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A STUDY OF  
THE ALTERNARIA LEAFSPOT COMPLEX  
ON POTATOES AND TOMATOES  
IN THE MANAWATU.

This Thesis is presented as partial fulfillment of the requirements for the  
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B.T. HAWTHORNE. September 1963.

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"YOU WILL FIND A FUNGUS AND DETERMINE ITS CHARACTERISTICS.  
YOU TURN TO THE BOOKS AND DECIDE ON ITS GENUS. THEN YOU  
LOOK FOR THE SPECIES. AND YOU LOOK, AND YOU LOOK, AND AFTER  
A WHILE YOU FIND IT!

IN ANOTHER GENUS!"

DR. J.J. DAVIS.

I N T R O D U C T I O N .

The fungus Alternaria (Macrosporium) solani is associated with a foliage disease of potatoes and tomatoes throughout the world. Although there are often several phases of attack on these hosts by the fungus e.g. tuber damage in the potato and seedling loss with tomatoes, the disease has been named on the basis of the foliage symptoms which are characteristic. Two names are commonly used (1) Target Spot

(2) Early Blight,

and of the two, 'Target Spot' is the more descriptive since foliage lesions are circular to irregular dark brown areas with a very characteristic zonation effect due to a series of more or less concentric rings within the lesion.

Early investigators studied the diseases of the respective hosts from a practical plant pathology standpoint, concerned, in the main, with tracing the Disease Cycle and formulating methods of control.

A number of independent studies of the disease(s) caused by A. solani on potatoes and tomatoes showed that the causal fungus was extremely variable in phenotype and genotype. This inherent variability has added to the confusion and complexity surrounding the taxonomy and nomenclature of the fungus through the years. An additional source of complication with respect to taxonomy and nomenclature has arisen from the slowly evolved conception of the genus Alternaria to its present day delimitations e.g. early workers placed the fungus in the genus Macrosporium on the basis of characters which, according to modern views, as put forward by Wiltshire in 1933, place the fungus in the genus Alternaria.

More particularly in the U.S.A. and Australia Alternaria solani seems capable of 'causing' widespread epiphytotics of 'Early Blight' of potatoes, which result in heavy loss. In addition, the several phases of attack, by the fungus, on tomatoes have proved very costly over the years due to seedling loss and reduction in fruit yield. In N.Z. in 1927 'Early Blight' was regarded as one of the most serious of potato diseases, latterly, however, the disease seems of no national importance although in localized areas it can cause heavy and often unsuspected loss e.g. the observation that 'Late Blight' resistant potato varieties Rua and Tahi in a recent season in the Manawatu were heavily affected with 'Early Blight' in later stages of growth - sufficient to cause local growers to doubt the claimed resistance to 'Late Blight' !

The consistent prevalence of leaf spotting and Early Blight on potatoes and tomatoes in the Manawatu area (of the N.Is., N.Z.) over several seasons together with the apparent absence of records of a comprehensive study of this disease in N.Z. has led to this study.

At the outset a straightforward study of the disease in the field and in the glasshouse was envisaged. This theme had to be considerably modified however because regular inspection of a representative 8 - 10 crops in the Manawatu showed that the incidence of 'Early Blight' on potatoes and tomatoes was extremely low in the two seasons covered by the duration of the work.

As a result the emphasis was shifted and the aims of this study became:

- (1) To make a study of the characteristics of the disease under glasshouse conditions.
- (2) To compare isolates of the fungus from three solanaceous hosts namely, Potato, Tomato and Black nightshade with reference to morphology, physiology and pathogenicity and to gauge the extent of variability between 'strains' and its importance in taxonomy.

MATERIALS and METHODS.

Materials and methods specific to particular parts of this study are described in detail in their respective sections. Materials and methods applicable to several sections of the work are, however, outlined below.

MATERIALS and METHODS USED  
in the LABORATORY.(a) PREPARATION OF MEDIA.

The detailed preparations of all media are described in Appendix I. Oxoid potato dextrose agar (P.D.A.) was used for all normal culturing work. This medium was prepared in 2 - 4 litre quantities as required, and stored after autoclaving in partly filled 250 ml. flasks stopped with cotton wool plugs.

(b) INOCULATION OF MEDIA.

Inoculum discs 5 mm in diameter, cut with a sterile cork borer from the edge of an actively growing colony on P.D.A. were used in all experimental work. The inoculum disc was always placed mycelium-side down on the media being inoculated. The diameter of the inoculum disc i.e. 5 mm, was subtracted from the measured diameter of all colonies recorded.

(c) MEASUREMENT OF GROWTH RATE ON ARTIFICIAL MEDIA.

Work by Brancato and Golding (1953) indicates that colony diameter is a valid measure of the effects of environmental factors such as medium constituents, pH, and temperature. Throughout this study where colony diameters were to be measured 3 - 4 colonies were used per treatment, 2 diameters at right angles were recorded for each colony and results expressed as an average. Since growth proved to be at a constant and linear rate records of colony diameters were made only once, normally 8 days after inoculation.

(d) INDUCTION OF SPORE PRODUCTION AND PREPARATION OF INOCULUM.

Prolific sporulation of Alternaria solani on artificial media is notoriously difficult to obtain and a wide variety of different methods involving various media, treatments with ultra violet light and combinations of light and high humidity, appear in the literature.

The technique used in this study is an adaptation of a method described in 1962 by Ludwig, Richardson and Urwin. The surface of approximately 2 week old cultures of the fungus growing on laboratory P.D.A., in petri dishes, was scraped with the end of a clean glass slide so that all the aerial mycelium was removed. The plates were then washed with lids removed, in running tap water for 24 hours. To prevent the agar floating away the plates were covered with a thin muslin cloth. Following washing the plates were stacked in an inverted, slanted position. The method of stacking ensured that conditions of high humidity obtained at the surface of all colonies.

This method provided heavy and consistently reliable crops of conidia from all isolates within 48 hours of stacking.

Spore suspensions used in all inoculation work were obtained quickly and easily.

A fine jet of distilled water from a plastic 'wash' bottle was directed onto the surface of the sporulating colony until there were about 7-8 ml. of water in the petri dish. A trace of TEEPOL was added to improve wetting and a smooth glass rod rubbed over the colony surface to further improve the release of conidia into the water. The concentrated spore suspension was poured into a measuring cylinder to determine the volume and then the actual concentration was measured with the aid of a Neubauer haemocytometer. Desired concentrations were obtained by adding the required volumes of distilled water.

(e) EXAMINATION OF DISEASED MATERIAL AND ISOLATION OF FUNGI FROM LESIONS.

Lesions from diseased material were placed on 2 glass slides in a petri dish containing a moist filter paper. The petri dishes were left on the laboratory bench. If the fungus was viable within the lesion 24-36 hours was sufficient time for production of conidia.

Conidia were identified by microscopic examination and then single spore isolations were made to laboratory P.D.A. slopes preparatory to subsequent culturing.

Since the conidia of Alternaria solani are comparatively large and readily discernible with a binocular microscope the technique used for isolation and identification was both simple and rapid.

The main contaminants found on naturally infected material were species of Stemphylium and Alternaria tenuis. These were readily distinguishable and caused no concern in making single spore transfers of the conidia of Alternaria solani.

MATERIALS and METHODS  
USED in the GLASSHOUSE.

(a) SOIL MIXTURES.

Sand, loam and peat were used, in conjunction with chemical fertilizers and dried blood, to make up 2 different soil mixtures.

- (i) Seedbox soil Mixture 2 Loam : 1 Peat : 1 Sand plus  $\frac{3}{4}$  oz lime per bushel of soil mixture and  $1\frac{1}{2}$ oz superphosphate per bushel.
- (ii) Pricking out soil Mixture 7 Loam: 3 Peat: 2 Sand plus 4 oz per bushel of a basic fertilizer mix containing the following ingredients:-  
1 part, by weight, Potassium sulphate; 2 parts dried blood; 2 parts superphosphate 1 oz per bushel of lime.

The peat and loam were partially sterilized before use. A thin layer of the peat and/or loam was spread on a concrete floor, dampened slightly, and covered with a polythene sheet which was securely held in position, around the edges, with heavy weights. Methyl bromide gas was then released under the sheeting which was left in place for 24 hours. Experience showed that a 1 lb. tin of Methyl bromide prevented subsequent growth of all weed seeds in approximately a cubic yard of peat or loam spread out in a thin layer. After treatment with Methyl bromide the peat and loam were stored in separate bins and used as required. The sand used was deep river sand and hence did not need methyl bromide treatment to kill weed seeds.

(b) PRODUCTION OF PLANTS.

Seeds of all plant species except potato were sown in small seed boxes (18"x12"x3"). When the seedlings were sufficiently advanced they were pricked out into pots. In most cases there was 1 plant per pot but with plants such as Zinnia, Carrot, Ageratum and Godetia, used in host-range studies, 3 seedlings were pricked out into each pot.

Potato plants were raised by planting seed potatoes in pots containing pricking out mix.

Where it was warranted, especially with tomatoes and black nightshade plants, staking and tying of the plants was carried out. This produced a

better plant and greatly facilitated movement of the pots plus plants together with economizing on bench space.

Watering of plants was carried out night and morning during the summer months (November to mid March.) since the tomato and black nightshade plants especially were prone to wilting. During cooler weather however the plants needed watering only every 2 - 3 days.

Maximum temperatures recorded in the glasshouse during hot weather were about 95°F but only for short periods. During the winter months an average temperature of approximately 70°F was maintained in the glasshouse by means of thermostatically controlled heaters placed under the benches.

Since potato seed proved difficult to obtain, in quantity, when required, and even more difficult to store in good condition most of the "Disease Cycle" studies were carried out with tomato plants. Hence, a constant supply of tomato plants was maintained by sowing seed every 2 - 3 weeks and pricking out the seedlings into pots.

(c) CONTROL OF PESTS.

Some trouble was experienced with White Butterfly Caterpillar damage but good control was obtained by dusting the plants with 'Derris Dust'. Occasionally an aphid population started to build up and this was controlled by shutting the glasshouse down at night and burning a NEXA strip inside. Carried out at weekly intervals this was an efficient control measure.

(d) INOCULATION OF PLANTS.

Plants were inoculated with spore suspensions sprayed onto the foliage in a fine misty spray from a patent "WINDEX" sprayer.

After inoculation plants were placed in a high humidity chamber constructed out of light, clear "Armathene" sheeting.

SELECTION AND DESIGNATION OF ISOLATES USED IN THIS STUDY.

In April 1962 crops of potatoes and tomatoes and volunteer plants of black nightshade, showing symptoms typical of attack by Alternaria solani, were examined and single spore isolates obtained.

The isolates were designated according to:

- (i) The host from which they were isolated. This was indicated by using 2 capital letters representing the botanical name. Thus an isolate from potato (Solanum tuberosum) was designated ST ---; from tomato (Lycopersicum esculentum), LE - - -; from Black nightshade (Solanum nigrum), SN - - -.
- (ii) The date on which the isolation was made. This was indicated by using the day and the month. Hence an isolate made on the 8th. of April (4th month) becomes
- |     |      |
|-----|------|
| ST) |      |
| LE) | 84 - |
| SN) |      |
- (iii) The locality where the diseased material was obtained. This was indicated by a letter of the alphabet. Usually 5 single spore isolations were made from each diseased specimen.

Eleven isolates were selected for study. These were:

<u>ex Black nightshade</u>	<u>ex Potato</u>	<u>ex Tomato</u>
SN84A	ST84B	LE84E
SN84D	ST84C	LE84G
SN264A	ST264B	LE264B.
SN264D	ST264C	

An additional isolate was obtained from the Auckland area through the services of Dr.F.J.Morton of Plant Diseases Division D.S.&I.R. This isolate was off potato and designated A4,6. This isolate was incorporated into the work part way through the study and hence it was not used in all studies.