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

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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).

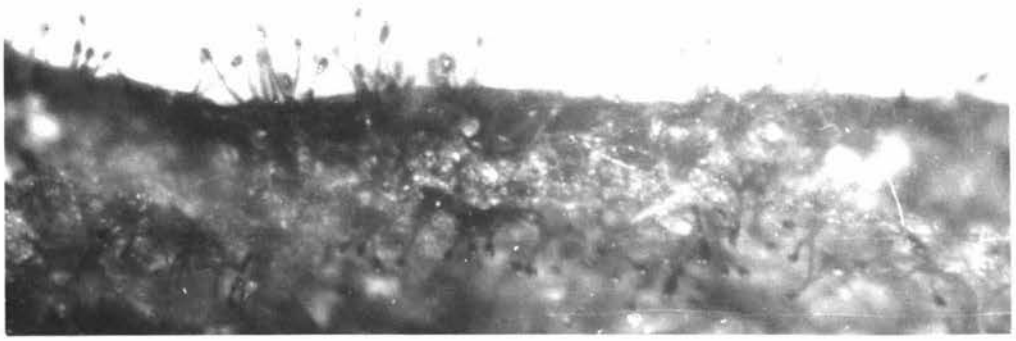
Further details of conidial dimensions are presented in Table 1. Although it was possible to identify the fungus by morphological characteristics and dimensions alone, its pathogenic association with carrot was an obvious aid to its identification.

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.

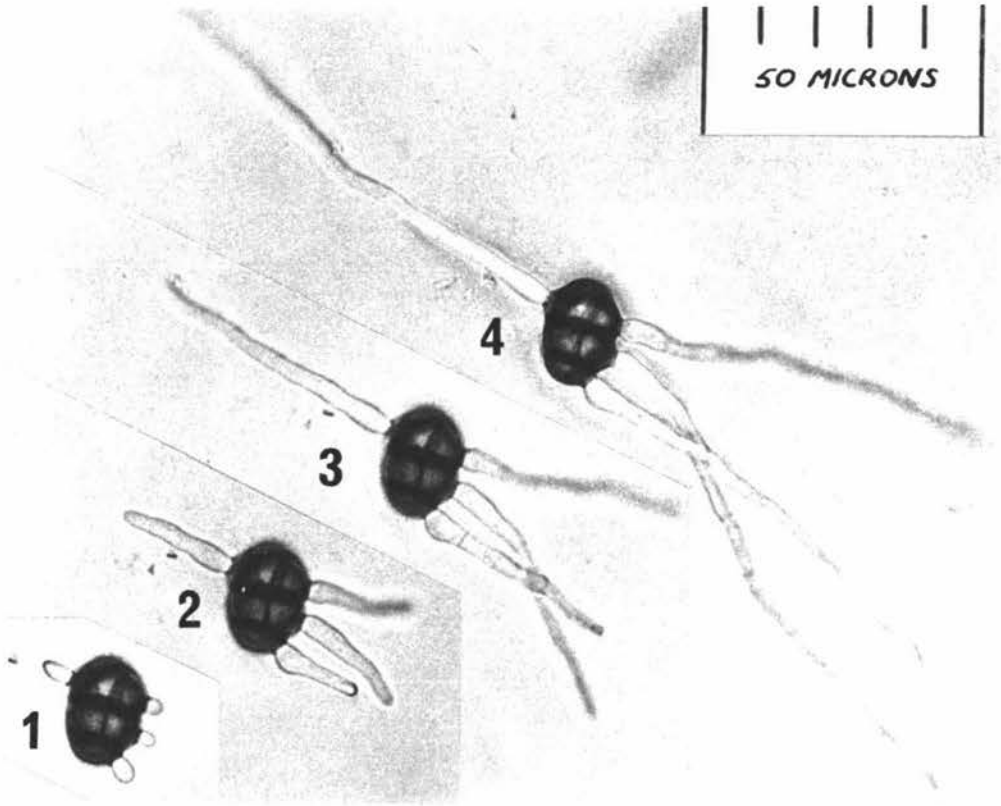
Cultures of A. radicina were also obtained by plating surface-treated, infected seeds to P.D.A., but were not used for pathogenicity or nomenclature studies.

(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.

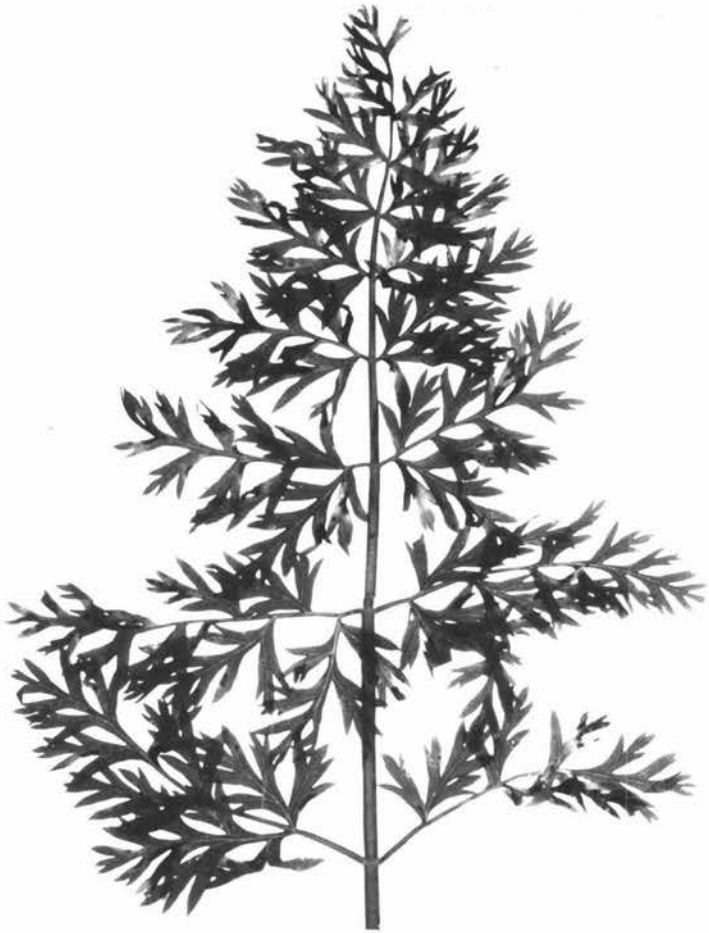
(ii) Foliage

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)

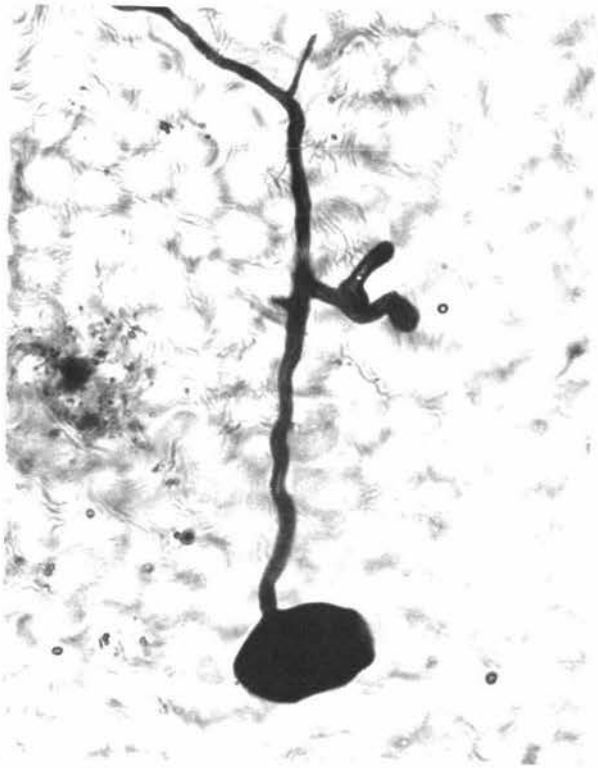


Pathogenic symptoms induced by artificial inoculation.

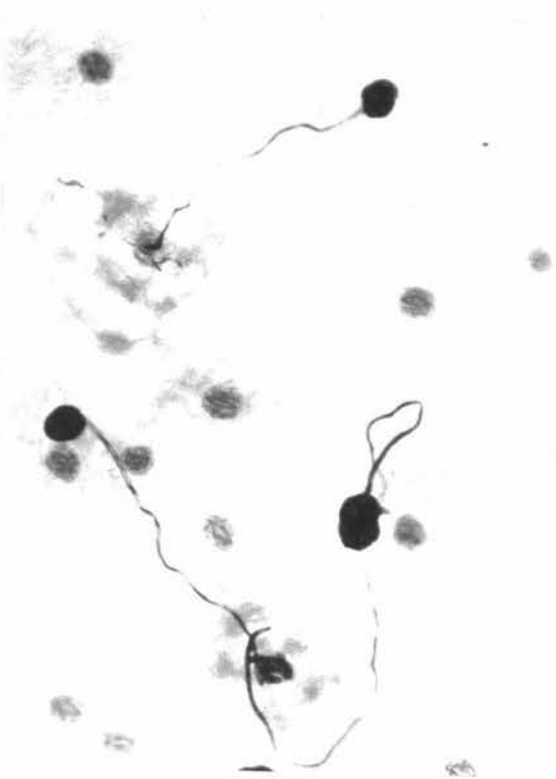
(a) On semi-mature carrot plants.

(b) Lesions on mature foliage.

Plate 5.



(a)



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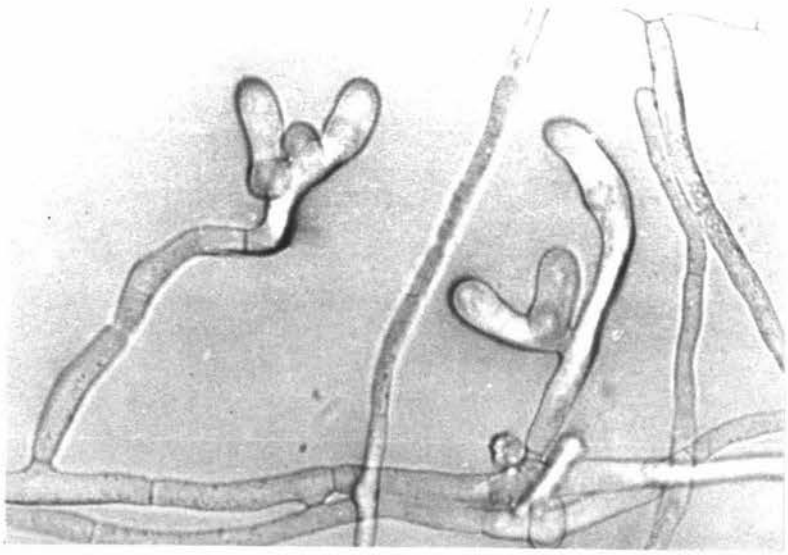


(c)

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.

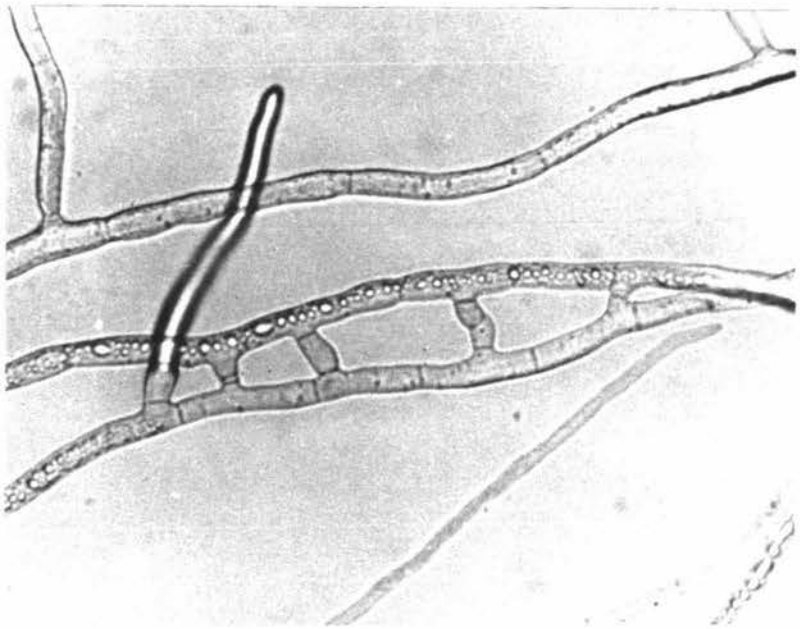
On the basis of the tests conducted on carrot seedlings, foliage and roots, the pathogenicity of all isolates of A. radicina used in this study was established beyond doubt.

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

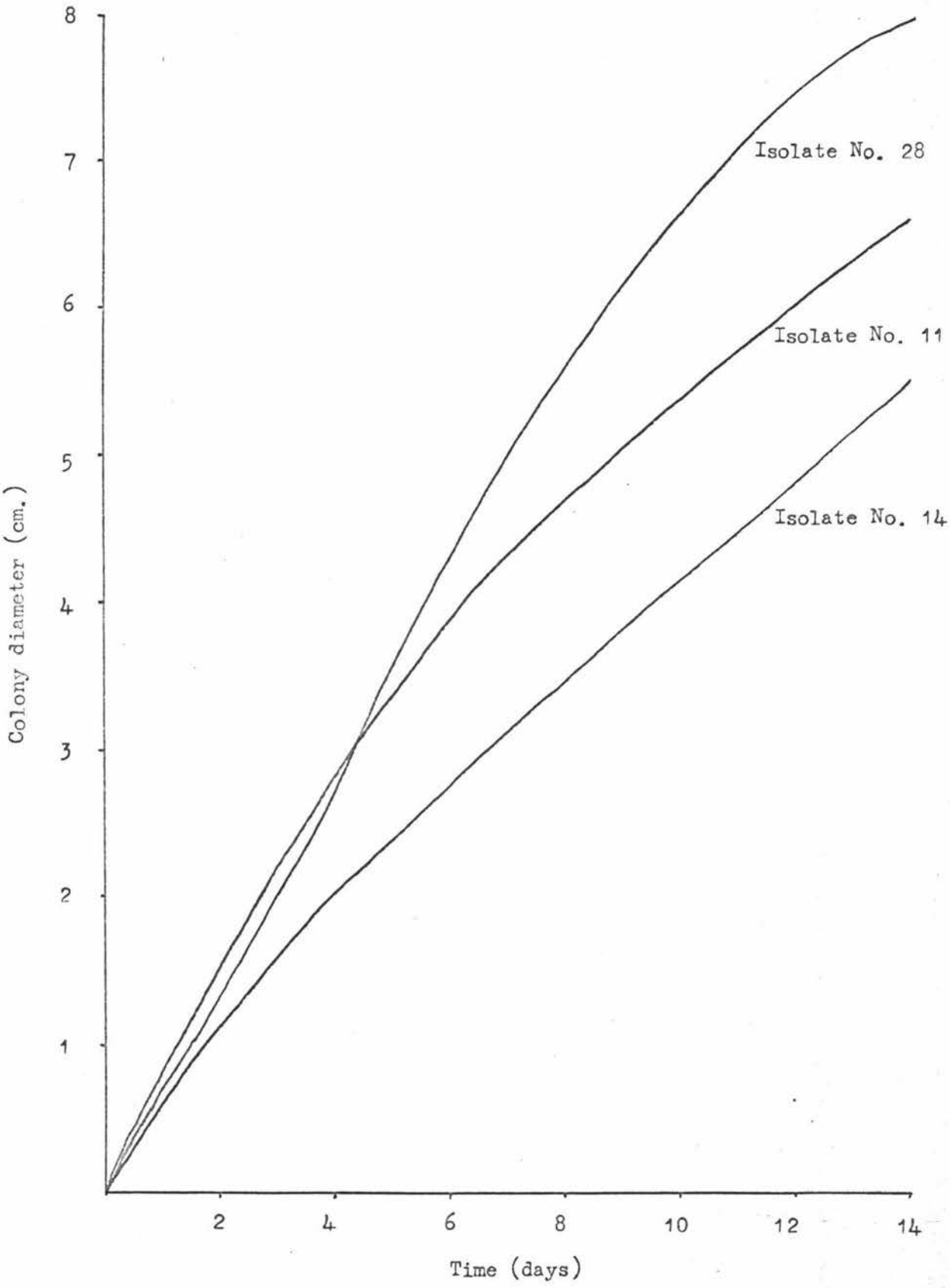


Fig. 1. Growth rate of three isolates of A. radicina on oxoid P.D.A. at 24°C.

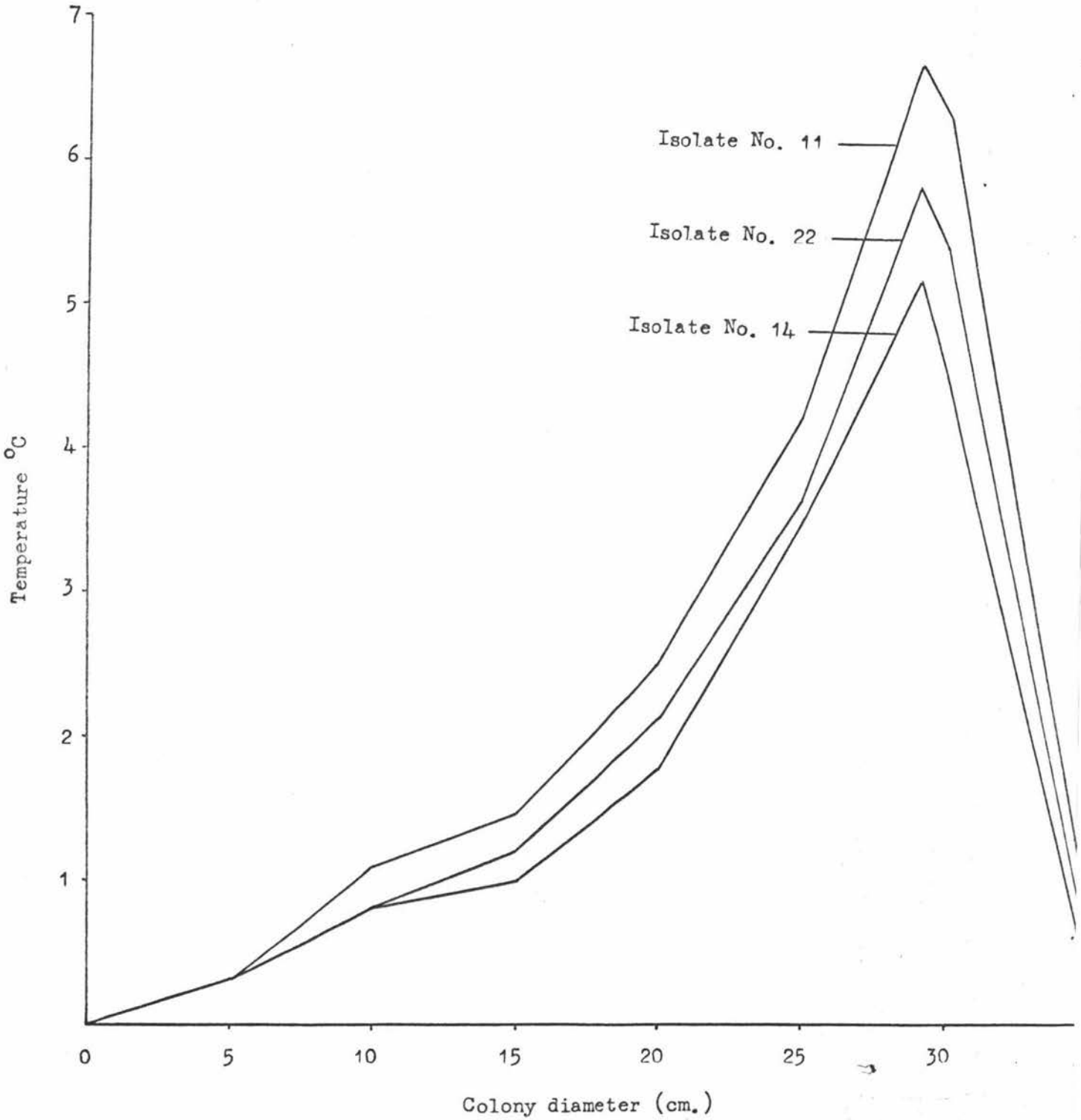


Fig. 2. Effect of incubation temperature on the growth of three isolates of A. radicina on P.D.A.

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.

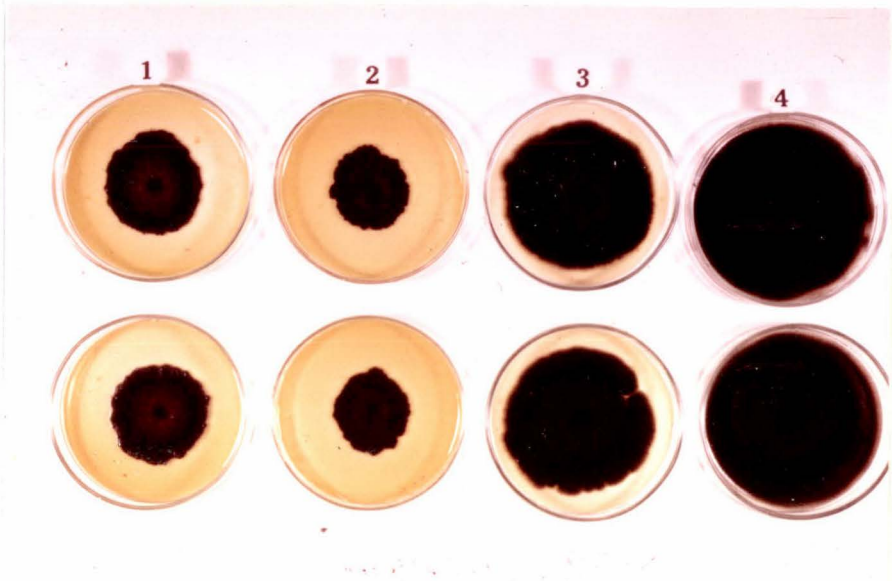
2. Non pigment-producing isolates. These isolates did not produce the yellow pigment and their growth rate was unaffected until they had grown to the edge of the plate.

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.

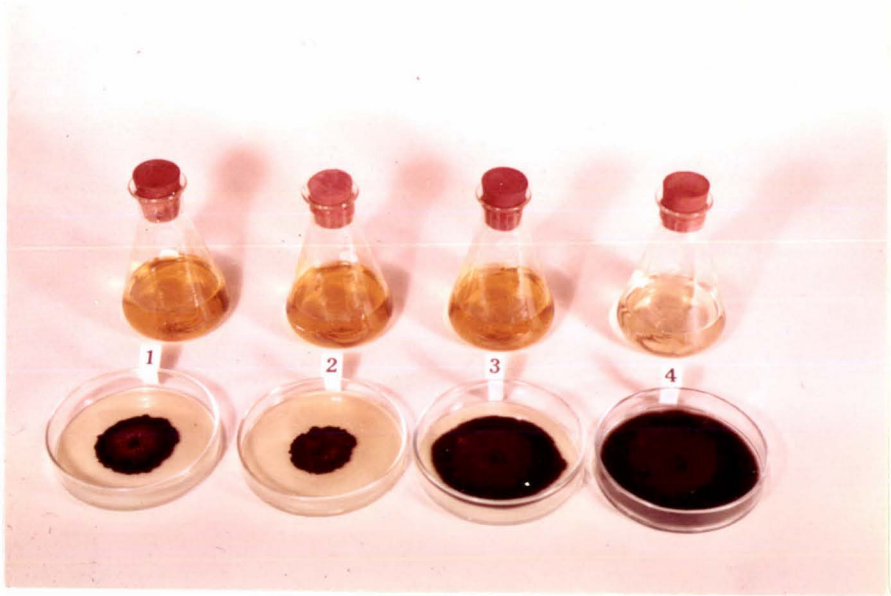
A further interesting observation was made of those plates

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.

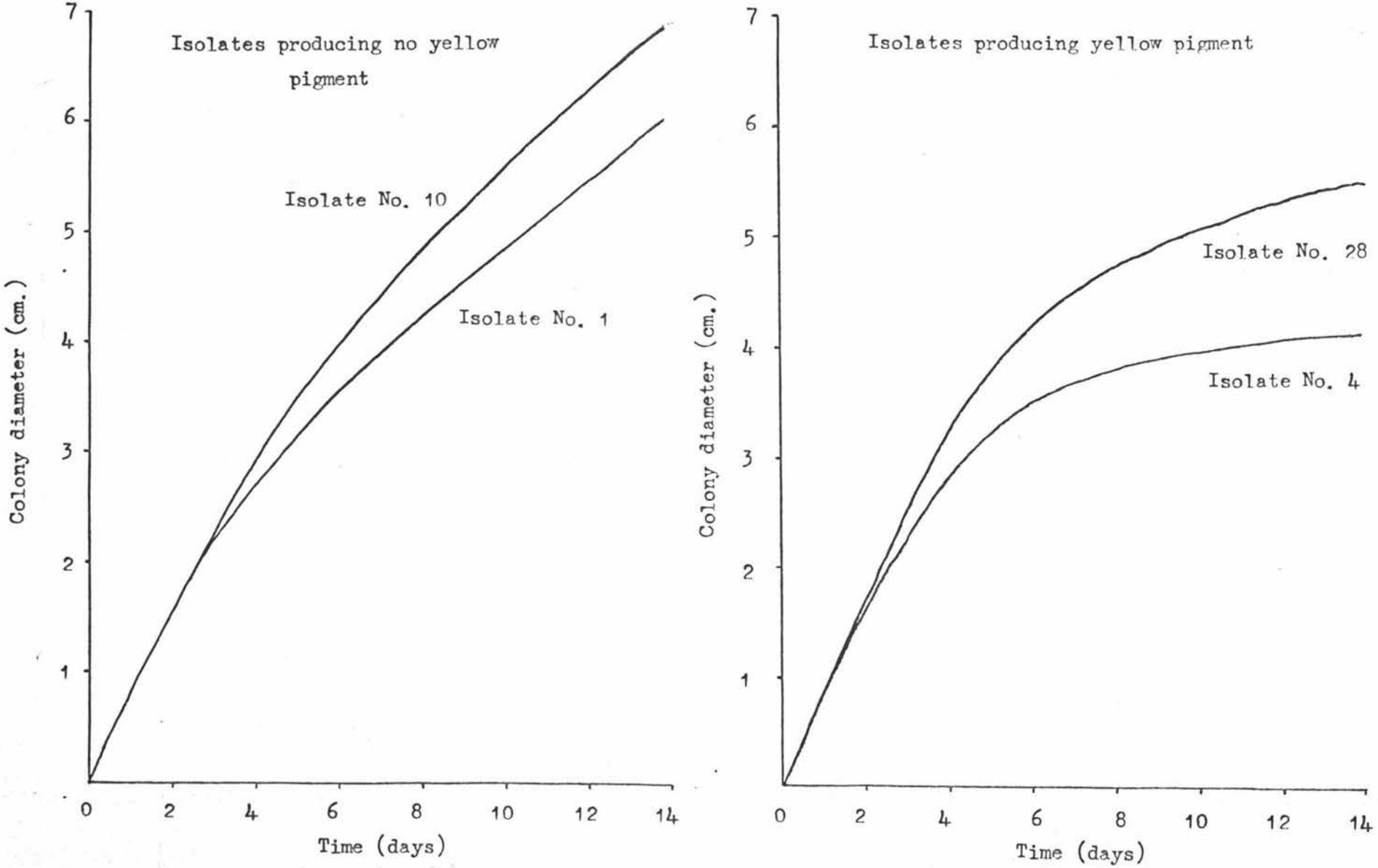


Fig. 3. Comparison of growth rate on lab P.D.A. between isolates of A. radicina that produced a yellow pigment and isolates that did not produce a yellow pigment (Incubation at 24°C).

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.

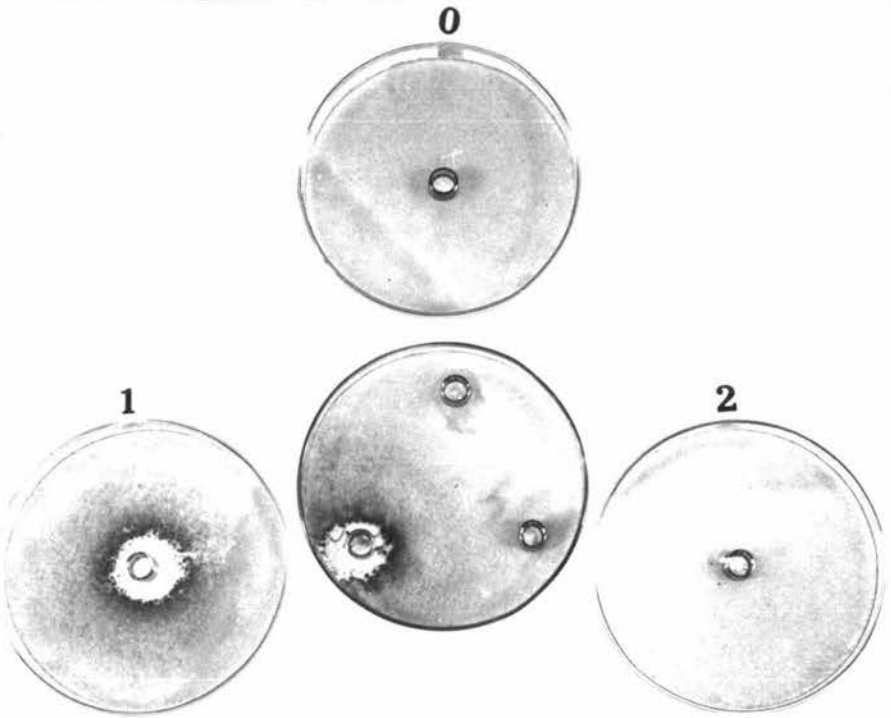
Stereum purpureum (Fr.) Fr., (Basidiomycetes). Mycelium from a fresh P.D.A. culture was fragmented by shaking vigorously in a McCartney bottle containing glass beads and sterile water.

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

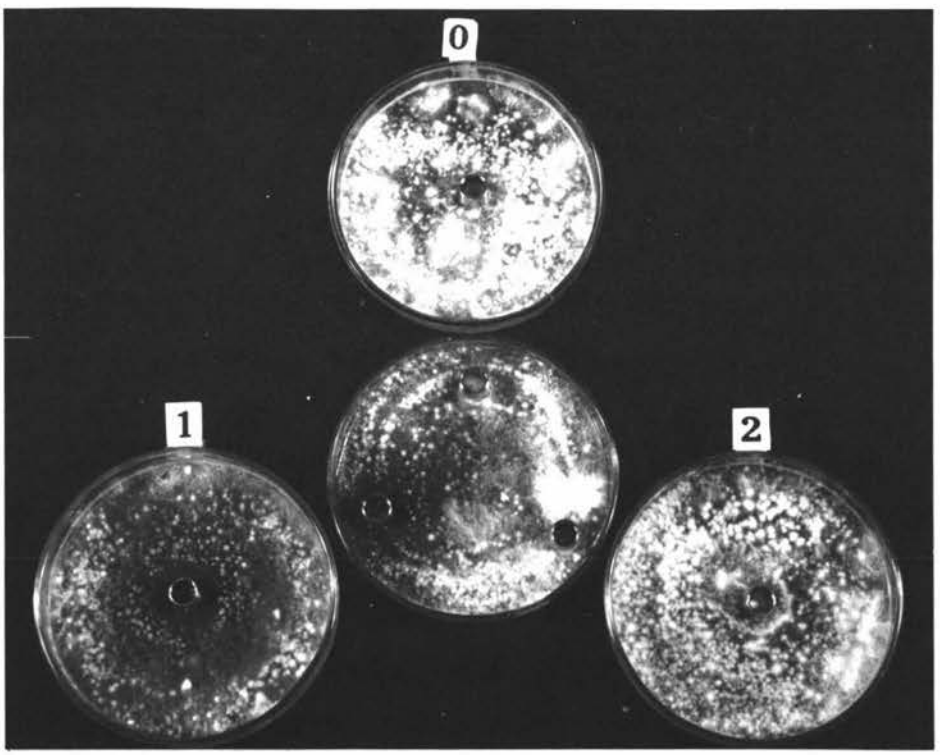
One ml. of inoculum was spread evenly over the surface of each oxoid P.D.A. test plate using a glass spreader. Hot stainless steel antibiotic assay rings were then partially embedded in the agar and allowed to cool. Five drops of concentrate from the appropriate isolate were placed within each antibiotic 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.