immersion. Two constant temperature water baths were set up, one at 20°C and the other at 30°C, and the seed samples immersed in them for between 15 seconds and 45 hours. After the

duration of treatment, each sample was removed from the water, shaken free of excess water and allowed to dry overnight between two sheets of blotting paper. From each treatment two representative samples of 100 seeds were subjected to an infection and germination assessment using standard testing blotters in the Copenhagen germinator.

The results of this experiment are presented in Figure 12 and for convenience the time intervals used were converted to a logarithmic scale. Soaking in water at 20°C for up to three hours caused an increase in apparent infection level, followed by a steady decline to 2.0% after 45 hours. The reason for the initial increase in level of infection has not been resolved, and the phenomenon obviously requires further investigation.

In the 30°C treatment no appreciable increase in apparent infection level was observed. A decline was observed beginning after one hour of water soaking, and after 45 hours the inoculum appeared to have been eliminated. This is in contrast to the findings of Maude(1966) who showed only an insignificant decline in infection after 24 hours soaking. The discrepancy suggests that inoculum may vary in its accessibility and susceptibility to treatment depending on the particular host/pathogen relationship. (For example, depth of infecting inoculum within the host tissues.) Until other seed-lines have been studied the use of water soaking as a means of control cannot be recommended. The results, however, suggest that part of the eradicating action of Maude's thiram soak treatment could be attributed simply to a water soak effect. Germination percentage was unaffected by treatment.

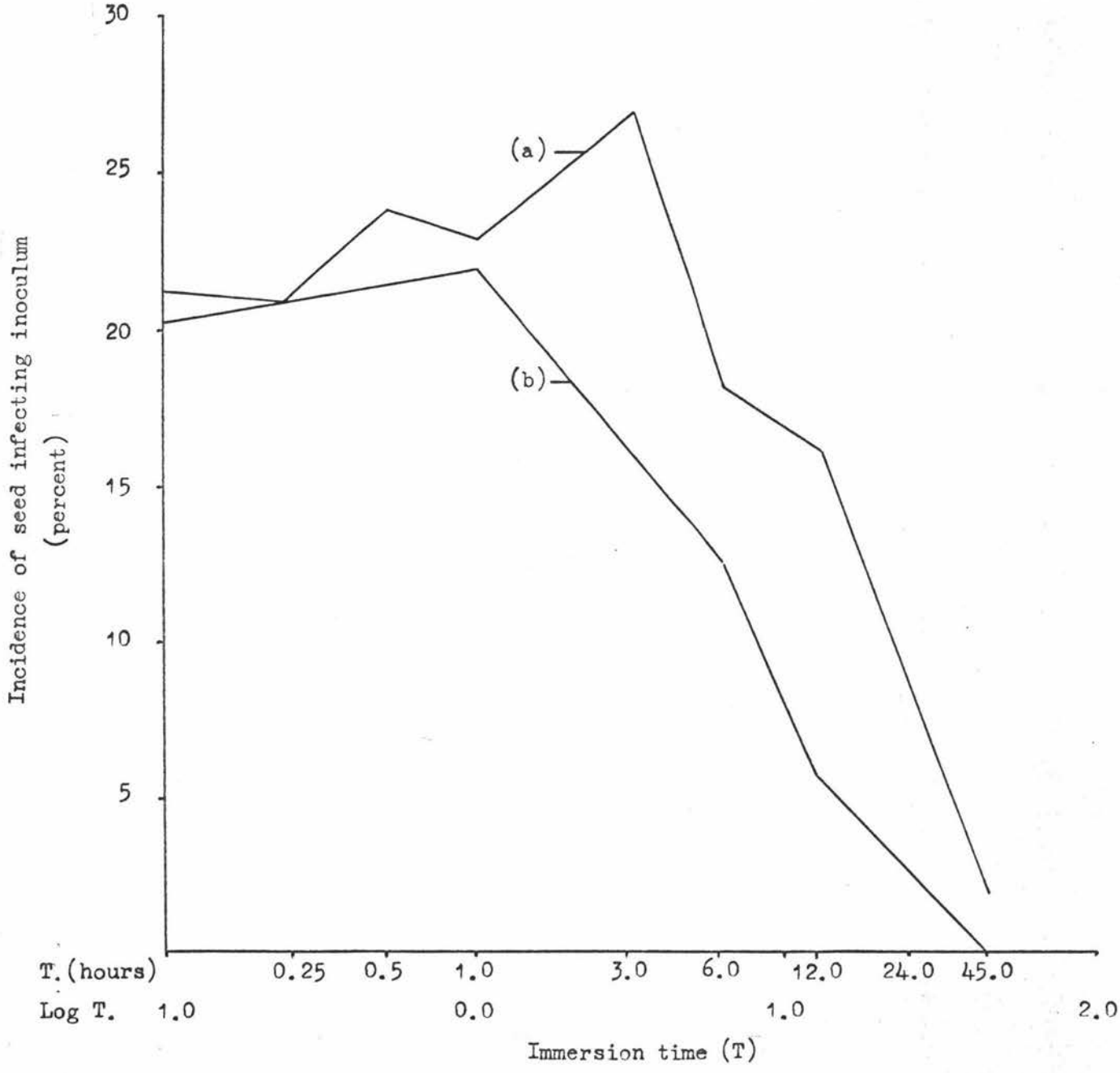


Fig. 12. Effect of duration of water soaking on viability of seed infecting inoculum. (a) water temperature 20°C.
(b) water temperature 30°C.

(b) Comparison of Methods of Seed Treatment

Although results of the previous section provided an indication of the absolute effectiveness of certain seed treatments, the need to compare them under glasshouse and field sowing conditions remained. The criterion of effectiveness depends on the type of pathogenic effect arising from the use of seed infected with A. radicina. In this connection it has been demonstrated that under moist glasshouse seedbed conditions seed infection causes pre-emergence damping-off whereas under dryer field conditions petiole base infection was important. The findings of the experiments described in the previous section were unknown at the time the glasshouse and field trials were initiated. For this reason treatments based on the recommendations of other workers were adopted, as follows:

Hot Water Treatment	50°C for 25 minutes (sensu Roberts, 1956)
Thiram soak treatment	0.2% thiram suspension at 30°C for 24 hours (sensu Maude, 1966)
Thiram dust treatment	1 oz. per 14 lbs seed (sensu Maude, 1966)
Benlate dust treatment	1 oz. per 14 lbs seed

Seed-line number four was the only one sufficiently infected (25%) on which the effectiveness of seed treatment could be determined.

(i) Glasshouse Trials

It has already been shown that seed infection with A. radicina can cause poor emergence under moist glasshouse seedbed conditions. In this experiment the objective was to confirm that certain seed treatments could improve the emergence of carrot seedlings by eliminating some or all of the seed infecting inoculum.

Seedbeds were prepared using sterilised peat-sand mix (Baker 1957) and 200 seeds per treatment were sown approximately one inch apart and half an inch deep. Throughout the experiment the seed beds were kept well watered by placing the trays in a shallow water bath. Overhead watering was avoided.

The relative effectiveness of each treatment method was assessed by comparing the percentage emergence of seedlings observed two weeks after the first seedlings emerged. The results of two replication's of this experiment are presented in Table 20.

Table 20 Effect of seed treatment on emergence of infected seed in the glasshouse.

Treatment	Percentage Seedling Emergence	
	Replicate 1	Replicate 2
Hot water	79.0	84.0
Thiram soak	84.5	81.5
Thiram dust	82.5	87.0
Benlate dust	69.0	72.5
Infected seed (control)	69.5	68.5

The results indicate that all the treatments used except benlate dust, equally improved the emergence of infected seed under the prevailing glasshouse conditions. Of those treatments which were effective against pre-emergence damping-off, seed dusting with thiram was undoubtedly the most attractive from a commercial growers point of view, since the method

is relatively cheap and easy to use.

(ii) Field Trial

The occurrence and build-up of petiole base infection was demonstrated in section C.2. of this chapter and shown to result from the sowing of seed infected with A. radicina. In conjunction with that experiment an investigation was conducted to determine the relative effectiveness of different seed treatments in reducing the amount of petiole base infection in the field. The treatments used were the same as those in the previous experiment, namely, hot water, thiram dust, thiram soak, and benlate dust, with an untreated infected sample as control.

Details of the cultivation, and sowing of the field trial are outlined in Appendix III. Three replicates of each treatment were sown and subsequent disease assessments were made in the same way as, and in conjunction with those described in section C.2. of this chapter.

The results of two assessments are presented in Table 21. The first assessment was conducted on 18/8/69 and the figures given are the averages of two replicates in each treatment. The second assessment was conducted on 13/10/69 and the figures given are the averages of three replicates.

Table 21 Effect of seed treatment on the incidence of petiole base infection in the field.

Treatment		Petiole Base Infection 1st Assessment (Av.) 18/8/69	2nd Assessment (Av.) 13/10/69 *
Non-treated seed	infection free	0	5
	infected	30	73
Hot water		3	10
Thiram soak		1	8
Thiram dust		2	12
Benlate dust		36	88

* A complete table of results is presented in Appendix III.

The main findings of this experiment can be summarised as follows:

(i) Hot water treatment, thiram soaking and thiram dusting of seed effectively reduced the amount of petiole base infection.

(ii) There was no difference between these methods of treatment at the first and second assessments. Part of the build-up of infection could have been due to spread from adjacent, infected rows.

(iii) Benlate dust was totally ineffective as a method of control; in fact there appeared to be more infection in these rows than in those grown from untreated infected seed. The reason for this was not determined but it is theorised that benlate may have favoured the development of A. radicina by

selectively eliminating competitive and antagonistic fungi.

Discussion

Although certain hot water treatments were found to effectively control seed-borne inoculum they frequently caused a depression in germination. In addition, the tedious nature of this method would tend to make it unacceptable to both the seed merchant and carrot grower.

The other alternative involved the use of a fungicide preparation, namely thiram 80% w/w. Both the thiram soak method (sensu Maude, 1968) and the external application of thiram dust to the seed effectively reduced seed-borne inoculum. Thiram dusting, at the rate of one ounce active ingredient per 14 lbs of seed was considered as the most satisfactory method for adoption by seed merchants or carrot growers because it was cheapest and quicker to use.

Whether or not it is necessary to achieve absolute control of a particular pathogen depends on the significance of the seed-borne inoculum to the host crop. Where even a small amount of inoculum can result in widespread crop infection through secondary spread from primary infection foci, then complete elimination of the seed-borne pathogen is essential; for example Septoria apiicola in celery (Bant & Storey, 1952). Alternatively if the pathogen is important because it reduces germination and / or stand establishment, then it may be more economical to sow extra seed than to attempt to eliminate the inoculum. In this latter case, if control measures were warranted they would be aimed at reducing the amount of seed-borne inoculum to an acceptably low level, but not necessarily eliminating it. In all cases complete elimination of the pathogen would

be desirable provided it could be done simply and cheaply.

In this connection there are two points to consider with regard to control of seed-borne A. radicina by the application of thiram dust. Firstly, seed-dusting with thiram was a simple, cheap and effective method of restoring the germination capacity of infected seed. Absolute control of pre-emergence damping-off was neither sought nor achieved, since the relatively few seeds remaining infected would be of no significance anyway. Seed dusting would also assist in the pre-emergence phase of host development by giving some protection from attack by other seed-borne and soil-borne pathogens. Secondly, seed dusting markedly reduced the amount of petiole base infection in both the young and the mature crop. Secondary spread of petiole base infection was minimal, and although complete control would have been desirable, the amount of inoculum available for subsequent infection of harvested carrots was effectively reduced as a result of treatment.

SUMMARY

1. The occurrence of black-rot infection of certain carrot crops in the Ohakune and Manawatu districts was established during this investigation. This is the first time the causal organism, Alternaria radicina M.D. & E., has been recorded in New Zealand.

2. The identity of the causal fungus was confirmed by comparing its morphological and cultural characteristics with original and more recent descriptions, and by demonstrating its pathogenicity to carrot.

3. Culture filtrates of the pathogen were found to exhibit certain hitherto unrecorded antibiotic properties. A yellow pigment, produced by some isolates of the pathogen on lab. potato-dextrose medium, was associated with fungal antibiosis. In addition all isolates appeared to produce a bacterial antibiotic in various culture media.

4. On the basis of his scheme for the delimitation of the genera Alternaria, Ulocladium and Stemphylium, Simmons (1966) maintained that the pathogen should remain in the genus Alternaria, as originally described by Meier, Drechsler & Eddy (1922). Photomicrographic evidence, presented in this study, supports Simmons' argument that the pathogen belongs to the genus Alternaria.

5. The transmission of A. radicina in some lines of imported carrot seed was demonstrated. A. dauci, a pathogen causing foliage blight of carrots, was also identified in some lines of commercially available seed. Infection levels for A. radicina ranged between one and thirty percent, and for A. dauci between one and twenty-seven percent.

6. Most of the seed lines infected with A. radicina originated from Australia, except for some of European origin. Seed originating from the United States of America was notably free of infection with A. radicina and A. dauci.

7. A. radicina was shown to be borne as a shallow infection of the testa. No natural conidial contamination of seed was found in any of the lines transmitting the pathogen.

8. This investigation confirmed that A. radicina is capable of causing pre-emergence damping-off under moist soil conditions in the glasshouse. Under dryer field conditions seedling infection developed as a direct consequence of sowing infected seed. The infection remained on the petiole bases until crop maturity, but had not detrimentally affected the harvestable product.

9. It was hypothesised that infected petiole bases, which commonly became part of the soil debris, constituted a substantial potential inoculum reserve for contamination of mature carrots during harvest. This inoculum would undoubtedly predispose stored crops to black rot infection.

10. Limited spread of petiole base infection was detected and appeared to be directly related to the initial amount of infection present, which, in turn, was related to the level of seed-borne inoculum.

11. Various approaches to the control of seed-borne A. radicina were investigated. From a practical viewpoint, the most satisfactory control method was one which involved dusting the seed with thiram wettable powder prior to sowing. This restored the germination capacity of the seed and almost eliminated the occurrence of petiole base infection.

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APPENDIX I

Composition and Preparation of Culture Media

Recipes are given below for the culture media used in this study. They were prepared using distilled water, autoclaved in 200 ml. quantities in 250 Erhlenmeyer flasks, and stored for use. All media was sterilised at 15 p.s.i. for 20 minutes immediately following preparation.

A. Potato Dextrose Agar (lab. P.D.A.)

Agar	12 gms.
Potatoes	200 gms.
Dextrose	10 gms.
Water	1000 mls.

The potatoes were peeled, sliced, and cooked slowly for one hour in 500 mls of water, and the filtrate retained after straining the mixture through cheese-cloth. Agar was melted in 500 mls of water, dextrose added, and the two solutions combined before autoclaving.

B. Potato Dextrose Broth

The same ingredients, except the agar, were used to prepare this liquid medium. The broth was autoclaved and stored in 250 ml. Erhlenmeyer flasks.

C. Oxoid P.D.A.

Reference : Oxoid Manual (1962) reprint second edition. The prepared dry medium was added to water as indicated on the label.

D. Malt Agar (M.A.)

Agar	25 gms.
malt extract (disco)	20 gms.
water	1000 mls.

The two constituents were dissolved in water by heating and stirring.

APPENDIX II

Routine Methods and Materials in the Glasshouse.

Flat perforated plastic trays (12" x 18" x 3"), embedded into a wet sand layer covering the bench tops were routinely used for the glasshouse experiments. A pre-sterilised medium fertility peat-sand mix (Baker 1957) was used throughout as the seed-bed. Frequent watering was carried out by placing the plastic trays in water, thus allowing seepage from underneath. This minimised the risk of spreading infection across the surface of the seed-bed when overhead watering was employed.

When necessary disease-free carrot seedlings were transferred from the flat trays to clay pots to allow the plants to develop to maturity, especially for inoculation purposes.

Various glasshouse pests, including caterpillars, aphids, leafhoppers and thrips were controlled by insecticides such as D.D.T., malathion, and sevin.

APPENDIX III

(a) Materials and Methods used in the Field Studies

A small plot of land (approximately 24 feet by 24 feet square) was cultivated in preparation for the field trials referred to in Chapter III, Sections C. & D. For each replicate a ridge six inches wide and twelve feet long was prepared in readiness for sowing. A "baking powder" tin punctured with small holes, was used as a seed drill. By placing sufficient seed in the drill and rolling it along the row, a six inch band of seed was sown fairly evenly along the length of the row. The seed was then covered with about half an inch of soil.

The rows were 18 inches apart and the positions of the three replicates of seven treatments were randomly selected.

The whole area of cultivated land, of which this plot was a part, had been chemically treated to control weeds. Any more weeds growing between the rows were removed manually.

(b) Field disease assessment of effectiveness of seed treatment

A complete table of results for the second assessment.

Treatment	Replicate 1 Percent infection	Replicate 2 Percent infection	Replicate 3 Percent infection	Average Percent infection
Non-treated infection-free	3	8	5	5
Non-treated infected	81	62	75	73
Hot water treated	10	11	9	10

APPENDIX III continued

Treatment	Replicate 1 Percent infection	Replicate 2 Percent infection	Replicate 3 Percent infection	Average Percent infection
Thiram soak treated	15	4	5	8
Thiram dust treated	17	10	10	12
Benlate dusted	83	90	91	88

APPENDIX IV

Effect of selected hot water treatments on germination of carrot seed.

A complete list of results

(a) Seed line 4. Germination percentage.

Treatment Temperature °C	15 MINS		20 MINS		25 MINS		30 MINS		35 MINS		(a)
	4DAY	7DAY	4DAY	7DAY	4DAY	7DAY	4DAY	7DAY	4DAY	7DAY	(b)
44	61	83	60	87	62	86	62	87	60	83	
46	60	85	70	87	61	84	56	85	61	82	
48	56	84	69	87	58	82	63	82	58	81	
50	61	77	53	74	52	83	43	78	45	75	
52	50	85	46	89	42	73	33	67	30	58	
54	39	86	20	60	13	48	11	49	4	25	

(b) Seed line 13. Germination percentage.

Treatment Temperature °C	15 MINS		20 MINS		25 MINS		30 MINS		35 MINS		(a)
	4DAY	7DAY	4DAY	7DAY	4DAY	7DAY	4DAY	7DAY	4DAY	7DAY	(b)
44	71	83	69	90	74	91	69	90	74	89	
46	66	84	70	88	63	84	58	80	59	85	
48	71	85	71	87	50	79	61	83	60	75	
50	70	88	56	79	42	75	55	71	34	62	
52	53	83	41	66	34	62	17	50	18	45	
54	27	69	14	45	16	46	3	20	4	15	

(a) duration of hot water treatment

(b) duration of germination test

APPENDIX IV continued

(c) Seed line 44. Germination percentage.

Treatment Temperature °C	15 MINS		20 MINS		25 MINS		30 MINS		35 MINS		(a)
	4DAY	7DAY	4DAY	7DAY	4DAY	7DAY	4DAY	7DAY	4DAY	7DAY	(b)
44	60	79	59	84	61	81	57	81	60	82	
46	56	74	53	74	69	81	57	75	66	85	
48	54	75	52	77	63	82	56	79	52	85	
50	60	83	57	78	54	78	58	77	52	72	
52	45	77	40	65	39	63	30	56	31	43	
54	43	70	28	50	19	42	1	11	2	11	

(a) duration of hot water treatment

(b) duration of germination test

APPENDIX V

Details of all carrot seed lines screened for the presence of A. radicina and A. dauci.

Line No.		Variety	Origin	Germination Percentage	Seed Infection A.radicina	Seed Infection A.dauci
1968	1	Spring Market Improved	Europe	48	-	-
	2	Manchester Table	U.S.A.	86	-	-
	3	Dividend	Australia	75	1	-
	4	Egmont Gold	"	83	30	-
	5	Selection 198	"	93	3	-
	6	Spring Market	France	82	-	-
	7	Topweight	Australia	90	5	-
	8	*	France	70	-	-
	9	*	Australia	91	3	-
	10	*	U.S.A.	82	-	-
	11	*	"	86	-	-
	12	*	Australia	88	1	-
	13	*	"	77	-	-
	14	*	U.S.A.	75	-	-
	15	*	Holland	91	-	1
	16	*	France	84	-	-
	17	*	"	83	-	-
	18	*	Australia	91	19	-
	19	Chantenay Long	"	92	1	-
	20	Chantenay Red Cored	U.S.A.	86	-	-
	21	Egmont Gold	Australia	82	17	-
	22	Nantes Cross	U.S.A.	90	-	-
	23	Tip Top	Holland	88	-	-
	24	Royal Chantenay	U.S.A.	79	-	-
	25	Spring Market Improved	Europe	83	-	-
	26	Topweight	Holland	83	1	-
	27	Touchon	France	73	-	-
	28	Autumn King	Europe	81	-	-
	29	Chantenay Long	U.S.A.	79	-	-
	30	Royal Chantenay	"	75	-	-

(* - variety unknown)

APPENDIX V continued

Line No.	Variety	Origin	Germination Percentage	Seed Infection A.radicina	Seed Infection A.dauci
31	Early Short Horn	U.S.A.	60	-	-
32	Gold Pak.	"	84	-	-
33	Geurande	"	46	-	-
34	Holmes Improved	Australia	87	24	-
35	Imperator	U.S.A.	81	-	-
36	Early Cross F.1	"	77	-	-
37	Nantes Improved	Europe	25	-	-
38	Touchon	U.S.A.	83	-	-
39	Hi-colour	"	87	-	-
40	Winter Perfection	Europe	54	-	-
41	Hafnia	"	81	-	-
42	Spring Market Improved	"	86	-	-
43	Spring Maxicrop	"	64	-	-
44	Touchon	U.S.A.	92	-	-
45	Manchester Table	"	83	-	-
46	Chantenay Long	"	50	-	-
47	Chantenay Red Cored	"	86	-	-
48	Red King	Europe	77	-	-
49	Quickie	Denmark	82	-	-
50	Chantenay Red Cored	U.S.A.	87	-	-
51	Egmont Gold	Australia	85	14	-
52	Manchester Table	France	97	-	-
53	Royal Red	Holland	73	3	-
54	Taranaki Strong Top	U.S.A.	89	-	-
55	White Belgian	France	86	-	-
1969					
101	Spring Maxicrop	Europe	88	-	22
102	Spring Market Improved	"	87	1	24
103	Manchester Table	U.S.A.	88	-	5
104	Touchon	"	85	-	-
105	Chantenay Red Cored	"	73	-	-
106	Red King	Europe	80	-	5
107	Topweight Improved	Australia	80	3	-
108	Amsterdam Forcing	Denmark	69	2	-

APPENDIX V continued

Line No.	Variety	Origin	Germination Percentage	Seed Infection A.radicina	Seed Infection A.dauci
109	Topweight	Holland	85	-	13
110	Royal Red	"	89	-	27
111	Nantes Early	France	84	-	-
112	Chantenay Royal	U.S.A.	89	-	-
113	Imperator	"	78	-	-
114	Geurande	"	77	-	-
115	Chantenay Red Cored	"	83	-	-
116	Chantenay Red Cored	"	70	-	-
117	Manchester Table	"	80	-	-
118	Holmes Improved	Australia	80	11	-
119	Selection 198	"	92	-	-
120	Egmont Gold	"	79	-	-
121	Topweight	"	84	5	-
122	Dividend	"	84	-	-
123	Oxheart	U.S.A.	74	-	-
124	Manchester Table	"	91	-	-
125	Chantenay Long	"	81	-	-
126	Manchester Table	"	88	-	-
127	Touchon	"	84	-	-
128	Chantenay Royal	"	75	-	-
129	Sugar Chub	France	83	-	-
130	White Belgian	"	82	-	-
131	Spring Market	"	71	-	-