STUDIES ON STEMPHYLIUM LEAF SPOT AND LEPTOSPHAERULINA PEPPER SPOT, TWO FOLIAGE DISEASES OF LUCERNE.

A thesis presented in partial fulfilment
of the requirements for the degree of
Masterate of Agricultural Science
at Massey University.

by

Phillip Donald Whitwell

1974

ACKNOWLEDGEMENTS

Grateful acknowledgement is made to Dr H.T. Wenham for his considerable assistance throughout the course of the study and in preparation of the manuscript.

I would also like to thank:

Dr K.S. Milne for his helpful discussions and interest,

Mr H. Neilson for technical assistance,

Mr R.W.S. Leitch and Mrs M.K. Scott of the Massey University Central Photographic Unit for the preparation of photographs,

Miss S. Bennett for typing this thesis.

SUMMARY

STEMPHYLIUM LEAF SPOT

1. The symptoms of Stemphylium leaf spot of lucerne in the Manawatu are described.

2. The morphological features of the imperfect and perfect states of the pathogen on the host conformed closely to those recorded overseas for Stemphylium botryosum Wallr. on lucerne. Conidia were sub-spherical to ovoid or oblong, light brown, echinulate, 17.6 x 29.4u, muriform, and with a major constriction at the median transverse septum. The globose, black pseudothecia contained several large (30.2 x 184.6u), cylindrical to clavate asci, with an obtuse apex tapering to a swollen or claw-like base. Ascospores were ellipsoid to clavate, yellow-brown, 16.3 x 37.2u, muriform, and with slight constrictions at all 7 transverse septa.

3. The cardinal temperatures for vegetative growth on PDA_L at 10 days were 4, 24 and 36°C. Of 9 media tested greatest growth at 24°C occurred on 20% V-8 juice agar. Gross colony characteristics changed with temperature and media.

4. Maximum conidial production occurred on PDA_L and 20% V-8 juice agar cultures exposed to continuous NUV light for 12 days at 23-27°C.

5. Greatest production of protopseudothecia occurred following exposure of actively growing colonies to NUV radiation for a minimum of 5 days at 23-27°C. Protopseudothecia matured when such cultures were incubated at 8-12°C for a further 6 weeks.

6. Germ-tubes produced from conidia streaked on PDA_L slides and incubated at 24°C were first evident within 2 hours, emerging initially from the lateral cells and later from the terminal cells of the muriform conidia. Within 4 hours 90% of the conidia had germinated, each producing between 4 and 10 germ-tubes.

7. Conidium ontogeny and morphology of mature conidia of an isolate initially identified as Stemphylium vesicarium (Wallr.) Simmons and a typical isolate of S. botryosum were compared; both isolates were considered identical and typical of the latter species.

8. Incubation temperature of isolates grown on 20% V-8 juice agar was shown to considerably affect morphology of mature conidia.

LEPTOSPHAERULINA PEPPER SPOT

1. The symptoms of Leptosphaerulina pepper spot of lucerne and red clover in the Manawatu are described.

2. Isolates from natural infections on lucerne and red clover were most readily obtained when infected tissue pieces were water-washed for 4-6 hours, plated to antibiotic PDA_L and incubated at 24°C.

3. Isolates from either host could not be differentiated on the basis of pathogenicity since in reciprocal cross-inoculations identical symptoms were produced. A species of Leptosphaerulina has not previously been recorded as a pathogen of red clover in New Zealand.

4. Viable inoculum was associated with two of 10 lucerne seed-lines screened, at levels of 0.4% and 1.2%. Following germination of seed of these two lines in a Copenhagen germinator, protopseudothecia of the pathogen were located on ungerminated seed and infected seedlings.

5. Protopseudothecia were not located in field infections, but they were readily produced in 20% V-8 juice agar or PDA_L cultures incubated at 24°C in the dark for 10 days. Protopseudothecia matured only after exposure to light for 5-7 days. Maturation was most intense under cyclic fluorescent light (12 hour light/12 hour dark) and least under natural/ diurnal light.

6. Germ-tubes produced from ascospores naturally ejected from lucerne agar isolates onto PDA_L slides and incubated at 24°C were first evident within 1 hour, emerging from either the lateral or terminal cells of the muriform ascospores. After 3 hours all ascospores had germinated, each producing between 3 and 7 germ-tubes.

7. The morphological features of the perfect state of isolates on artificially inoculated excised lucerne leaves and on agar were essentially similar, and conformed with overseas descriptions of the pathogen on lucerne. On host tissue pseudothecia were black, globose, erumpent with several large (140.1 x 78.7u), saccate, thick-walled bitunicate asci. Ascospores were oblong, ellipsoid or clavate, hyaline, 14.2 x 35.3u, phragmosporous or muriform, and surrounded by a thin gelatinous sheath.

8. Lucerne and red clover isolates on agar could not be separated on the basis of gross colony characteristics or dimensions of pseudothecia, asci and ascospores.

9. The majority of ascospores from lucerne isolates were transversely 4-septate; those of red clover isolates were predominantly 3-septate. However, this distinction in itself was considered insufficient to warrant recognition of the two series of isolates as separate species.

10. *Leptosphaerulina trifolii* (Rost.) Petr., published in 1959, has priority over *L. briosiana* (Poll.) Graham & Luttrell and is proposed as the correct binomial for the species pathogenic to lucerne and red clover.

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INTRODUCTION

Over recent years the area of lucerne (*Medicago sativa* L.) grown in New Zealand has rapidly increased (Table 1) as farmers and commercial firms have realised the many and varied virtues of this crop in farming enterprises.

TABLE 1. Areas of lucerne grown in New Zealand used for hay, silage and seed production.

Year	Area (acres)	Authority
1958-59	144,516	N.Z. Year Book, 1963
1963-64	164,860	N.Z. Year Book, 1969
1968-69	202,770	N.Z. Year Book, 1971
1970-71	300,000	Meeklah & Allen, 1971

Lynch (1967) estimated that if the present trend continued a lucerne area of at least 300,000 acres could be expected by 1977. However, this area was attained by 1970, and the increased rate of expansion can be accounted for in several ways:

1. Due to its agronomic features lucerne is primarily grown in free-draining soils subject to an unreliable summer rainfall, so ensuring a continued feed supply over this period.

2. Lucerne has a greater versatility than most crops within a farming enterprise in that it can be utilised in several ways; for example, hay and silage, grazing, seed production, and more recently for lucerne meal or pellets, and protein extraction.

3. A gross margin analysis of lucerne as a cash crop indicates a return comparable with, or better than, such other popular crops as wheat or peas (Lamb, 1969; Tocker, 1970; Anon, 1970; Anon, 1972).

Because lucerne must be treated as a cash crop, factors causing a decrease in foliage yield and quality must be minimised to ensure the greatest gross returns. Factors implicated are:

1. Agronomic. In a survey of farmer practice in relation to lucerne, Blair (1965) found the average duration of stands was nine years and that deterioration was mainly due to grass and weed invasion. This can be encouraged by a soil pH divergent from the optimum of 6.2, inefficient inoculation of seed with Rhizobium meliloti Dangeard, unsatisfactory seed-bed conditions and poor crop management.

2. Disease. This factor appears to operate in varying degrees in the decline of older stands (Close, 1967). Diseases can affect lucerne at all stages of development and so influence establishment, herbage yields, seed quantity and quality, and the longevity of stands.

Several soil and seed-borne fungi can cause pre- and post-emergence damping-off of seedlings, such as Phoma medicaginis Malbr. and Roum., Thanatephorus cucumeris (Frank) Donk, and species of Fusarium and Pythium (Close, 1967). Foliage diseases primarily cause a reduction in herbage quality and premature leaf fall, especially when infections are severe, thereby reducing the potential yield of a crop. In a study in Canada to determine the effect of leaf and stem diseases on yield, defoliation, protein and carotene content, Willis, Stuteville and Sorensen (1969) found that plots sprayed with mancozeb (Dithane M-45) yielded up to 27% more hay than unsprayed plots, increased carotene content up to 45% and decreased stem defoliation up to 27%. A similar increase in yield (up to 42%) due to leaf disease control was also demonstrated by Wilcoxson and Bielenberg (1972). Recent work with lucerne (Loper and Hanson, 1964; Loper, Hanson and Graham, 1967) and with white clover (Wong and Latch, 1971) indicated that an accumulation of coumestrol was more likely to occur in plants infected with foliar pathogens, with possible oestrogenic effects when fed to livestock. All these reports serve to emphasise the potential detrimental effects of foliar pathogens on both herbage yield and herbage quality.

Dingley (1969) records eight foliar diseases of lucerne in New Zealand (Table 2), of which common leaf spot (Pseudopeziza medicaginis (Lib) Sacc.) and Stemphylium leaf spot (Pleospora herbarum (Fr.) Rab.) are the most prevalent (Close, 1967).

The latter disease is world wide in distribution on lucerne (Benedict, 1954; Nelson, 1955; Focke, 1966; Perisic and Stojanovic, 1967) and the imperfect stage is invariably referred to as Stemphylium botryosum Wallroth. In the course of preliminary studies an isolate considered by the author to be S. botryosum was tentatively identified by Laundon (pers. comm.) as Stemphylium vesicarium (Wallr.) Simmons. This information stimulated studies on the Stemphylium disease in New Zealand, and mycological studies of the causal organism.

TABLE 2. A list of foliage pathogens recorded on lucerne in New Zealand.

Pathogen	Authority
Colletotrichum trifolii Bain & Essary	Dingley, 1965
Leptosphaeria pratensis Sacc. & Briard	Smith, 1955
Peronospora trifoliorum de Bary	Cunningham, 1922b
Phoma medicaginis Malbr. & Roum.	Cunningham, 1956
Pleospora herbarum (Fr.) Rab.	Brien & Dingley, 1959
Pseudopeziza jonesii Nannf.	Cunningham, 1922b
Pseudopeziza medicaginis (Lib.) Sacc.	Cunningham, 1922b
Uromyces striatus Schroet.	Cunningham, 1922b

In reviewing the diseases of lucerne in New Zealand, Close (1972) included a previously unreported disease first located at Putaruru in 1969 and subsequently found at Murupara, Hastings and in Mid-Canterbury. The causal organism he cited as Leptosphaerulina trifolii (Rost.) Petr., and commented that it is also common on white clover. What is apparently the same pathogen was located by the author in late 1971 in an eight acre lucerne stand in the Manawatu. This disease, commonly known overseas as pepper spot, is one of the major diseases of lucerne in the humid, temperate areas of the United States (Martinez and Hanson, 1963). Graham and Luttrell (1961) in their study of Leptosphaerulina species on forage plants considered that two distinct species existed on the Leguminosae, namely L. briosiana (Poll.) Graham and Luttrell and L. trifolii, both of which were pathogenic to Medicago and Trifolium species. Subsequently Booth and Pirozynski (1967a) listed L. briosiana as a synonym of L. trifolii

with the resulting confusion amongst plant pathologists as to the correct epithet to apply to the pathogen on lucerne. This raises the question as to whether in fact the diseases as present on lucerne and clover species in New Zealand are caused by the one morphological species.