

ETIOLOGY AND CHEMICAL CONTROL OF CERCOSPORA LEAF SPOT
OF THE ORNAMENTAL BELLS OF IRELAND (Molucella laevis).

BY

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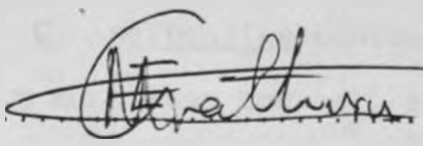
DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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This thesis has been submitted with our approval as University supervisors.

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A B S T R A C T

Production of bells of Ireland (Molucella laevis) in Kenya is currently threatened by *Cercospora* leaf spot, a highly devastating disease. Results showed that this disease is incited by Cercospora molucellae Bremer and Petrak. Investigations on C. molucellae with respect to the effects of culture media, temperatures and light regimes on sporulation commenced after collection of the isolate from Updown Agricultural Development Corporation farm. Benlate, Mancozeb and Kocide 101 were tested for control of the disease.

C. molucellae was found to sporulate best in molucella decoction agar but poor on carrot leaf decoction agar. Sporulation occurred at 20, 24 and 28°C with an optimum at 24°C. At 32°C growth was restricted and no sporulation occurred. Sporulation was induced by the 24 hr light, 24 hr dark and 12 hr light/ 12 hr dark regimes. However, 24 hr light regime induced most conidial production.

C. molucellae conidia produced germ tubes that emerged from more than one cell and penetrated host tissue passively through the stomata. Koch's postulates were verified to the effect that C. molucellae was the causal agent of cercospora leaf spot of M. laevis.

Benlate and Mancozeb effectively controlled the *Cercospora* leaf spot while Kocide 101 proved ineffective. The average flower length was highest in plots sprayed with Mancozeb (61.11cm.), followed by Benlate (58.89cm), Kocide 101 (53.28cm) and the control plot had the lowest (29.36cm).

In conclusion, *C. molucellae* should be cultured on molucella decoction agar at 24°C under 24hr light for 12 days. *Cercospora* leaf spot on *M. laevis* can be controlled by use of Benlate at 250 ppm. or Mancozeb at 2000 ppm. sprayed at two weeks interval.

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LIST OF CONTENTS

	Page
Abstract.....	1
Acknowledgements.....	iii
LIST OF CONTENTS.....	iv
LIST OF TABLES.....	ix
LIST OF PLATES.....	xi
LIST OF FIGURES.....	xiii
INTRODUCTION.....	1
LITERATURE REVIEW.....	4
2:1 HISTORY AND DISTRIBUTION OF CERCOSPORA LEAF SPOTS.....	4
2:2 SYMPTOMATOLOGY OF CERCOSPORA LEAF SPOTS.....	5
2:3 ECONOMIC IMPORTANCE OF CERCOSPORA LEAF SPOTS.....	6
2:4 CONTROL OF CERCOSPORA LEAF SPOTS.....	7
2:5 MORPHOLOGICAL CHARACTERISTICS OF <u>Cercospora molucellae</u>	9
2:6 CULTURAL STUDIES OF <u>Cercospora</u> SPECIES.....	10
2:7 HISTOPATHOLOGICAL RELATIONSHIPS.....	13

MATERIALS AND METHODS.....	15
3:1 ISOLATION OF SINGLE SPORE CULTURE OF <u>Cercospora molucellae</u>	15
3:2 INOCULUM PREPARATION	16
3:3 EFFECT OF THREE MEDIA ON SPORULATION OF <u>Cercospora molucellae</u>	16
3:3:1 Media preparation.....	16
3:3:2 Plate inoculation and incubation.....	17
3:3:3 Colony sampling and conidia counting...	17
3:3:4 Sporulation of <u>Cercospora molucellae</u> under varying light regimes and incubation temperature on a selected medium.....	18
3:5 PROOF OF PATHOGENECITY	19
3:6 THE EFFECT OF INOCULUM CONCENTRATION AND HOST PLANT AGE ON DISEASE SEVERITY.....	21
3:6:1 Disease assessment in the greenhouse...	21
3:7 EFFECTS OF THREE FUNGICIDES ON CERCOSPORA LEAF SPOT OF <u>Molucellae</u> <u>laevis</u> UNDER FIELD CONDITIONS.....	22
3:7:1 Cultural practices.....	22
3:7:2 Spray application.....	23
3:7:3 Data collection.....	24

RESULTS.....	27
4:1 CONIDIAL INOCULUM PRODUCTION.....	27
4:1:1 Sporulation of <u>Cercospora molucellae</u> in three different media.....	27
4:1:2 Sporulation of <u>Cercospora molucellae</u> at four different temperatures and three light regimes.....	30
4:2 HOST - PATHOGEN RELATIONSHIP.....	35
4:2:1 Conidial germination and penetration...	35
4:2:2 Symptomatology.....	38
4:2:3 Effect of inoculum concentration and host age on disease severity under greenhouse conditions.....	43
4:2:3:1 Inoculum concentration.....	43
4:2:3:2 Host age.....	43
4:3 EFFECTS OF BENLATE, MANCOZEB AND KOCIDE 101 ON CERCOSPORA LEAF SPOT OF <u>Molucella</u> <u>laevis</u> UNDER FIELD CONDITIONS.....	49
4:3:1 Assessment of disease incidence.....	49
4:3:2 Assessment of disease severity.....	58
4:3:3 Flower length.....	63
DISCUSSION.....	66
5:1 SPORULATION OF <u>Cercospora molucellae</u> IN MOLUCELLA DECOCTION AGAR, POTATO DEXTROSE AGAR AND CARROT LEAF DECOCTION AGAR.....	66

5:2	SPORULATION OF <u>Cercospora molucellae</u> AT FOUR DIFFERENT TEMPERATURES AND THREE LIGHT REGIMES.....	67
5:2:1	Effect of temperature on sporulation.....	67
5:2:2	Effect of light on sporulation.....	68
5:2:3	Effect of light, temperature and in- cubation time on sporulation.....	69
5:3	HISTOPATHOLOGICAL RELATIONSHIP OF <u>Cercospora molucellae</u> AND <u>Molucella</u> <u>laevis</u>	71
5:3:1	Conidial germination and penetration.....	71
5:3:2	Effects of inoculum concentration and host age on disease intensity.....	71
5:3:2:1	Effects of inoculum concentration.....	71
5:3:2:2	Effects of host age.....	73
5:4	EFFECTS OF THREE FUNGICIDES ON <u>CERCOS-</u> <u>PORA</u> LEAF SPOT OF <u>Molucella laevis</u> UNDER FIELD CONDITIONS.....	74
5:4:1	Effect of spray interval on disease incidence and severity.....	75
5:4:2	Disease incidence and severity in control plots.....	76

5:4:3	Disease incidence and severity in plots sprayed with Kocide 101.....	76
5:4:4	Disease incidence and severity in plots sprayed with Benlate and Mancozeb.....	77
5:4:5	Overall effect of fungicides on disease incidence and severity.....	78
5:4:6	Overall trend in disease incidence and severity.....	78
5:5	EFFECT OF FUNGICIDES AND SPRAY INTERVAL ON FLOWER LENGTH.....	79
5:5:1	Flower length in the control and Kocide 101 treated plots.....	79
5:5:2	Flower length in plots treated with Mancozeb and Benlate.....	80
	CONCLUSIONS.....	81
	REFERENCES	82

LIST OF TABLES

Page

Table 1:		
	Infection grades based on percentage of diseased leaf area.....	25
Table 2:		
	Effect of three different media and incubation period on sporulation of <u>Cer-</u> <u>cospora molucellae</u>	31
Table 3:		
	Effect of the three different temperatures, and three light regimes on sporulation of <u>Cercospora molucellae</u>	
Table 4:		
	Effect of three different temperatures, incubation period and three light regimes on sporulation of <u>Cercospora molucellae</u> ...	36
Table 5(a):		
	Effect of four different inoculum concen- trations and host plant age on percentage leaf area diseased.....	45

Table 5(b):	
Effect of four different inoculum concentrations and host plant age, on the number of lesions per leaf.....	47
Table 6(a):	
Effects of fungicides, time in days after inoculation and spraying interval on percentage of diseased plants.....	50
Table 6(b):	
Effects of fungicides, time in days after inoculation and spraying interval on percentage of diseased leaves.....	51
Table 7(a):	
Effects of fungicides, time in days after inoculation and spraying interval on lesions per leaf.....	61
Table 7(b):	
Effects of fungicides, time in days after inoculation and spraying interval on Grade (percentage of diseased leaf area).....	62
Table 8:	
Effects of fungicides and spraying interval on flower length of harvest.....	65

LIST OF PLATES

Plate 1:	Inoculation of <u>Molucella laevis</u> plants in the greenhouse using a Baygon atomiser...	20
Plate 2:	Illustration of the above infection grades based on percentage leaf area diseased.....	25
Plate 3:	Conidiophore and conidia of <u>Cercospora</u> <u>molucellae</u> showing variation in number of septa and length.....	29
Plate 4:	<u>Cercospora molucellae</u> conidium germinating on <u>Molucella laevis</u> leaf surface three hours after inoculation.....	37
Plate 5:	A germ tube penetrating the leaf through a stomata 24 hours after leaf inoculation....	39
Plate 6(a):	Symptoms of cercospora leaf spot of <u>Molucella laevis</u> leaf.....	40

Plate 6(b): Defoliation, stunting and poor tillering on diseased plant (right) as compared to the control (left).....	41
Plate 6(c): (A) Diseased bells of Ireland (infected by <u>Cercospora molucellae</u>) 21 days after inoculation. (B) Healthy bells of Ireland (control).....	42
Plate 7(a): Plants in plots sprayed with water without fungicides (control).....	54
Plate 7(b): Plants in plots sprayed with Kocide 101.....	55
Plate 7(c): Plants in plots sprayed with Benlate.....	56
Plate 7(d): Plants in plots sprayed with Mancozeb.....	57
Plate 8: The five market grades of <u>Molucella</u> <u>laevis</u> cut flower.....	64

LIST OF FIGURES

Page

Figure 1:

Effect of three different media and incubation period on sporulation of Cercospora molucellae..... 32

Figure 2:

Effects of three different temperatures, and three light regimes on sporulation of Cercospora molucellae 34

Figure 3(a):

Effect of conidial concentrations of Cercospora molucellae and host plant age on percentage diseased leaf area 12 days after inoculation..... 44

Figure 3(b):

Effect of conidial concentrations of Cercospora molucellae and host plant age on number of lesions per leaf 12 days after inoculation..... 46

Figure 4:

Effects of fungicides, time in days after inoculation and spraying interval on percentage of diseased leaves..... 52

Figure 5:

Effects of fungicides, time in days after inoculation and spraying interval on percentage of diseased plants..... 53

Figure 6:

Effects of fungicides, time in days after inoculation and spraying interval on lesions per leaf..... 59

Figure 7:

Effects of fungicides, time in days after inoculation and spraying interval on Grade (Percentage of diseased leaf area)..... 60

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

Cut flower production in Kenya is becoming increasingly popular as a source of income from both domestic and export markets. Cut flowers form a major export market commodity within the horticultural industry. They represent about 24% of the market by volume and 56% by earnings (Anon. 1987). Kenya exports over 25 different ornamentals among which carnations, Alstroemeia, dillies, euphorbia, roses, stattice, ornithogolums, lilies and Molucella laevis are most important.

Molucella laevis (also known as 'bells of Ireland', 'Molucella, balm' and 'shell flower') is ranked among the ten most important cut flowers in the country (Anon. 1987). The cut flower is characterised by a long stem covered with light green bell-shaped calyx. Bells of Ireland belong to the family Labitae and it's primary center of diversity was Western Asia (Larson, 1980). This ornamental has been grown in Kenya since 1957 (Jex-Blake, 1957), although commercial production started in the 1970's. Currently, the two major producers of this cut flower are Oserian and Sulmac companies. However, Updown Agricultural Development Corporation (ADC) farm in Tigoni and a few scattered small scale farmers do make some contribution to the national

production. The ornamental is contributing much to the needed foreign exchange earnings. The major export markets include. Holland, West Germany, Netherlands, Switzerland, Saudi Arabia and United States.

M. laevis has a yield potential of 90-100 stems per 100 m² (Sahim, 1987). This is barely realised in Kenya. Low crop yield is normally attributed to poor agronomic practices, insufficient or lack of inputs, diseases and pest problems. Considering that producers employ effective horticultural practices, disease problems can be singled out as the most outstanding of these factors. In Kenya diseases reported on 'bells of Ireland' include, crown rot caused by Myrothecium species, (Ondiek, 1973), bacterial wilt incited by Erwinia species (Anon. 1985) and Cercospora leaf spot incited by Cercospora molucellae (Gatumbi, 1985). Of these, Cercospora leaf spot is the most threatening and was first reported in Kenya in 1984 in all Molucella growing areas.

Although 'bells of Ireland' is a hardy plant, Cercospora molucellae appears to cause considerable damage. Diseased plants first exhibit small, gray to brown circular or irregular necrotic spots 5-10 mm in diameter on leaves, bells and stems. Lesions enlarge and coalesce thus causing a blight. In severe cases, the bells dry up leading to acute defoliation problem.

The necrosis and defoliation reduce the photosynthetic area of the plant. Consequently, this affects the product quality. Cut flower grading is based on freedom from disease, pests and injuries. Stem length and proportion of the stem covered by the bells are also taken into account.

In 1984, the *Cercospora* leaf spot problem was so severe that the National Agriculture Laboratory Advisory Section recommended destruction of the ornamental in all affected fields. The immense crop damage, waste of scarce resources and consequent loss of foreign exchange earner attributed to the leaf spot prompted the commencement of this investigation.

The present investigation was undertaken with the following objectives:

- I. Study the etiology of *Cercospora molucellae* with respect to:
 - (a) the effect of various media on sporulation
 - (b) the effect of incubation time, temperature and light on sporulation.
 - (c) the histopathological relationship of *C. molucellae* and *M. laevis*.
- II. Field screening of fungicides for the control of *Cercospora* leaf spot of *Molucellae laevis*.

LITERATURE REVIEW

2:1 HISTORY AND DISTRIBUTION OF CERCOSPORA LEAF SPOTS

Cercospora leaf spots are widely distributed and have been reported in Africa, North and South America, Asia and Europe. From the list compiled by Chupp (1953), the disease occurs on 148 different plant families. In Kenya it has been reported on 60 hosts (Nattrass, 1961; Ondiek, 1973).

Cercospora leaf spot of M. laevis, caused by Cercospora molucellae Bremer and Petrak was first reported in Turkey in 1943. It was reported in Kenya in Tigoni, Kiambu and Naivasha in 1984 (Gatumbi, 1985). Ondieki had mentioned the presence of C. molucellae in 1973. Proof of pathogenicity was never carried out despite the occurrence of the disease in all M. laevis growing areas in Kenya.

Generally, *Cercospora* leaf spot is a late disease. On maize, it normally strikes at silk stage (Rupe et al., 1982; Hilty et al., 1979) and on groundnuts, it strikes 11 weeks after planting (Hemingway, 1954, 1955). On M. laevis, it normally occurs at the inflorescence stage. Like any other fungal disease, it's effect is most devastating in the presence of high relative humidity and low temperature.

2:2 SYMPTOMATOLOGY OF CERCOSPORA LEAF SPOTS

Leaf spots varying in distinctness from a faint discoloration to characteristically marked necrotic lesions are typical of cercospora leaf spots (Chupp, 1953). These turn ashy grey if environmental conditions favour sporulation.

Lesion shape varies from irregular, circular, elliptical to rectangular as dictated by leaf venation. Rectangular lesions measure 0.5 to 1.0 to 2.5 cm, whereas the others have a diameter of 2-7 mm (Kingsland, 1963; Latch and Hanson, 1962; Hemingway, 1954; Baxter, 1956). Coalescence produce aggregate lesions of greater dimensions and in corn and cassava, severe leaf blight has been reported (Kingsland, 1963; Teri et al., 1980). With severe pathogen attack, dying portions get dehydrated and in shrinking tear away from the living tissues leaving a shot-hole effect. Complete defoliation is caused by virulent species. Many Cercospora species also infect the blossoms, fruits, pods, succulent petioles and young stems (Chupp, 1953).

On M. laevis the cercospora leaf spot is characterised by grey to brown circular or irregular necrotic spots 5-10 mm in diameter on leaves, calyxes and stems. The lesions coalesce to form a blight and in severe attack, defoliation follows. Infected calyxes dry up.

2:3 ECONOMIC IMPORTANCE OF CERCOSPORA LEAF SPOTS

Crop damage attributed to Cercospora species attack are both direct and indirect. They extend from the field to storage (Garry and Ruppel, 1971; Hemingway, 1954; Hilty et al., 1979).

A decline in photosynthetic area due to both necrosis and defoliation is the initial effect of Cercospora leaf spot on the host plant. On groundnuts, duration of growth is determined by defoliation rate (Elston et al., 1976) and complete defoliation results in premature death of the crop (Hemingway, 1954).

On corn, crop losses ranging from 50 to 100% have been reported (Hilty et al., 1979; Nevill, 1981; Roane et al., 1974). In most cases, leaf blight is followed by severe stalk rot and breakage. The breakage does not only result in additional ear damage but also causes inefficiency in the harvesting operation. (Hemingway, 1954; Hilty et al., 1979)

On Sugar beet, 50% loss due to rot during storage has been reported (Garry and Ruppel, 1971). Disease incidence in the field closely paralleled the number of harvested roots that rotted in storage.

There is no documented report on the effects of Cercospora leaf spot on M. laevis but field visits have

revealed total crop failure due to the attack. In 1984, there was a general outcry from farmers in M. laevis growing areas due to the disease. The situation was so devastating that the National Agriculture Laboratory Advisory Section recommended destruction of the crop in all infected areas (personal communication from Gatumbi).

2:4 CONTROL OF CERCOSPORA LEAF SPOTS

No investigation has been carried out in an attempt to control Cercospora leaf spot of M. laevis. However, various control measures have been recommended for control of other Cercospora leaf spots.

Use of resistant varieties has been singled out as the most economical measure in the control of Cercospora leaf spots. In maize, cassava, peanut and groundnuts, sources of resistance have been identified and resistant cultivars developed (Hemingway, 1957; Grover et al., 1976; Hilty et al., 1979; Nevill, 1981). Immediate application of this disease control method is limited by the long gestation period required in development of resistant varieties.

A wide range of cultural practices is commonly used in the disease control. Crop losses have been significantly reduced by burning and deep ploughing infected plant debris. A two year crop rotation has also proved effective

(Hemingway, 1954; Latch and Hanson, 1962; Jackson, 1980; Smith and Littrell, 1980). On maize, the disease is associated with continuous production under minimum tillage. To reduce crop loss, deep ploughing of plant residues before planting has been recommended while the advantages of minimum tillage under severe infection by Cercospora zea maydis were being re-evaluated (Roane et al., 1974; Hilty et al., 1979).

A few Cercospora species are seed-borne (Fawcett and Klotz, 1938; Hemingway, 1954). Use of clean certified seeds especially in areas where the disease has not been reported is a potential exclusion measure.

Application of fungicides is a common practice which has given encouraging results. Harrison (1969) reported 100% increase in peanut yield and 90% reduction in defoliation when he used Danonil 2787 80 wp at 1.5 pounds per acre and Benlate (methyl-1-(butylcarbonyl)benzimidazol-2-carbamate) at 0.3 pounds per acre. Both fungicides were sprayed at two week intervals. In 1980, Smith and Littrell, in an attempt to control peanut leaf spot used Benlate, copper hydroxide, chlorothalonil (tetrachloisophthalonitrile) and Mancozeb, a zinc ion and manganese ethylenebisdithiocarbamate. Their results were very promising. They realised 35.4% reduction in defoliation and 60% increase in yield.

They also carried out a two year trial in control of leaf spot on Vigna unguiculata and achieved 83.8% and 72.7% control. Cercospora leaf blight of cassava has been controlled by spraying 2.5-5.0 gm of Benlate in 19 litres of water at 7 day intervals (Teri et al., 1980).

Harrison (1969) doubted the effectiveness of Mancozeb whereas Elston et al (1976) realised 65% increase in total groundnut weight by using 1.8 kg Mancozeb in 400 ml of water at 7 day intervals. 50% increase in maize yield has been realised by spraying Mancozeb at 2 gm per litre at 10-14 day interval (Hilty et al., 1979), Various fungicides have been recommended for leaf spot control on ornamental plants. Among these are Maneb, Mancozeb, Ferbam, Zineb, Ziram and Benlate (McCain, 1975).

2:5 MORPHOLOGICAL CHARACTERISTIC OF Cercospora molucellae

Cercospora molucellae Bremer and Petrak, is the causal agent of cercospora leaf spot of Molucella laevis (Chupp, 1953). It belongs to the form genus Cercospora which is placed in the form-family dematiaceae and in the form-order Moniliales of the form-class Deuteromycetes and subdivision Deuteromycotina (Alexopolous and Minns, 1979).

Morphological characteristics of the conidia and conidiophore provide the main taxonomic criteria for species delimitation (Hughes, 1953). Conidiophore of C. molucellae are light brown, paler near the apex, subtruncate, straight to curved, unbranched, rarely geniculate and of the dimensions $4-5.5\mu \times 50-500\mu$. Conidia are hyaline, acicular, straight to curved, with truncate base and acute tips. They are multiseptate measuring $3-5\mu \times 80-250\mu$ (chupp, 1953).

2:6 CULTURAL STUDIES OF Cercospora SPECIES

Extensive work has been carried out worldwide on a number of Cercospora species but virtually none has been done on C. molucellae. Germ tubes emerge from any of the conidial cells within 4 hours (Cooperman and Jenkins, 1986; Jenkins, 1938, Alderman and Beute, 1986). In 1969, Sober noted that on Potato Dextrose Agar (PDA), the genus is characterised by gray colonies with or without a pink pigment. Sporulation is not easy to achieve and a series of workers have reported on the fastidious requirements for in vitro sporulation (Nagel, 1934; Diachun and Valteau, 1941; Goode and Brown, 1970; smith, 1971). Owing to this, it has been common practice to subject a few isolates of a given species to various cultural manipulations. This involves such variables as nutrition, light and temperature (Calpouzos

and Stallknecht, 1965,1967; Berger and Hanson,1963; Miller, 1969; Vathakos and Walters, 1979; El-Gholt et al., 1982).

As early as 1934, Nagel found that the nature of the nutrient substratum has a strong bearing on conidial production. He further observed that a medium suitable to a particular species for optimal sporulation may not prove satisfactory for other species of Cercospora.

Profuse sporulation of various Cercospora species on their respective host decoction agar has been reported by a series of workers to occur 7-21 days from time of seeding. This has been observed on C. nicotianae, C. beticola C. zebrina and C. arachidicola (Diachun and Valleau, 1941; Berger and Hanson, 1963; Calpouzos and Stallknecht, 1965; Smith, 1969 and El-Gholt et al., 1982). Despite this, erratic sporulation on same has been reported by Cooperman and Jenkins (1986) and Vathakos and Walters (1979) while working with C. asparagi and C. Kikuchii respectively. The latter pair observed abundant conidiophore formation and extremely sparse sporulation.

Carrot leaf decoction agar is yet another medium which has proved useful in induction of sporulation of Cercospora species. Abundant sporulation on the carrot medium has been attained with C. asparagi, C. kikuchii, C. Zebrina and C. davisii (Kilpatrick and Johnson, 1956;

Latch and Hanson, 1962; Berger and Hanson, 1963; Cooperman and Jenkins, 1986)

PDA is a common medium for culturing fungi but success in inducing sporulation of Cercospora species has been reported only in a few cases (Latch and Hanson, 1962; Sober, 1969; Nagel, 1934).

In 1965, Calpouzos and Stallknecht, not only emphasised the importance of selection of an appropriate medium but also the interaction between light and temperature for maximum sporulation. Continuous darkness has been found as the optimum condition for sporulation of C. nicotianae, C. davisii and C. kikuchii (Vathakos and Walters, 1979).

Alternative dark and light period favours sporulation of C. zebrina, C. asparagi and C. kikuchii (Berger and Hanson, 1963; Cooperman and Jenkins, 1986; Kilpatrick and Johnson, 1956). There are yet other Cercospora species which require exposure to continuous light for sporulation to occur. This is the case with C. beticola as reported by Calpouzos and Stallknecht (1967).

Cercospora species grow and sporulate within a temperature range of 15-30°C. The optimum for growth and sporulation reported for C. zebrina, C. asparagi, C. davisii is 24°C while for C. gossypina, C. beticola, and

C. nicotianae it is 21-29°C, 15°C, 26°C respectively. This was reported by, Calpouzos and Stallknecht (1967), Stavely and Nimmo (1968), Miller (1969), Cooperman and Jenkins (1986).

2:7 HISTOPATHOLOGICAL RELATIONSHIPS

Infection through artificial inoculation of host plants has been accomplished at ages between two weeks and ten months using varying conidial concentrations. On leaf surfaces, conidia germinate from one or more cells within 3-6 hours (Jenkins, 1938; Baxter, 1956; Berger and Hanson, 1963). Passive ingress (through stomata) has been observed on C. medicaginis, C. zebrina and C. davisii with the earliest penetration occurring 24-48 hours after inoculation. Except in the case of C. beticola, no appressoria are formed over stomatal opening (Baxter, 1956; Latch and Hanson, 1962). Chupp (1953) and Solel and Minz (1971) stated that the fungus kills the infected part as it penetrates the host tissue. Thus, the lesion size depends upon the host or the ability of the fungus to overcome mechanical obstructions such as veins.

The incubation period ranges from 6-22 days depending on the isolate, inoculum concentration, host plant and prevailing temperature (Latch and Hanson, 1962; Berger

and Hanson, 1963; Cooperman and Jenkins, 1986). With favourable conditions, the pathogen sporulates on infected plant tissues. On leaves, stromata form beneath the epidermis in substomatal chambers or between guard cells. As the stromata enlarges, they force guard cells apart and rupture the epidermis. In some cases, the stromata emerge through the stomata and thus are slightly erumpent on leaf surfaces (Berger and Hanson, 1963; Beckman and Payne, 1981). In some cases, stromata remain buried beneath the epidermis and emerge through stomata.

MATERIALS AND METHODS

3:1 SINGLE SPORE CULTURE OF Cercospora molucellae

In March 1987, Molucella laevis leaves exhibiting characteristic cercospora leaf spot symptoms were collected from Updown ADC farm in Kiambu District in Central Province.

With the aid of a dissecting microscope, conidia were picked from the leaves by use of a fine sterile blade. These were planted in water agar and incubated at room temperature (22 - 24°C). Colonies formed were carefully cut out and transferred into Molucella leaf decoction agar (MDA). Onto a six day old plate culture, 10 ml of sterile distilled water was added and by use of a bent glass rod, the culture surface was gently scraped to make a conidial suspension. The conidial concentration was determined by use of a Neubaure improved haemocytometer. This was then adjusted to 10 conidia per ml by serial dilution. One ml of this suspension was poured onto a water agar plate, spread evenly and incubated at room temperature. With a dissecting scope, a small agar block containing a single germinating conidium was cut out after ten hours. This was transferred into an agar plate and incubated at 20 - 22°C for seven days. A conid-

dial suspension was then prepared and stored in vials at 4-6°C.

3:2 INOCULUM PREPARATION

One ml of conidial suspension was pipetted and evenly spread onto plates of MDA. These were incubated at room temperature for 7 days. Twenty ml of sterile distilled water was then added to each plate culture and conidia were detached by gently rubbing the culture surface with a bent glass rod.

3:3 EFFECTS OF THREE MEDIA ON SPORULATION OF Cercospora molucellae

3:3:1 Media preparation

Carrot leaf decoction agar (CDA), PDA and MDA were tested for their effect on sporulation. To prepare CDA, 300 gm of plant tissue were macerated by use of a blender (National Solid State Control Model) for two minutes and then added to 500 ml of distilled water. The mixture was boiled for five minutes, then strained through two layer of cheese cloth. The strained decoction was added to 12 gm of agar and the volumes adjusted to one litre. The media was autoclaved for 15 minutes at 121°C. MDA was similarly prepared from 500 gm of plant tissue.

3:3:2 Plate inoculation and incubation.

A conidial suspension was prepared and the spore concentration was adjusted to 20,000 per ml. One ml of suspension was pipetted into petri-dishes containing agar media and evenly spread by use of a bent glass rod. For each of the three media, 12 cultures were used. Plates were individually wrapped with parafilm and incubated at room temperature on a 12 hr light and 12 hr darkness schedule. Conidial counts were taken on the 5th, 9th, 12th and 15th day after inoculation. This experiment was carried out in a completely randomized design with three replicates. The treatments were the four sampling periods and the three media (PDA, CDA and MDA).

3:3:3 Colony sampling and conidia counting.

After each incubation period, three 18 mm disks were randomly cut from each of the three agar cultures for each medium. Each colony piece was placed in 10 ml distilled water in a pyrex test tube and shaken for five minutes on a Netz motor oscillating shaker (model IKA-Schuttler S 50) at the highest level of speed control.

For each colony sample, four counts were made each consisting of nine values from the nine 1 mm² areas of the

haemocytometer .

Analysis of variance was carried out and significantly different treatment effects at the 5% level of the F-test were separated using Duncan's Multiple Range Test.

3:4:2 SPORULATION OF Cercospora molucellae UNDER VARYING LIGHT REGIMES AND INCUBATION TEMPERATURE ON A SELECTED MEDIUM

Molucella leaf decoction agar was prepared and agar plate inoculation carried out. The temperatures used were 20°C, 24°C, 28°C and 32°C. Four Gallenkamp illuminated cooled incubators were used. Some plates were under 24 hr darkness, others were under 24 hr light and others were under 12 hr light/12 hr dark. The light source within the incubator consisted of 2 fluorescent tubes (Cadillac cool white, 8W-159 VL, 26 cm long) per shelf. Tubes were located 10-15 cm above the shelf, one on the left and the other on the right side of the incubator. Twelve plate cultures were incubated for each light-temperature treatment. Plates incubated in 24 hr dark were wrapped in aluminium foil while those under 12 hr light/ 12 hr dark had the foil removed every 12 hours. All the plates were randomly placed on the shelves. Conidial counts were made on the 5th, 9th, 12th and 15th day after inoculation and statistical analysis carried out.

3:5 PROOF OF PATHOGENICITY

Molucella laevis seeds obtained from Updown ADC farm were seeded in pots containing soil, sand and manure in the ratios 2:1:1 respectively. Six weeks later, a Cercospora molucellae conidial suspension (20,000 con/ml) was sprayed on both surfaces of all leaves present on the seedling. This was achieved by use of a half litre Baygon atomiser (Bayer East Africa Ltd) held at a distance of 10-15 cm away until runoff (Plate 1). Plants were then subjected to high relative humidity which was attained by covering them with moist polythene bags for 5 days. The bag was supported by a stick in the center of the pot and fastened at the bottom with a rubber band. Plants were left to stand in the greenhouse where the average temperature was 24.5°C. After 14 days, leaves showing characteristic Cercospora leaf spot symptoms were detached and reisolation of the pathogen carried out.

Pathogen growth on the host was monitored so as to observe time of germination and mode of penetration. To facilitate this, several leaves were cut into small pieces and dropped into vials containing Farmer's fluid. This was done at 3, 6, 12, 24, 36 and 48 hours after inoculation. Each harvest was placed in a different vial which had time of harvest marked on it. Twenty four hours after leaf material was added to Farmer's fluid, it was transferred into



Plate 1:

Inoculation of Molucella laevis plants with Cercospora molucellae conidial suspension in the greenhouse, using a Baygon atomiser.

vials containing lactophenol for clearing for same period of time. The same specimens were transferred into fuchsin stain for 48 hours for the purpose of staining fungal cells. The specimens were then mounted in 50% glycerine on glass slides for microscopic observations.

3:6 THE EFFECT OF INOCULUM CONCENTRATION AND HOST PLANT AGE ON DISEASE SEVERITY

In this experiment seeding was programmed so that at inoculation time, plants 5, 7 and 9 weeks old were available.

A conidial suspension was prepared and its concentration was adjusted to 5.4×10^6 , 2.7×10^6 , 1×10^6 and 0.5×10^6 conidia per ml using a haemocytometer. Inoculum was sprayed on both surfaces of all leaves present using a half litre Baygon atomiser. Plants were then subjected to high relative humidity for 5 days and left to stand in the greenhouse where the average temperature was 24.5°C .

3:6:1 Disease assessment in the greenhouse.

Twelve days after inoculation, disease intensity was assessed on the inoculated leaves using two methods. The first one was based on the number of lesions per leaf. The second one focused on percentage leaf area diseased which

was determined by use of a leaf area meter.

A completely randomised design was used, with four replicates. Treatments were plant age and inoculum concentration. Leaves sampled for pathogen development observations were obtained from plants different from the above. The significantly different effects at the 5% level of the F-test were separated using Duncan's Multiple Range Test.

3:7 EFFECTS OF THREE FUNGICIDES ON CERCOSPORA LEAF SPOT OF Molucella laevis UNDER FIELD CONDITIONS.

3:7:1 Cultural practices.

A trial was made at Field Station, Faculty of Agriculture, University of Nairobi with the aim of investigating the effects of three fungicides on Cercospora leaf spot of Molucella laevis. Clean M. laevis seeds were obtained from Updown ADC farm and were planted on 10th November, 1987 in a randomised complete block design with three replicates. Each block consisted of eight experimental units each measuring 2.5 x 2.25 m. Direct sowing was employed with three seeds per hole (3 cm depth) at a spacing of 20 x 20 cm. Seven days later, two lines of oats were planted around each experimental unit to act as a barrier to chemical drift and minimise inoculum dissemination among experimental units.

Plants were irrigated twice per week and three hand weedings maintained the field weed-free. Three weeks after planting thinning and gapping was done to leave one plant per hole. The field was uniformly top dressed with calcium ammonium phosphate twice at a rate of 1kg per 50 m² bed (200 kg/ha). Fifty two days after sowing, the plants were disbudded every two weeks so as to leave the main shoot and three axilaries from the first node.

3:7:2 Spray application.

Three fungicides were applied at one and two week interval, starting eight weeks after planting. They were applied at the following rates: Benlate 0.5 kg/1000 litres of water, Mancozeb 1.5kg/400 litres of water and Kocide 101 0.9kg/455 litres of water.

Eight weeks after planting, seven of the experimental units in each block were sprayed with the respective fungicides by use of a one litre Baygon atomiser. The two controls were sprayed with water. A day before spraying, the field had three hours of continuous irrigation.

One day after fungicide application, inoculum was prepared and its concentration adjusted to 10,000 conidia per ml. Inoculation of seven of the experimental plots per block was accomplished by use of an atomiser. Following

inoculation, the three blocks were covered with transparent polythene sheet for five days to ensure high relative humidity.

3:7:3 Data collection.

A centrally located area measuring 0.5 x 0.5 m with a population of 25 plants was demarcated for data collection in each experimental unit. Disease assessment exercise was carried out four times (on day 14, 19, 23 and 27) after inoculation. Disease incidence was taken as the percentage plants and leaves showing symptoms.

Unlike the case of incidence assessment where all plant units in the sampling were considered, only the most severely diseased leaf from each plant was assessed for severity rating. This was estimated by use of a scale consisting of eight grades based on percentages of leaf area diseased (Table 1, Plate 2). This was a modification of the scale devised by Horsfall and Barratt (1945).

Disease severity was also estimated by the number of lesions per leaf. At harvest, the flowers were graded by measuring the total flower length and the diseased length of the cut flower.

Analysis of variance (ANOVA) was computed for:-
grade, lesions per leaf, percentage leaves and plants di-

Table 1:

Infection grades based on percentage, of diseased leaf area.

GRADES	PERCENTAGE OF DISEASED LEAF AREA
1 (A)	0
2 (B)	1 - 3
3 (C)	4 - 10
4 (D)	11 - 20
5 (E)	21 - 40
6 (F)	41 - 60
7 (G)	61 - 80
8 (H)	>80 and/or defoliation



Plate 2:

Illustrations of the above infection grades based on percentage of diseased leaf area.

seased, and flower length with respect to the three fungicides treatments and spray interval.

R E S U L T S

4:1 INOCULUM PRODUCTION

4:1:1 Sporulation of Carcospora molucellae in three different media.

Within 10 hours, conidia germinated by means of germ tubes. These grew to form tiny colonies by the 4th day. Grey colonies with a pink pigment were formed on MDA while those on CDA, were dark grey with a black pigment. The white colonies formed on PDA, showed variation in pigmentation ranging from pink, yellow and orange.

Sporulation occurred 5 days after plate inoculation. Conidia were borne in sympodula arrangement on conidiophore (Plate 3a). The conidia were hyaline, acicular, with truncate base and acute tips. The number of septa varied from 5, 8, 10 and 25. Conidia length ranged from 60u to 195u (Plate 3b, 3c, 3d and 3e).

Sporulation occurred in all the three media tested. For PDA and MDA, maximum sporulation occurred on the 12th day after which there was a decline. In CDA, sporulation increased from the 6th day upto the 15th day (Table 2 and Fig. 1). Germinating conidia were noted on the 12th day yet

Plate 3: Conidiophore and conidia of Cercospora
molucellae showing variation in the
number of septa and length.

A: Conidiophore showing hilum structures.
Magnification 1800 times.

B: Conidia with 10 septa.
Length: 195 μ
Magnification: 1800 times.

C: Conidia with 25 septa.
Length: 153 μ
Magnification: 1800 times.

D: Conidia with 8 septa.
Length: 75 μ
Magnification: 7200 times.

E: Conidia with 5 septa.
Length: 70 μ
Magnification: 7200 times.

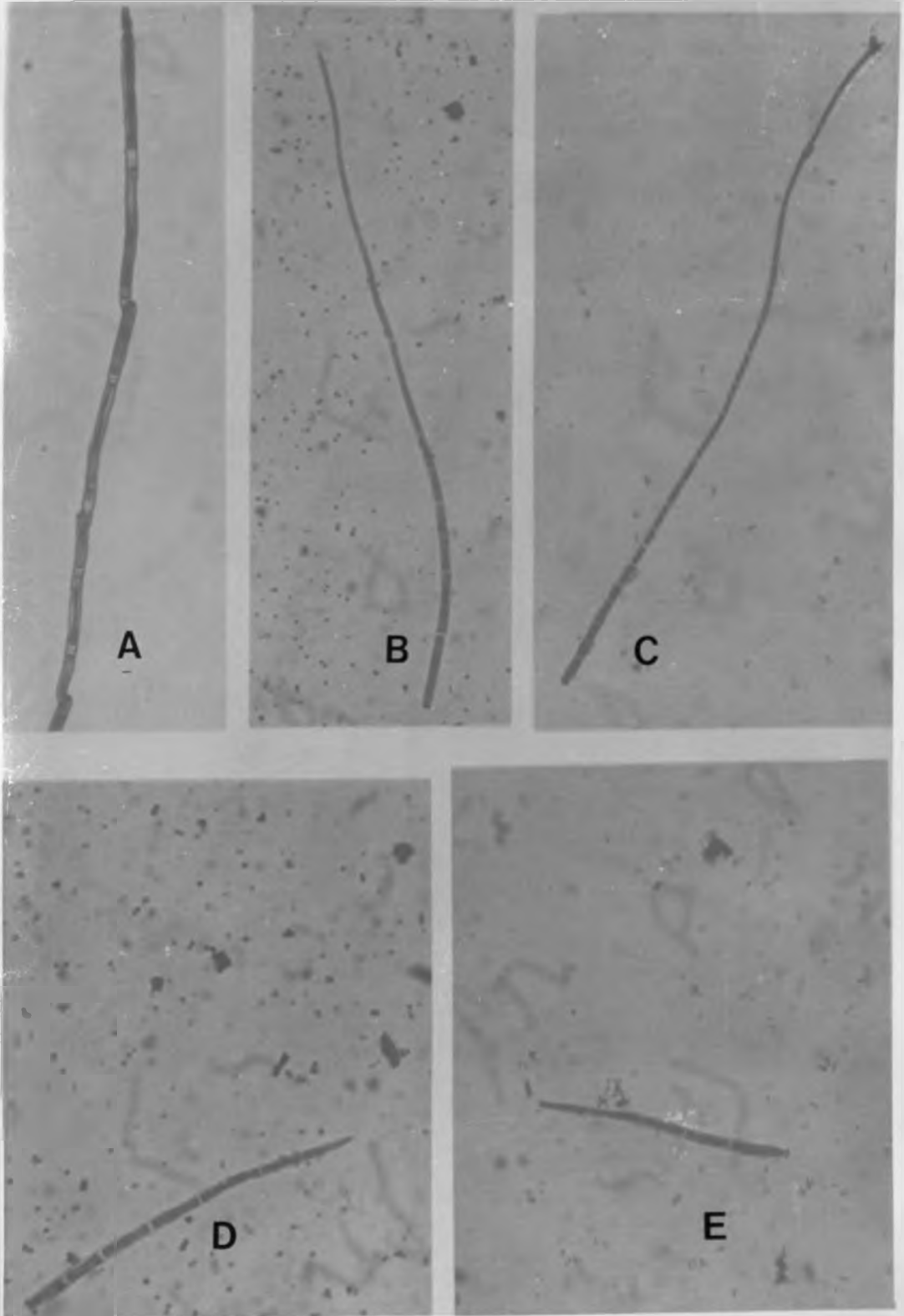


Plate 3:
 Conidiophore and conidia of Cercospora
molucellae showing variation in number of
 septa and length.

sporulation was observed 5 days after inoculation.

41112 Sporulation of Cercospora molucellae at four different temperatures and three light regimes.

Results on sporulation of C. molucellae at 20, 21 and 28 C under the three light regimes are summarised in Table 3. At 32 C, growth was restricted and sporulation did not occur, thus results at this temperature are omitted from Table 3.

At 20 C, under 24 hr light and 24 hr dark sporulation increased with time and peaked on the 12th day after which there was a decline. Under 12 hr light/12 hr dark sporulation increased throughout the four sampling days (Figure 2).

At 24 C, under 24 hr light, sporulation increased with time and peaked by the 12th day after which there was a decline. There was a gradual increase in sporulation under both the 24 hr dark and 12 hr light /12 hr dark treatments.

At 28 C, maximum sporulation occurred by the 12th and 9th day under 24 hr light and 24 hr dark conditions respectively. There was a gradual increase in sporulation under 12 hr light/12 hr dark conditions.

Table 2: Effect of three different media (M) and incubation period (T) on sporulation (conidia/ml $\times 10^3$) of Cercospora molucellae.

TIME IN DAYS AFTER PLATE INOCULATION (T)					
MEDIA (M)	6	9	12	15	M MEANS
MDA	5.51	7.50	8.65	3.69	6.35 a*
PDA	3.27	4.54	5.81	4.18	4.45 b
CDA	0	0.18	1.82	3.63	1.41 c
T MEANS	2.93	4.07	5.42	3.83	

* Means with same letter within a column are not significantly different (Duncan's multiple range test).

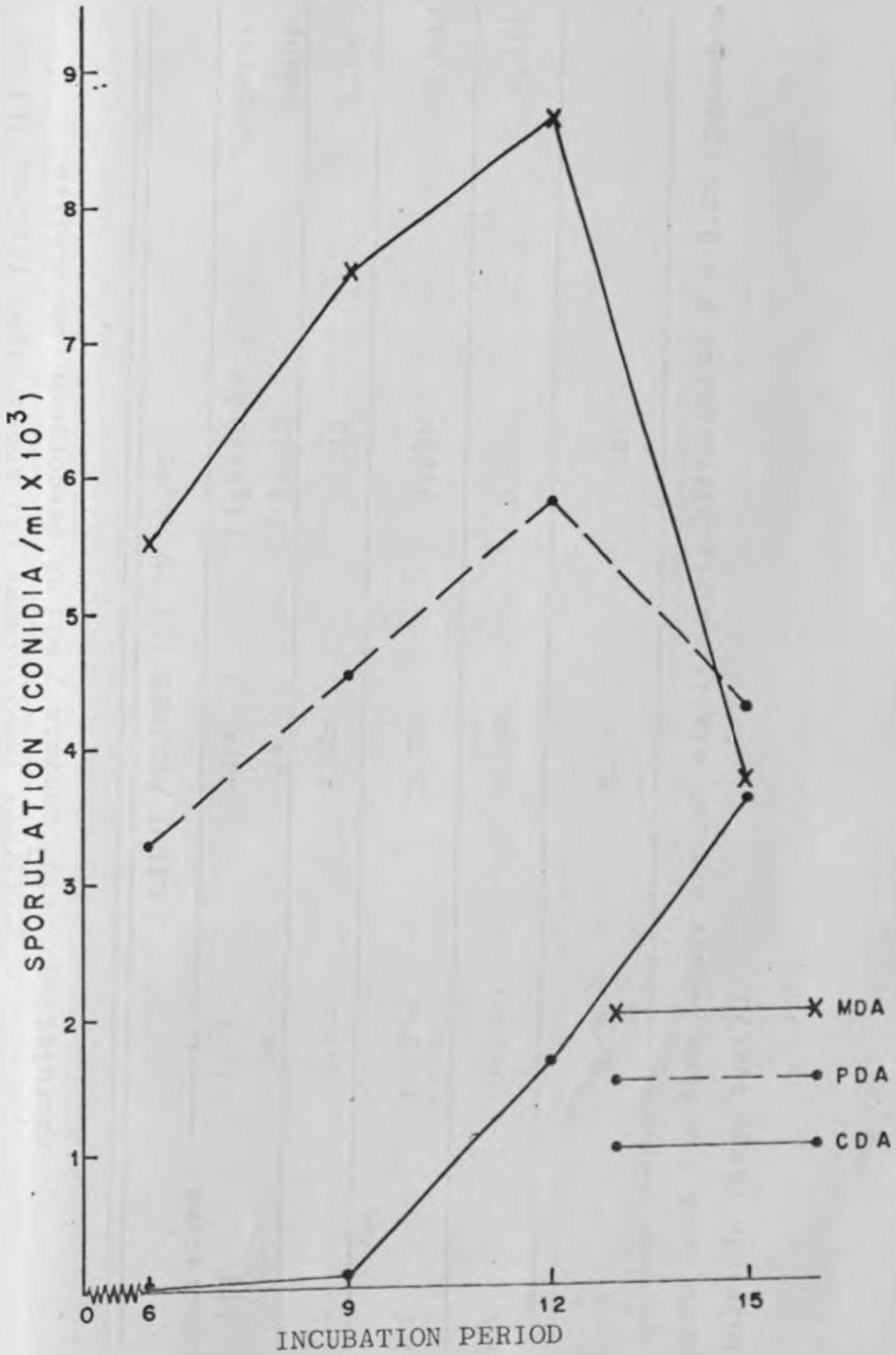


Fig. 1: Effect of three different media and incubation period on sporulation of Cercospora molucellae.

Table 3: Effect of 3 different temperatures (T) and 3 light regimes (L) on sporulation (10^3 conidia / ml) of Cercospora molucellae.

Temperature (T)	LIGHT REGIMES (L) IN HOURS			Temperature means
	Light 24	Dark 24	Light/Dark 12/12	
20°C	8.90b	4.04d	3.06d	5.33g
24°C	10.21a	2.79d	3.29d	5.43g
28°C	5.29c	4.59c	3.57d	4.48h
Light Means	8.13e	3.80f	3.30f	

*Means with the same letter are not significantly different at $P = 0.05$ (Duncan's Multiple range test).

X ——— X LIGHT (24 HR)
 ● ——— ● DARK (24 HR)
 ——— ● LIGHT/DARK (12 HR / 12 HR)

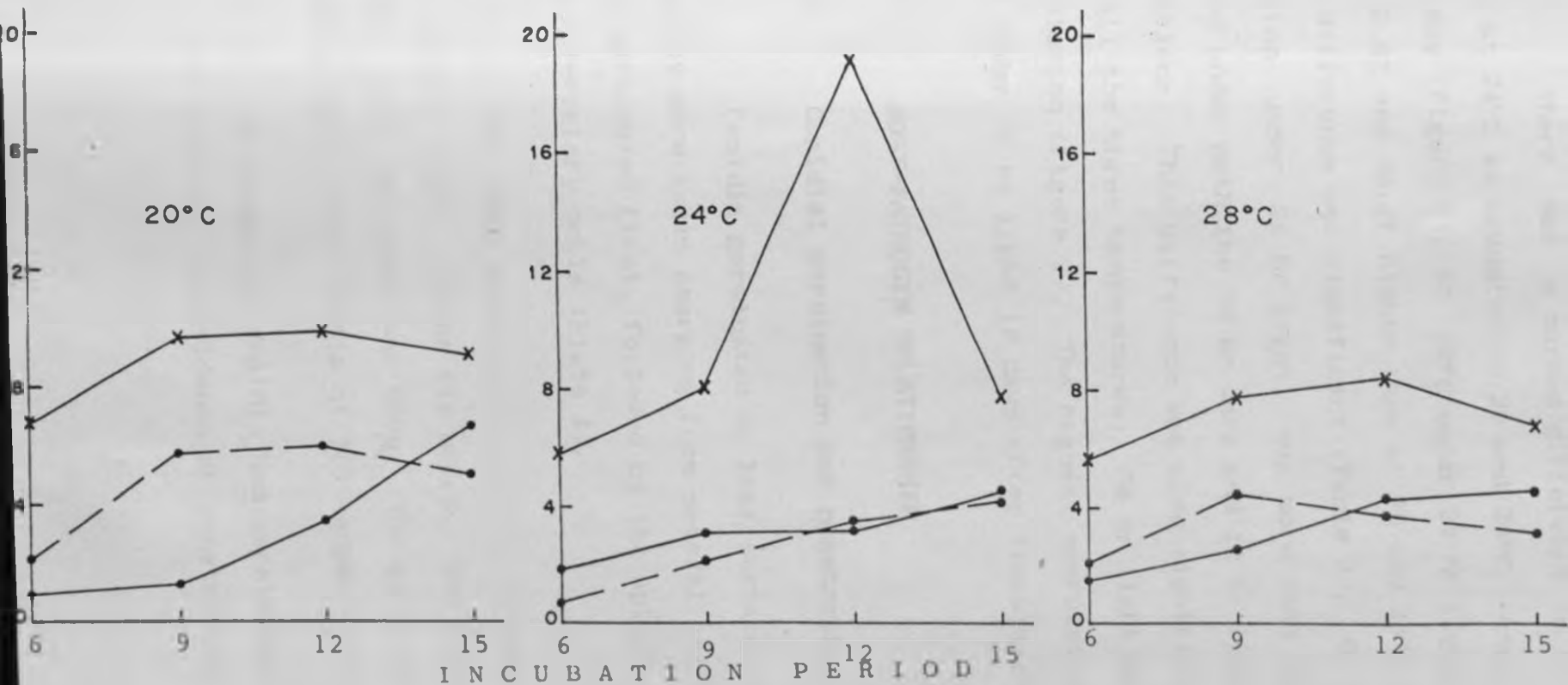


Fig. 2: Effects of three different temperatures, and three light regimes on sporulation of Cercospora molucellae.

There was a marked difference in the effect of light at 24°C as compared to 20 and 28°C, especially on the 12th day (Figure 2). At 24°C under 24 hr light, sporulation was 12.8% and 48.2% higher than at 20 and 28°C respectively. This difference was significant (Table 3). On average, sporulation under 24 hr light, was more than twice that achieved under both the 24 hr dark and 12 hr light/12 hr dark conditions. This difference was also significant (Table 3). At all the three temperatures, 24 hr light had the highest sporulation (Figure 2). The highest sporulation occurred at 24°C under 24 hr light 12 days after inoculation (Table 4).

4:2 HOST-PATHOGEN RELATIONSHIP

4:2:1 Conidial germination and penetration

Conidia germinated on leaf surfaces within three hours by germ tubes emerging from several cells. The basal cell germinated first, followed by the apical cell and then the intercalary cells (Plate 4).

The germ tubes had a uniform diameter throughout the entire length. After six hours, the average length was 5000u while the width was 500 μ . The germ tube and stomatal opening had a width ratio of 1:3 respectively.

Although all conidia had germinated by the end of 12 hour period, no evidence of penetration was observed

(L) on sporulation (Conidia/ml $\times 10^5$) of *Cereospora minutellae*

		TIME IN DAYS AFTER PLATE INOCULATION (D)											
		6			9			12			15		
Temperature (T)	LIGHT REGIMES IN HOURS (L)												
	Light 24	Dark 24	Dark/ light 12/12	Light 24	Dark 24	Light/Dark 12/12	Light 24	Dark 24	Light/Dark 12/12	Light 24	Dark 24	Light/Dark 12/12	
10°C	6.81 c-g*	2.36 L-q	1.00 pq	9.71 b	5.45 e-k	1.36 opq	10.0 b	6.00 d-i	3.72 h-o	9.09 bc	5.00 f-k	6.17 d-h	
20°C	5.81 d-j	0.82 q	1.91 m-q	8.08 bcd	2.26 L-q	3.18 j-q	19.24 a	3.63 h-p	3.54 h-p	7.72 b-e	4.45 g-m	4.54 g-m	
30°C	4.18 g-n	3.63 h-p	2.09 L-q	6.17 d-h	5.54 f-k	3.36 J-q	7.36 b-f	6.08 d-i	5.72 d-k	3.45 i-q	3.09 k-q	3.09 k-q	
Means	5.60	2.27	1.67	7.99	4.42	2.63	10.44	3.75	4.33	6.75	3.30	4.60	
Means	3.18t			5.01s			6.17r			4.88s			

-36-

Means with the same letter are not significantly different at P = 0.05 (Duncan's multiple range test).
The "—" between letters means inclusive.



Plate 4:
Cercospora molucellae conidia germinating
on Molucella laevis leaf surface 3 hours
after inoculation.

A - Apical cell

B - Basal cell

G - Germ tube

I - Intercalary cell

Magnification: 200 times.

until 24 hours after inoculation. Penetration was accomplished through stomata without the formation of appresoria (Plate 5). Some germ tubes passed near the stomata without penetrating through them. There was no evidence of direct penetration.

4:2:2 Symptomatology

The incubation period was 5, 7, 10 and 11 days for plants inoculated with 5.4×10^4 , 2.7×10^4 , 1.0×10^4 and 0.5×10^4 inoculum levels respectively. Symptoms first appeared on the oldest leaves and subsequently on the younger ones. They were exhibited as small, gray to brown circular or irregular necrotic spots 6 - 12mm in diameter. Initially, pathogen spread on the leaf surface was restricted by veins (Plate 6a), but lesions later coalesced to form a blight and severe defoliation followed. There was pronounced stunting and hardly any tillering (Plate 6b). As bells formed, they too got diseased. Diseased bells were characterised by circular brown necrotic lesions which coalesced to give the drying up effect (Plate 6c). Cercospora molucellae was found to be the causal agent of cercospora leaf spot of M. laevis as verified by Koch's postulate.

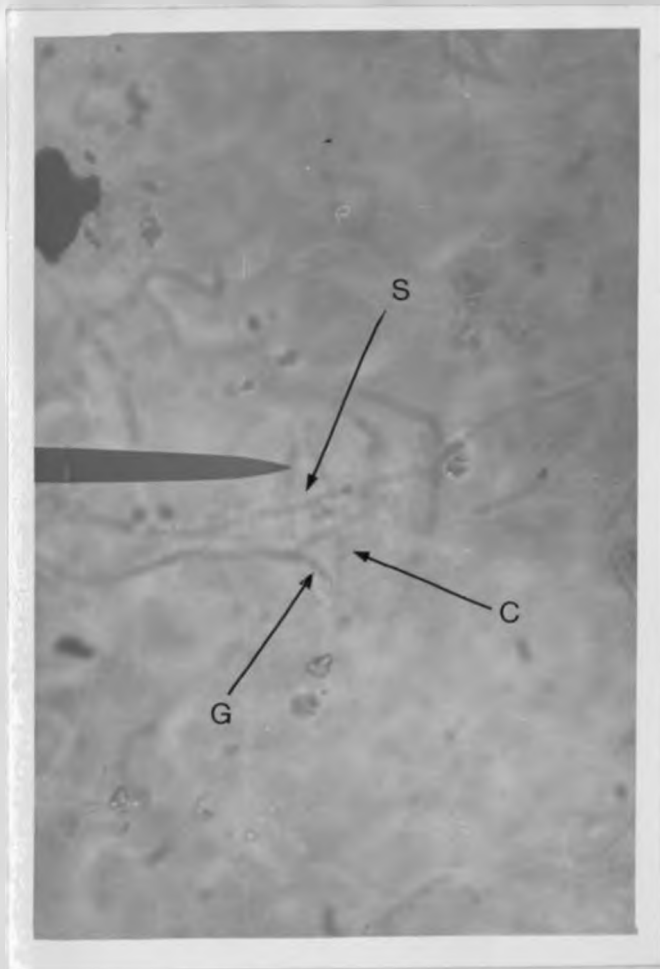


Plate 5:
A germ tube penetrating the leaf through a
stoma 24 hours after leaf inoculation.

C - Guard cell

G - Germ tube

S - Stoma

Magnification: 1134 times



Plate 6(a):
Symptoms of *Cercospora* leaf spot on
molucella laevis. Note the necrotic spots.



Plate 6(b):
Defoliation, stunting and poor tillering
on a diseased Molucella laevis plant (right)
as compared to the control (left).

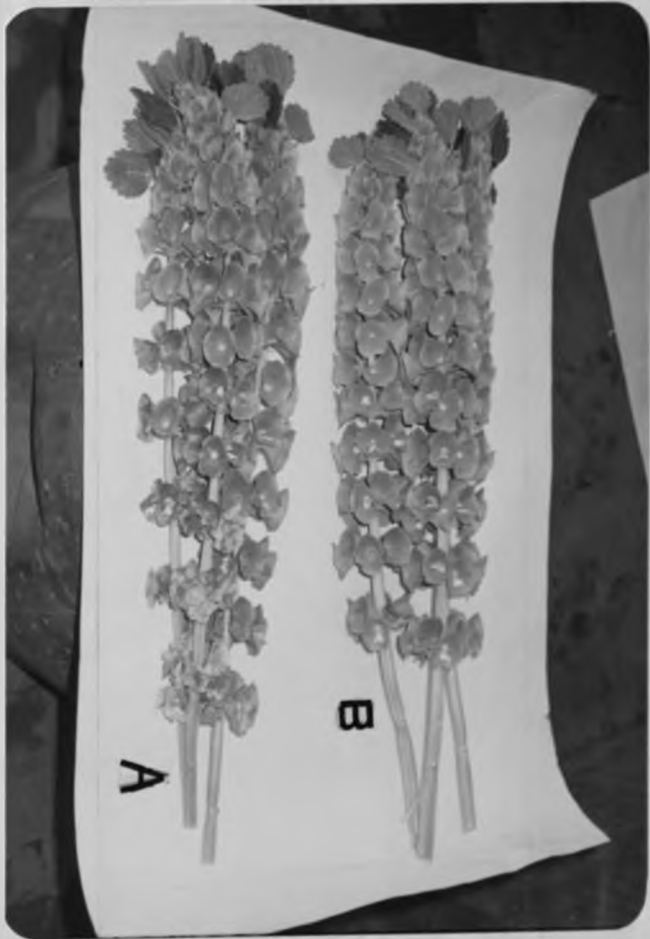


Plate 6(c):
(A) Diseased bells of Ireland (infected by Cercospora molucellae), 21 days after inoculation.
(B) Healthy bells of Ireland (control).

4:2:3 Effect of inoculum concentration and host age on disease severity under greenhouse conditions.

4:2:3:1 Inoculum concentration.

The control plants remained healthy while the inoculated ones showed varying degree of disease severity. There was a consistent decline in the percentage leaf area diseased as the inoculum concentration decreased (Figure 3a). Between the highest and the lowest inoculum levels, there was a 41.01 difference in the percentage leaf area diseased. This difference was highly significant (Table 5a).

The general trend in the number of lesion per leaf is as shown in Figure 3b. This showed no consistency in the number of lesions per leaf following decrease in inoculum concentration. No significant difference in the number of lesions per leaf was observed when inoculum level was reduced from 5.4×10^4 to 0.5×10^4 conidia/ml. However there was a significant increase of 86.99% in the number of lesions per leaf when inoculum level was reduced from 5.4×10^4 to 2.7×10^4 con/ml (Table 5b).

4:2:3:2 Host age.

There was an increase in disease severity both in terms of lesions per leaf and percentage leaf area diseased as the plant age increased (Figures 3a and 3b). Between the 5 and 7

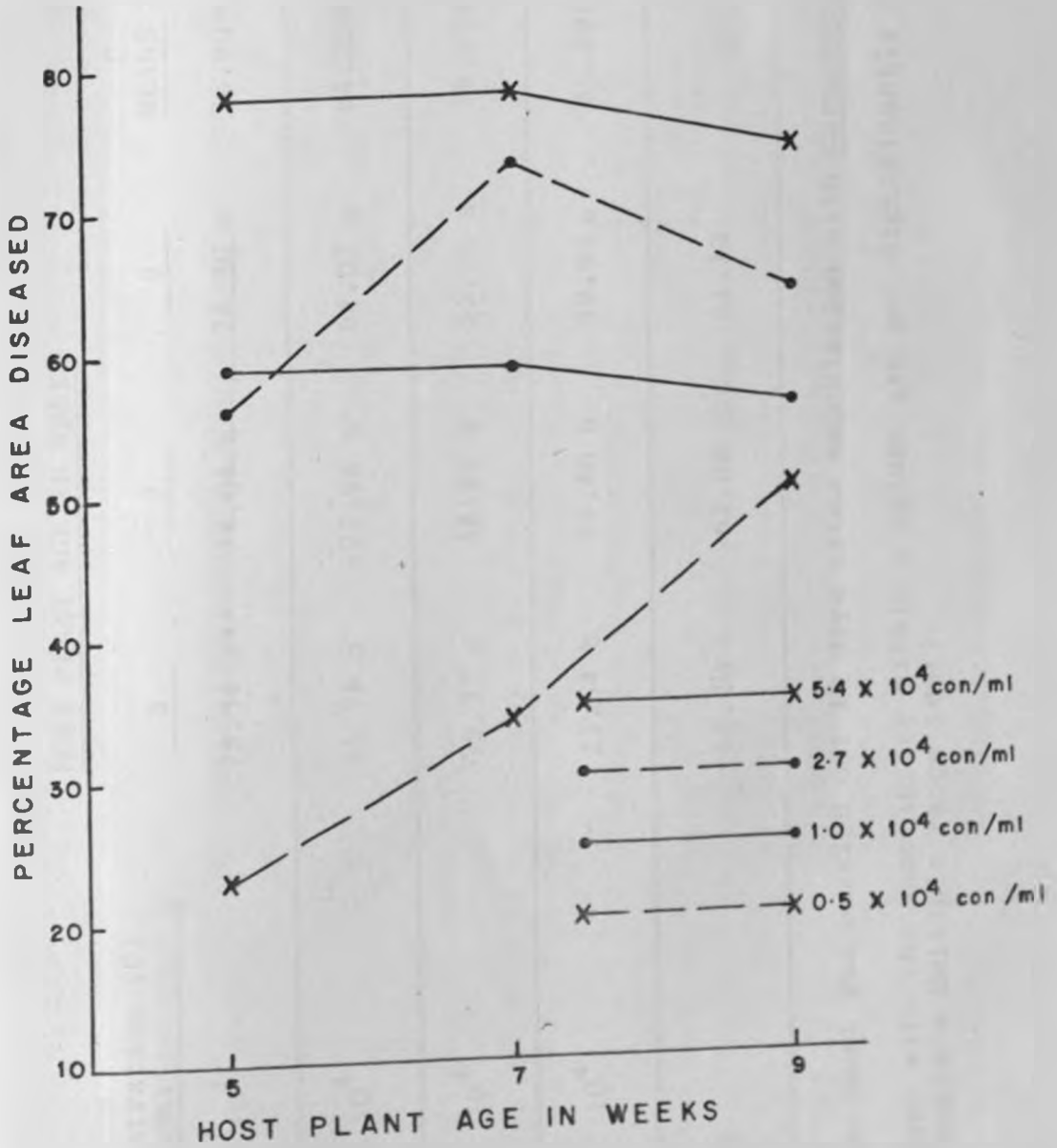


Fig. 3(a):

Effect of conidial concentrations, of *Cercospora molucellae*, and host plant age on percentage diseased leaf area 12 days after inoculation.

age (H) on percentage leaf area diseased.

Inoculum concentration (C) in con/ml	HOST PLANT AGE IN WEEKS			
	5	7	9	C MEANS 5
5.4×10^4	78.34 a**	78.06 a	74.30 a	76.90 _a
2.7×10^4	55.76 a	72.98 a	64.07 a	64.27 _a
1.0×10^4	59.37 a	58.87 a	56.21 a	58.15 _a
0.5×10^4	23.34 b	34.40 b	49.93 a	35.89 _b
H MEANS	54.20	61.08	61.13	

*Assessment was carried out 12 days after inoculation with Cercospora molucellae

**Means with the same letter within a column are not significantly different (Duncan's Multiple range test).

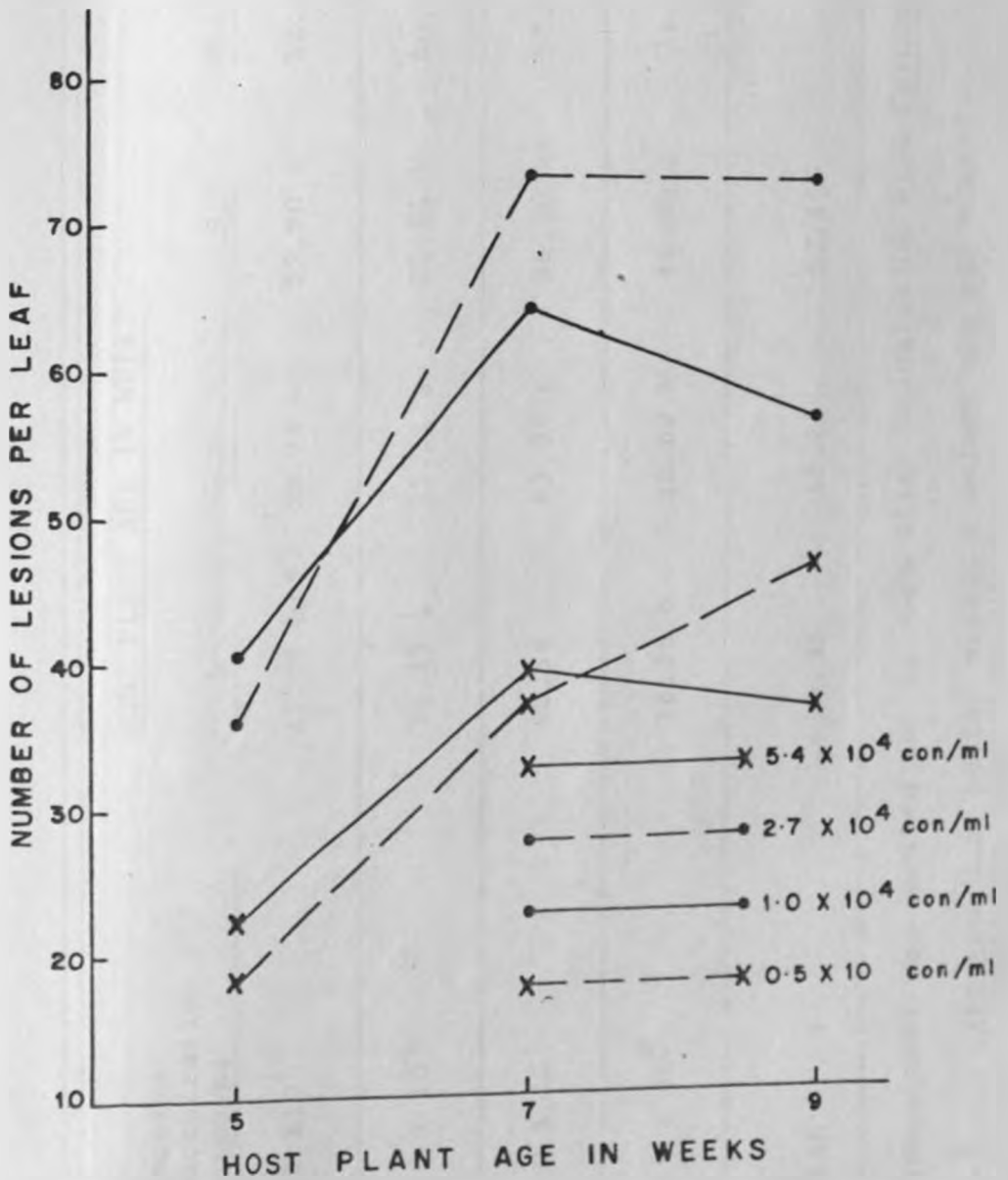


Fig. 3(b)
Effect of conidial concentrations, of *Cercospora molucellae*, and host plant age on the number of lesions per leaf 12 days after inoculation.

Table 5(b): Effect of four different inoculum concentrations (C) and host plant age (H) on the number of lesions per leaf.*

Inoculum concentration (C) in con/ml	HOST PLANT AGE IN WEEKS			C MEAN 5
	5	7	9	
5.4 X 10 ⁴	22.25 b**	38.94 b	35.90 a	32.36 b
2.7 X 10 ⁴	36.33 a	73.31 a	71.85 b	60.50 a
1.0 X 10 ⁴	40.58 a	63.94 a	56.20 bc	53.57 a
0.5 X 10 ⁴	18.25 b	36.69 b	46.00 c	33.65 b
H MEAN	29.35	53.22	52.49	

*Assessment was carried out 12 days after inoculation with Cercospora molucellae

**Means with the same letter within a column are not significantly different (Duncan's Multiple range test).

week old plants, there was a 23.87 increase in the number of lesions per leaf. This difference was significant. Between the 7 and 9 week plants, there was less than 1% difference in percentage leaf area diseased and number of lesions per leaf (Tables 5a and 5b).

4:2:3:3 Host age and inoculum concentration interaction

There was 55 and 43.66 increase in percentage leaf area diseased when inoculum concentration was increased from 0.5×10^4 to 5.4×10^4 con/ml on the 5 and 7 week plant respectively. This difference was highly significant. With the 9 week plants, there was 24.37 percentage increase in leaf area diseased using the same inoculum levels. The increase was not significant (Table 5a).

When the inoculum concentration was increased from 1.0×10^4 to 2.7×10^4 con/ml, the number of lesions per leaf increased as the host plant became older (Figure 3b). However, this increase was not significant (Table 5b).

On the 5 and 7 week plants, there was no significant difference in the number of lesions per leaf when the inoculum level was increased from 0.5×10^4 to 5.4×10^4 con/ml. With the 9 week plants, the difference in the number of lesions at these two inoculum levels was significant (Table 5b).

4:3 EFFECTS OF BENLATE, MANCOZEB AND KOCIDE 101 ON CERCOSPORA LEAF SPOT OF Molucella laevis UNDER FIELD CONDITIONS.

4:3:1 Assessment of disease incidence.

The incubation period was 14 days. Results on disease assessment are in Tables 6a and 6b, Figures 4 and 5. Disease incidence in the control plots (inoculated, sprayed with water without fungicide) was high. 15 days after inoculation, the percentage leaves and plants diseased was 7.46 and 86.20 respectively. By the 23rd day, there was 53.54% increase in the leaves diseased (Table 6b). At the end of disease assessment exercise (27 days after inoculation), the percentage plants and leaves diseased was 90 and 50.87% respectively.

In plots sprayed with Kocide 101 disease incidence increased with time although the percentage leaves diseased was lower than in the control (Figures 4 and 5, Plates 7a and 7b). Between 14 and 27 days after inoculation, there was 45.72 and 46.73 increase in the percentage plants and leaves diseased respectively. This difference was significant (Tables 6a and 6b). Both Benlate and Mancozeb treatments maintained disease incidence low throughout (Figures 4 and 5 Plates 7c and 7d) and the difference in disease incidence in the plots sprayed with the two fungicides was

Table 6(a) Effect of fungicides (F), time in days (T) after inoculation and spraying interval in weeks (S) on percentage of diseased plants.

Fungicides (F)	TIME IN DAYS AFTER INOCULATION								F MEANS
	15		19		23		27		
	SPRAYING INTERVALS IN WEEKS (S)								
	1	2	1	2	1	2	1	2	
No fungicides	86.20		90.00		90.00		90.00		89.05
Kocide	54.52	34.04	70.70	69.12	79.10	80.85	90.00	90.00	71.05g
Mean	44.28c		69.91b		79.99ab		90.00a		
Benlate	0.00	5.28	6.76	5.48	0.00	4.31	0.00	4.31	3.29b
Mean	2.74d		6.12d		2.15d		2.15d		
Mancozeb	4.04	4.04	13.60	4.08	10.75	13.33	8.27	9.48	8.45b
Mean	4.04d		8.82d		12.04d		8.88d		
S									
Means	19.52	14.52	30.35	26.21	29.95	32.86	32.86	34.58	
T									
Means	17.02f		28.02e		31.39e		33.67e		

*Means with the same letter are not significantly different at P = 0.05 (Duncan's multiple range test).

Table 6(b) Effect of Fungicides (F), Time in days(T) after inoculation and spraying interval in weeks (S) on percentage of diseased leaves.

TIME IN DAYS AFTER INOCULATION (T)									
FUNGICIDES (F)	15		19		23		27		F MEANS
	SPRAYING		INTERVAL IN		WEEKS (S)				
	1	2	1	2	1	2	1	2	
NO FUNGICIDES	7.46		7.92		61.00		58.47		33.71
KOCIDE MEAN	4.41 4.14 c*	3.87	6.11 5.57 c	5.02	47.74 45.14 b	42.54	50.66 50.87 a	51.08	26.43 f
BENLATE MEAN	0.71 0.78 c	0.85	0.88 0.79 c	0.71	0.00 0.92 c	1.84	0.00 0.90 c	1.79	0.85 g
MANCOZEB MEAN	0.81 0.80 c	0.79	1.15 0.98 c	0.80	3.90 4.46 c	5.02	3.83 4.36 c	4.89	2.65 g
S MEANS	1.98	1.84	2.71	2.18	17.22	16.47	18.17	19.26	
T MEANS	1.91 e		2.45 e		16.85 d		18.72 d		

* Means with the same letter are not significantly different at P = 0.05 (Duncan's Multiple range test).

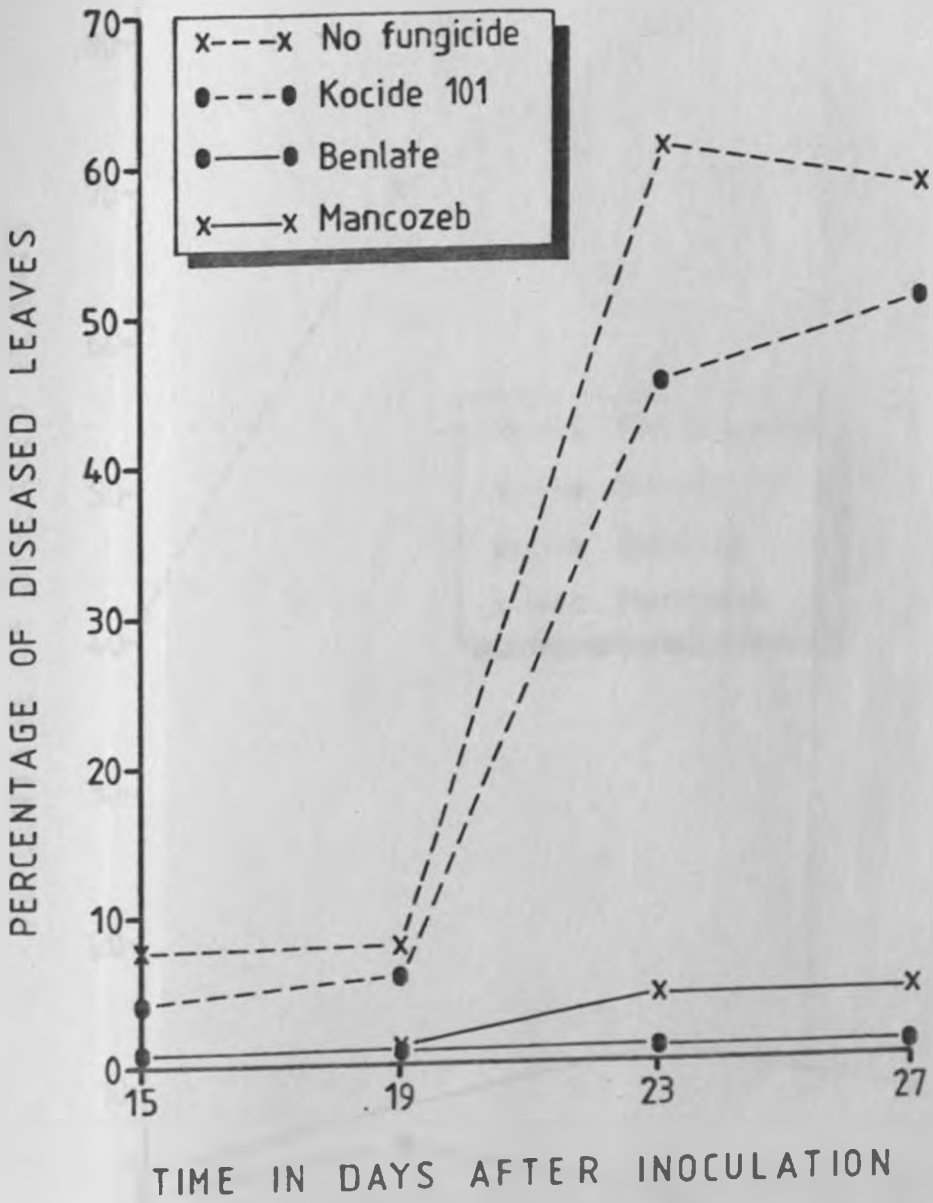


Fig. 4. Effect of fungicides, time in days after inoculation and spraying interval on percentage of diseased leaves.

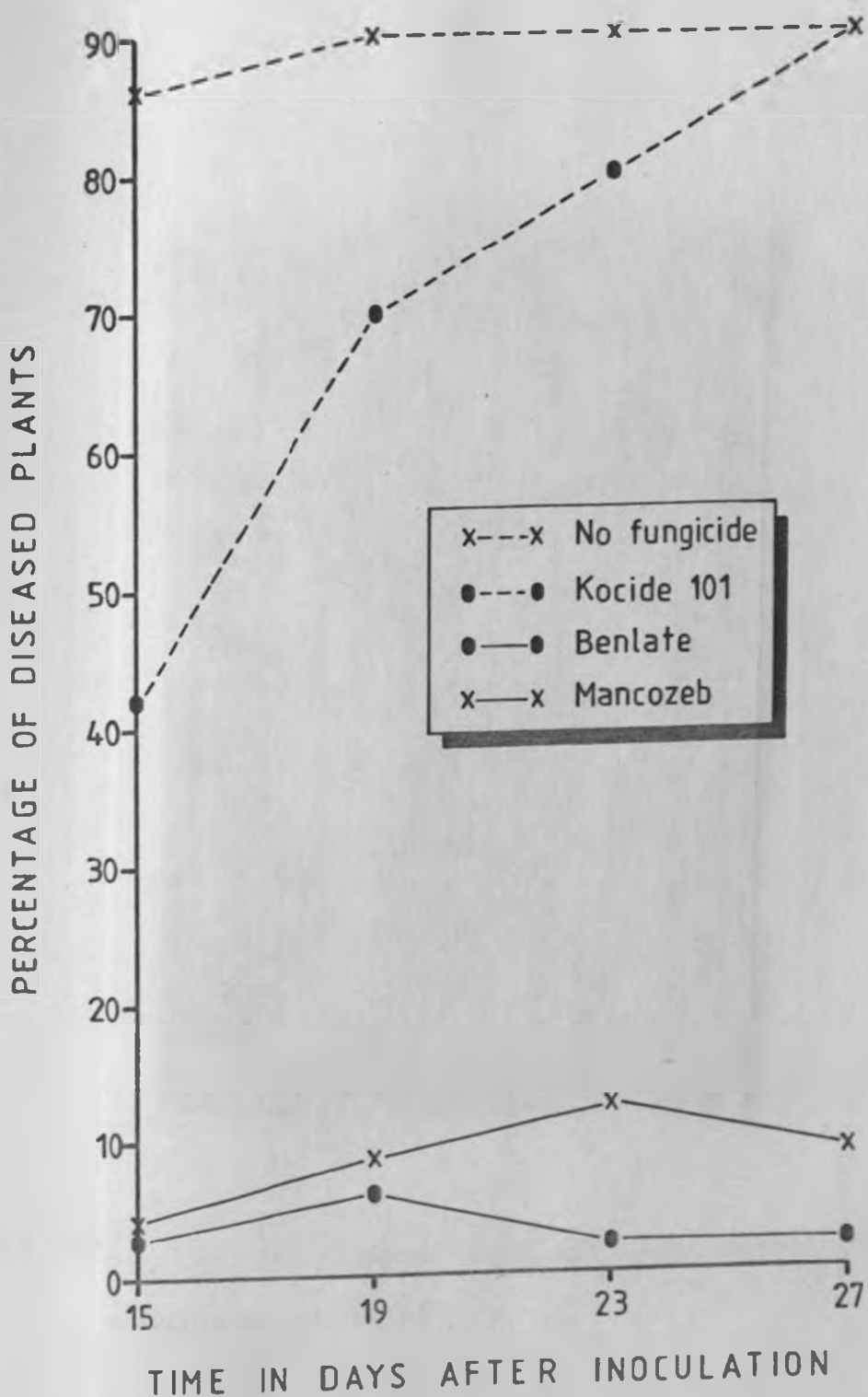


Fig. 5. Effects of fungicides, time in days after inoculation and spraying interval on percentage of diseased plants.



Plate 7(a):
Plants in plots sprayed with water without
fungicides (control).



Plate 7(b):
Plants in plots sprayed with Kocide 101.



Plate 7(c):

Plants in plots sprayed with Benlate.



Plate 7(d):
Plants in plots sprayed with Mancozeb.

not significant. On average, the percentage leaves and plants diseased in plots sprayed with Kocide 101 was 25.58 and 67.76% higher than those in plots sprayed with Benlate. This difference was highly significant (Tables 6a and 6b).

4:3:2 Assessment of disease severity.

Results on disease severity ratings throughout the assessment period are shown in Figures 6 and 7 and Tables 7a and 7b. The number of lesions per leaf and grade increased with time in both the control and Kocide 101 sprayed plots although in the latter they remained relatively lower. In the plots sprayed with Benlate and Mancozeb, both the lesions per leaf and grade remained much lower than in the other two treatments (Figures 6 and 7).

On average, the number of lesions per leaf in plots sprayed with Kocide 101, Benlate, Mancozeb and water without fungicide were 2.36, 0.76, 0.77 and 4.71 respectively. Disease severity in the control plots was significantly different from the other three treatments. It was also significantly higher in plots sprayed with Kocide 101 than in plots sprayed with either Benlate or Mancozeb (Table 7a). Assessment of the percentage leaf area diseased showed a similar trend (Table 7b).

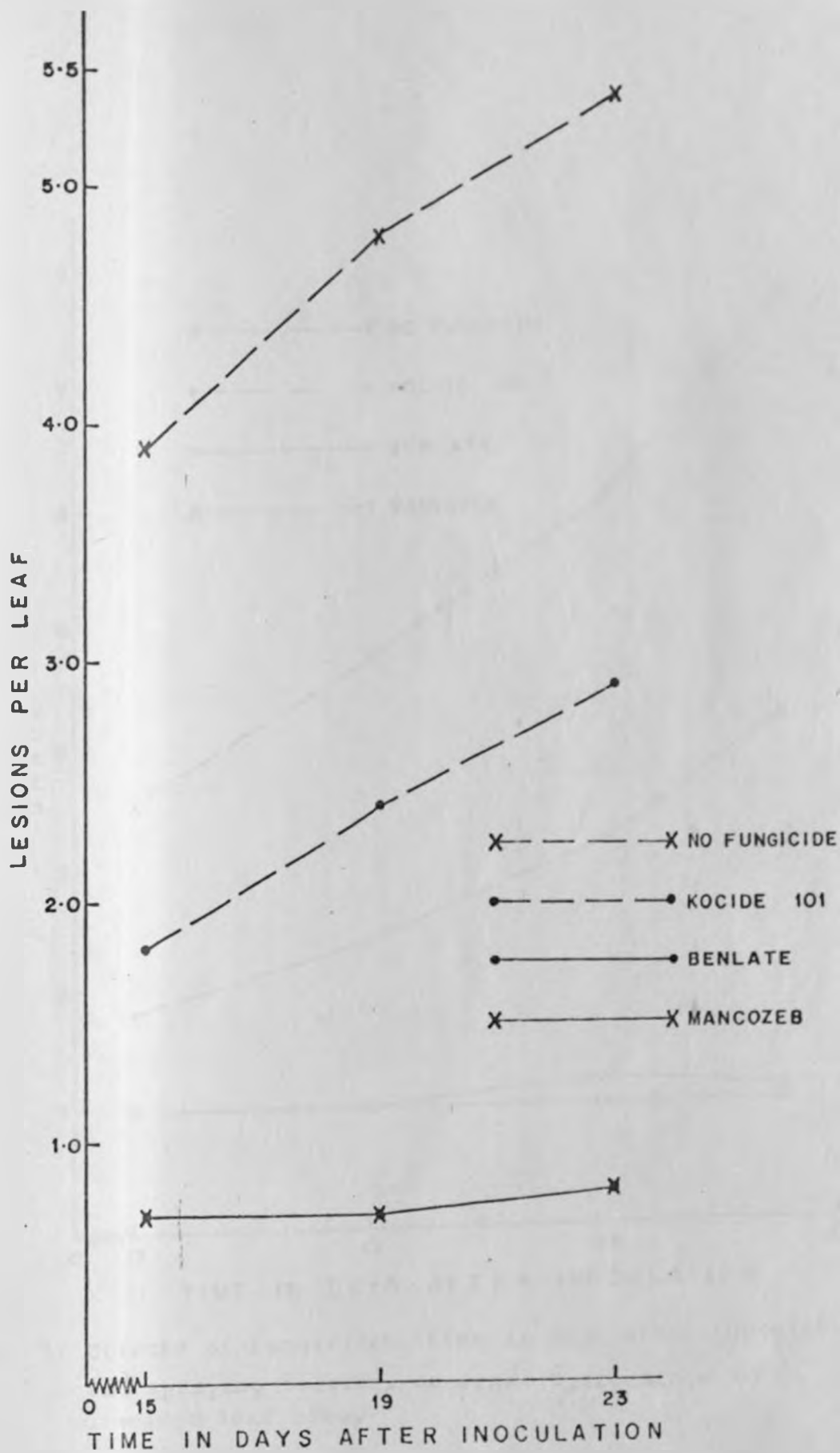


Fig. 6: Effects of fungicides, time in days, after inoculation and spraying interval on lesions per leaf.

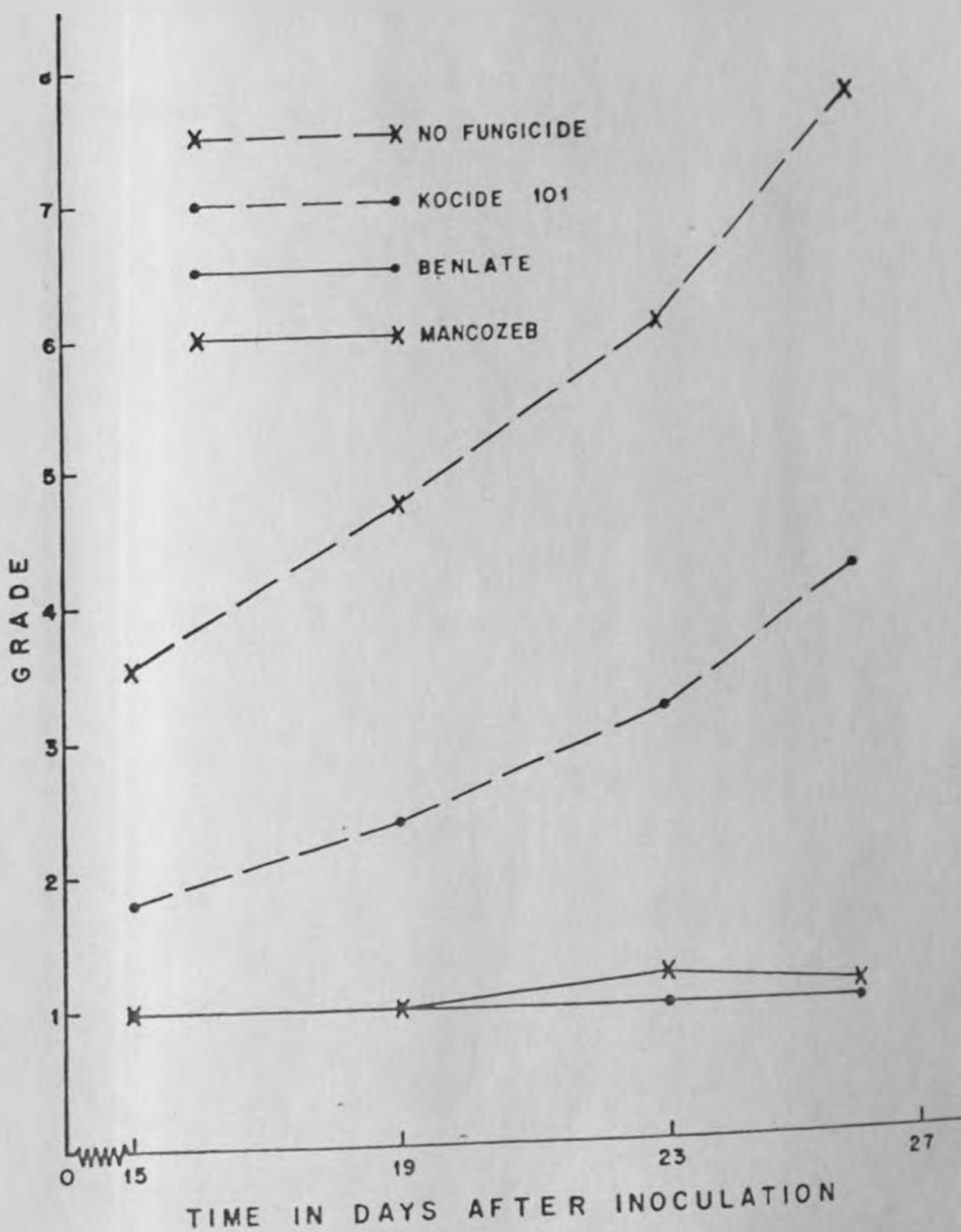


Fig. 7: Effects of fungicides, time in days after inoculation and spraying interval on Grade (percentage of diseased leaf area).

Table 7 (a) Effect of fungicides (F), Time in days (T) after inoculation and spraying intervals in weeks (S) on lesions per leaf.

FUNGICIDES (F)	TIME IN DAYS AFTER INOCULATION (T)						F MEANS
	15		19		23		
	SPRAYING INTERVAL IN WEEKS (S)						
	<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>	
NO FUNGICIDES	3.90		4.83		5.41		4.71
KOCIDE MEAN	1.71 1.77 c*	1.83	2.58 2.44 b	2.32	2.84 2.86 a	2.87	2.36 g
BENLATE MEAN	0.73 0.73 d	0.73	0.71 0.71 d	0.71	0.71 0.83 d	0.94	0.76 h
MANCOZEB MEAN	0.75 0.73 d	0.72	0.75 0.74 d	0.72	0.77 0.84 d	0.92	0.77 h
S MEAN	1.06	1.09	1.34	1.25	1.44	1.58	
T MEAN	1.08 f		1.30 ef		1.51 e		

* Means with the same letter are not significantly different at P = 0.05 (Duncan's Multiple range test).

Table 7(b) Effect of Fungicides (F), Time in days (T) after inoculation and spraying interval in weeks (S) on Grade (Percentage of diseased leaf area).

FUNGICIDES (F)	TIME IN DAYS AFTER INOCULATION (T)										F MEANS		
	15		19		23		27		SPRAYING INTERVAL IN WEEKS (S)				
	<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>			
NO FUNGICIDES	3.55		4.72		6.06		7.80				5.53		
KOCIDE MEAN	2.05 1.82 cd*	1.59	2.62 2.42 c	2.22	3.51 3.18 b	2.86	4.69 4.19 a	3.68			2.90 h		
BENLATE MEAN	1.00 1.01 d	1.03	1.04 1.02 d	1.00	1.00 1.02 d	1.03	1.00 1.02 d	1.03			1.09 i		
MANCOZEB MEAN	1.01 1.01 d	1.01	1.04 1.03 d	1.01	1.33 1.22 d	1.10	1.08 1.09 d	1.09			1.02 i		
S MEANS	1.35		1.21		1.57		1.41		1.95		1.66	2.26	1.95
T MEANS	1.28 g		1.49 fg		1.81 ef		2.10 e						

* Means with the same letter are not significantly different at P = 0.05 (Duncan's Multiple range test).

4:3:3 Flower length.

The five market grades are based on flower length as in Plate 8. Results on flower length are summarised in Table 8. On average, the control plots gave the lowest flower length. The highest length was obtained in plots sprayed with Mancozeb followed by Benlate and Kocide 101 was next. The difference in flower length due to the four treatments was significant (Table 8). There was no significance difference in flower length in the 1 and 2 weeks spray interval. There was a significant difference in flower length due to the four treatments (control, Kocide 101, Benlate and Mancozeb).

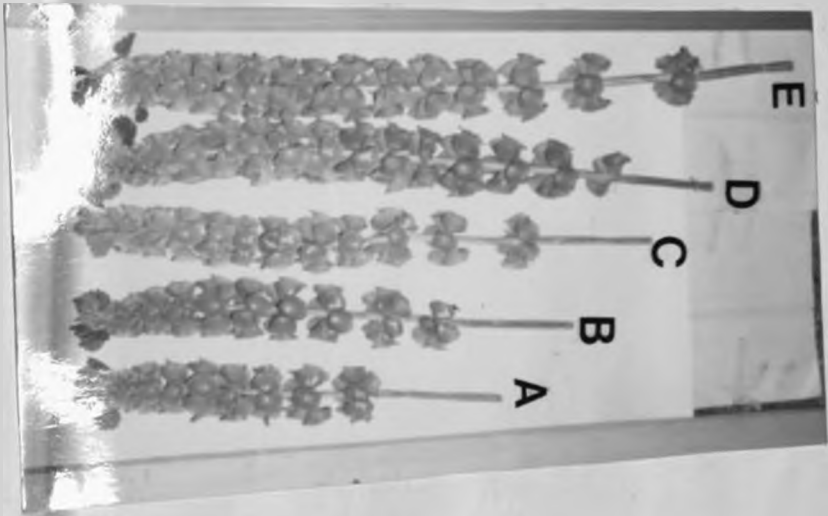


Plate 8: The five market grades of Molucella laevis cut flower.

Symbol	Grade	Length in cm
E	White	75 and above
D	Blue	65-74
C	Yellow	55-64
B	Red	45-54
A	Brown	35-44

Table 8. Effect of fungicides (F) and spraying interval in weeks (S) on flower length in cm at Harvest

F U N G I C I D E S (F)					
SPRAYING INTERVAL IN WEEKS	NO FUNGICIDE	KOCIDE	BENLATE	MANCOZEB	S MEANS
1		55.79	57.81	61.81	58.47 e
2		50.77	59.96	60.41	57.05 e
F MEANS	29.36 d*	53.28 c	58.89 b	61.11 a	

* Means with the same letter are not significantly different (Duncan's multiple range test).

D I S C U S I O N

511 SPORULATION OF Cercospora molucellae IN MOLUCELLA DECOCTION AGAR (MDA), POTATO DEXTROSE AGAR (PDA) AND CARROT LEAF DECOCTION AGAR (CDA).

The objective of this experiment was to identify which of the three media, induces abundant sporulation of Cercospora molucellae within the shortest time.

MDA and PDA showed a gradual increase in sporulation with the peak being realised on the 12th day (Figure 1). This was followed by a sudden decline. This could attributed to exhaustion of nutrients in the media, germination and disintegration of produced conidia as reported on other Cercospora species (Nagel, 1934; Cooperman and Jenkins, 1986). On the 12th day, maximum sporulation occurred on MDA.

On CDA, sporulation was not only delayed but was also sparse (Figure 1). This is contrary to the findings of Berger and Hanson (1963) and Latch and Hanson, (1962). They worked with different cercospora species (Cercospora zebrina, Cercospora davisii) which may be the cause of the discrepancy.

When all the sampling days were considered, there was a significant difference in sporulation in the three

media. Maximum sporulation was attained with MDA, followed by PDA. CDA had the least sporulation (Table 2). Abundant sporulation of Cercospora species on their respective host decoction agar has been reported by other workers (Nagel, 1934; Diachun and Valleau, 1941; Berger and Hanson, 1963; Calpouzos and Stallknecht, 1965). On PDA, a fair amount was produced. This has been observed in other Cercospora species (Sober, 1969 ; Nagel, 1934).

5:2 . SPORULATION OF Cercospora molucellae AT FOUR DIFFERENT TEMPERATURES AND THREE LIGHT REGIMES.

The objective of this experiment was to identify the incubation temperature and light regime which supports abundant sporulation of Cercospora molucellae within the shortest time.

5:2:1 Effect of temperature on sporulation.

Sporulation at 20 and 24 C, were not significantly different when all light regimes were considered. However, maximum sporulation occurred at 24 C, followed by 20 C and was least at 28 C (Table 3). Sporulation at 28 C was

significantly lower than at 20 and 24 C. At 32 C, there was restricted growth and no sporulation occurred. Poor and delayed conidial germination at 31 C has been reported in other Cercospora species (Alderman and Beute, 1986).

At high temperatures, especially at 32 C, evaporation of water from the culture plates could lead to dehydration of the media. Since it has been shown (Berger and Hanson, 1963) that high relative humidity is required for conidiophore and subsequent conidia formation on host tissue, it is possible that high moisture is required also in vitro. Since fungi absorb their food from solution, it follows that an abundant supply of water is essential for growth and sporulation.

The fact that lower temperatures (20 and 24 C), favoured abundant production of conidia in vitro is in agreement with field observations (personal communication, Too) and might explain to some extent why the fungus requires periods of lower temperatures and high relative humidity to produce an epiphytotic of cercospora leaf spot.

5:2:2 Effect of light on sporulation.

When all the three temperatures were considered, conidia production under 24 hr light was significantly different from the others. The 24 hr light treatment gave the

highest conidia, followed by 24 hr dark and the 12 hr light/12 hr dark treatment had the lowest (Table 3). Abundant sporulation of Cercospora species under 24 hr light has been observed (Berger and Hanson, 1963; Kilpatrick and Johnson, 1956; Cooperman and Jenkins, 1986). This could be attributed to light inducing production of sporogenic substances in fungi (Trione and Leach, 1969) and hence promotion of conidiophore and conidia formation (Barnett and Lilly, 1950).

There was no significant difference in sporulation between the 24 hr dark and the 12 hr light/12 hr dark (Table 3). It would appear that light duration was too short to induce production of sporogenic substances.

5:2:3 Effect of light, temperature and incubation time on sporulation.

At all the three temperatures, under 24 hr light, there was an increase in sporulation from the 6th day (Figure 2). This peaked on the 12th day after which, there was a decline, possibly, due to conidia germination and disintegration. The interaction between light, temperature and sampling day was significant. The highest sporulation was obtained at 24 C, under 24 hr light, on the 12th day (Figure 2). This was significantly different from the others (Table 4).

At all the three temperatures, there was a general gradual increase in sporulation under both the 24 hr dark and 12 hr light/12 hr dark (Figure 2). A significant difference in sporulation between these two light regimes was not reached at any time or temperature. This is contrary to findings of Calpouzos and Stallknecht, (1967) who reported a significant difference in sporulation of Cercospora beticola in culture under continuous dark and those exposed to light for a period of 24 hours. Trione and Leach, (1969) observed that light induces production of sporogenic substances in fungi and their synthesis continues in the dark. Perhaps the 12 hr light duration was too short to induce production of these substances and hence the insignificant difference in sporulation under 24 hr dark and 12 hr light/12 hr dark treatments.

In this experiment, the environmental factors (media, light regime and temperature) which support abundant sporulation of Cercospora molucellae have been identified. This made it possible to proceed to the next experiment which required large quantities of conidial inoculum.

5:3 THE HISTOPATHOLOGICAL RELATIONSHIP OF Cercospora molucellae and Molucella laevis

5:3:1 Conidial germination and penetration.

Conidia germinated on leaf surface by germ tubes emerging from several cells and penetration was accomplished through stomata (Plate 4 and 5). Passive ingress through stomata has been observed on C. medicaginis, C. zebrina, C. davisii, C. beticola and C. arachidicola (Baxter, 1956; Latch and Hanson, 1962 and Solel and Minz, 1971). The germ tube and stomata opening had a width ratio of 1:3 respectively. Severity of cercospora leaf spot on sugar beet and groundnut depend on stomatal density and their opening size, which determine the chance of penetration (Hemingway, 1957; Solel and Minz, 1971). In breeding for resistance, breeders could focus on manipulating stomatal density and size among other factors.

5:3:2 Effects of inoculum concentration and age of the host on disease intensity.

5:3:2:1 Effects of inoculum concentration.

With a low inoculum level, the incubation period was long (11 days) as opposed to 5 days with the highest concentration. Plants inoculated with 2.7×10^4 and 1.0×10^4 con/ml had an incubation period of 5 and 7 days respec-

tively. Plants inoculated with these two inoculum levels exhibited small grey to brown, irregular to circular necrotic lesions 6-12 mm in diameter. These are symptoms typical of cercospora leaf spots. Plants inoculated with these two inoculum levels showed no significant difference in disease development (Tables 5a and 5b). Similar symptoms were obtained with 0.5×10^4 inoculum level. Plants inoculated with 5.4×10^4 con/ml showed total blight as the initial symptoms. With the higher inoculum levels, the probability of more conidia accomplishing the infection process was higher. This may have resulted in competition for nutrients, faster cell necrosis and hence the shorter incubation period. Re-isolation of the pathogen revealed that Cercospora molucellae is the causal agent of cercospora leaf spot of molucella laevis

Inoculum concentration had a significant effect on disease development. This factor has to be closely controlled. It must be sufficiently high to result in strong selection pressure of the host and prevent escapes in screening tests. However, it should not be so high as to miss utilisable resistance and to make disease rating difficult.

Increasing inoculum concentration gave increasing disease intensity (percentage leaf area diseased) but with

diminishing returns (Table 5a and Figure 3a). This may suggest existence of antagonism between Cercospora molucellae conidia in high concentration on the host. This became more pronounced when the number of lesions per leaf was considered.

The number of lesions per leaf at 5.4×10^4 con/ml was extremely low (Table 5a). At this level, there may have been acute antagonism. Also, there was merging of the lesions by the end of the incubation period. When such high inoculum level is used, assessment of the percentage leaf area diseased would give a more accurate measure of disease situation

5:3:2:2 Effects of host age.

With respect to host age, it appears that the 5 weeks plants were less severely diseased than either the 7 or 9 week plants. Under field conditions, it has been observed that Cercospora molucellae normally causes epiphytotic when the plants are not less than 8-9 weeks of age. This cannot be due to delay in appearance of initial inoculum since adjacent younger plants are normally healthy. While the effect on plant canopy on microclimate could be attributed to this late attack in the field, it could not have been so under greenhouse conditions which were uniform. Tr

plants physiologic age appears to influence disease development. Similar observations have been made on cercospora leaf spot of groundnuts and corn (Hemingway, 1954, 1955, Hitly et. al., 1979). Young plants may possess some chemical defence mechanism which inhibit pathogen infection. Schneider and Sinclair (1975) reported inhibition of conidial germination and germ tube growth of C. canescens by cowpea leaf diffusates. In disease resistance screening tests, it would be essential to consider this host pathogen interaction. Seven week old plants should be inoculated with a conidial suspension whose concentration is 1.0×10^6 con/ml. to ensure that the resulting disease intensity is neither too low nor too high for appropriate testing for resistance.

5:4 EFFECTS OF THREE FUNGICIDES ON CERCOSPORA LEAF SPOT OF Molucella laevis UNDER FIELD CONDITIONS.

The objective of this experiment was to determine which of the three fungicides (Kocide 101, Benlate and Mancozeb) and two spray intervals (1 and 2 weeks) are most effective in controlling cercospora leaf spot. The selection was based on the fungicides effect on disease incidence (percentage plants and leaves diseased), severity (lesions per leaf and percentage leaf area diseased) and flower length.

In the field, incubation period was twice (14 days) that under greenhouse conditions. This may have been as a result of the differences in temperature and relative humidity. Cooperman and Jerkins (1986) made similar observations on asparagus.

5:4:1 Effect of spray interval on disease incidence and severity.

There was no significant difference in either disease incidence or severity due to spraying interval. Manufacturers recommend use of Mancozeb at 7 to 10 days interval (Rohm and Haas and Dupont chemical Co.). The disease was effectively controlled at 14 days interval. This is much more economical as it reduces spraying expenditure by 50%. Since secondary inoculum was produced 5 days after incubation, then Mancozeb must be quite persistent. However, there is need to ensure complete coverage of the foliage owing to the protectant nature of the fungicide.

Benlate, which is a systemic fungicide effectively controlled the disease at 14 days intervals. Plant tissues must have contained the fungicide in high enough concentrations to inhibit pathogen infection.

5:4:2 Disease incidence and severity in control plots

Disease incidence in the plots which received no fungicides increased at a high rate (Figure 4 and 5). The percentage leaves diseased was highest on the 23rd day after which there was a decline. At this stage, the rate at which new leaves emerged was rather fast and young leaves appeared to possess some resistance to pathogen attack. Young leaves may possess a chemical defence mechanism as reported by Schneider and Sinclair (1975). They reported inhibition of conidial germination and germ tube growth on young cowpea leaves due to production of diffusates. Disease severity in these plots increased with time and reflects the growth of the pathogen in undisturbed environment.

5:4:3 Disease incidence and severity in plots sprayed with Kocide 101.

Plots sprayed with Kocide 101, showed a general increase in percentage leaves and plants diseased, lesions per leaf and percentage leaf area diseased (Figures 4, 5, 6 and 7). A significant increase in percentage plants diseased was reached by the 19th day (Table 6a). The Kocide 101 may have altered the pathogen's growth rate and thus prolonged incubation period in some of the plants. Had it been a result of secondary inoculum, it would have been reflected

by a significant increase in lesions per leaf and percentage leaf area diseased. However, this did not increase significantly until the 23rd day. After the 19th day, there was a significant increase in percentage leaves diseased, lesions per leaf and percentage leaf area diseased. Kocide 101, does not cause total inhibition of mycelial growth and sporulation of the pathogen. This was reflected in symptom development in emerging leaves, increase in number of lesions and percentage leaf area diseased. Inoculum inciting disease on emerging leaves must have originated from within the experimental units as the oats barrier ensured that inoculum dissemination between plots was minimal if not non-existence.

5:4:4 Disease incidence and severity in plots sprayed with Benlate and Mancozeb.

In plots sprayed with Benlate and Mancozeb, disease incidence and severity remained low relative to those sprayed with Kocide 101 and the control (Figures 4, 5, 6 and 7). There was no significant difference in either disease incidence or severity in Mancozeb and Benlate sprayed plots over time (Tables 6a, 6b, 7a and 7b). This reflected their effectiveness in controlling the disease. When leaves sampled from plots sprayed with Benlate and Mancozeb were viewed under the microscope, they revealed ungerminated conidia. The fungicide must be inhibiting conidial germina-

tion.

5:4:5 Overall effect of fungicides on disease incidence and severity.

When all the four sampling days were considered, the disease incidence (percentage leaves and plants diseased), in the control plots was found to be significantly different from the plots sprayed with fungicides. Plots sprayed with Benlate had the lowest disease incidence followed by Mancozeb and Kocide 101 was next (Table 6a and 6b). The lower disease incidence in plots sprayed with Benlate may have been due to its systemic mode of action and thus its ability to destroy inoculum landing on unsprayed emerging leaves. There was no significant difference in incidence and severity (lesions per leaf and percentage leaf area diseased) results (Tables 7a and 7b).

5:4:6 Overall trend in disease incidence and severity.

There was a consistent increase in disease severity with time, (Figures 6 and 7). A significant increase in both the percentage area diseased and number of lesions per leaf was achieved on the 23rd day (Tables 7a and 7b). After every 8 days there was a significant difference in disease severity. This could have been a result of the increasing inoculum potential in the field as the pathogen multiplied.

5:5 EFFECT OF FUNGICIDES AND SPRAY INTERVAL
ON FLOWER LENGTH.

Flower length was used as the final measure of the fungicides effectiveness in disease control. Spray interval had no significant effect on flower length. There was a significant difference in flower length among the four treatments (control, Kocide 101, Benlate and Mancozeb). The highest mean flower length was attained in plots sprayed with Mancozeb, followed by Benlate then Kocide 101 and the control had the least (Table 8).

5:5:1 Flower length in the control and Kocide 101 treated plots.

In the plots sprayed with Kocide 101 and in the control, a large proportion of the bells was diseased and hence the low average. The extensive leaf necrosis (indicated by percentage area diseased) reduced the photosynthetic surface area and may have contributed to the short flower length. Similar observations have been reported on ground nuts (Elston et al 1976). The bells on these plots were ready for harvest slightly earlier (7 days) than those in plots sprayed with Benlate and Mancozeb. This left the apical bells compact and premature. This may have been the

plant's response to **pathogen** attack and may have influenced flower length. The average length from the unsprayed plots fell far below the lowest market requirement (Plate 6). These were too short for any aesthetic purpose. This clearly indicates how devastating cercospora leaf spot on Molucella laevis could be.

5:5:2 Flower length in plots treated with Mancozeb and Benlate.

Although there was no significant difference in either disease incidence or severity in plots sprayed with Benlate and Macozeb, there was a significant difference in flower length. Mancozeb is a coordination product of manganese 16%, zinc 2% and ethylene bisdithiocarbamate 62%. Sulphur ions are known for their high affinity for metals and complex with ions and coenzymes within the fungal body. The same ions may have acted as a nutrient and hence the difference in flower length between Mancozeb and Benlate treatments. Similar reports have been made by Smith and Litrell (1980).

Benlate would be most effective in controlling the leaf spot after onset, owing to its systemic nature. However, its extensive and continuous use should be avoided. This would minimise development of Benlate-tolerant stains as observed by Smith and Littrell (1980).

C O N C L U S I O N S

For abundant sporulation, Cercospora molucellae should be cultured on MDA incubated at 24°C, under 24 hr light regime. Conidia should be harvested, 12 days after plate inoculation to ensure a high number of viable conidia.

Cercospora molucellae conidia germinate by germ tubes emerging from two or more cells and penetrates host tissues, passively through the stomata. C. molucellae is not merely associated with M. laevis but is the causal agent of cercospora leaf spot of the ornamental.

Results from the control plots showed that the flower length fell below the minimum market requirement. Thus the Cercospora leaf spot can result in 100% crop loss. Kocide 101 proved ineffective in controlling Cercospora leaf spot on M. laevis. Benlate at 250ppm and Mancozeb at 2000ppm effectively controlled the disease at 2 weeks spray interval by inhibiting conidial germination. Mancozeb resulted in higher flower length and would be more economical to use in control of cercospora leaf spot.

R E F E R E N C E S

- Alderman, S.C. and M.K. Beute, 1986: Influence of temperature and moisture on germination and germ tube elongation of Cercospora arachidicola. *Phytopath.* 76:715- 719.
- Alexopolous, C. J and C. W. Minns. 1979: Introductory mycology. Wiley international edition, 3rd edition New York pp. 566-567.
- Beckman, P. M. and G. A. Payne. 1981: Penetration, infection and development of Cercospora zeaе maydis in corn leaf. *Phytopath.* 71:202.
- Berger, R. D. and E. W. Hanson 1963: Pathogenecity, host parasite relationship and morphology of some forage legume cercosporae and factors related to disease development. *Phytopath.* 53:500-508.
- Berger, R. D. and E. W. Hanson 1963: Relationship of enviromental factors to growth and sporulation of Cercospora zebrina. *Phytopath.* 53:286-294.
- Barnett, H. L and V. G. Lilly 1950: Influence of nutrition and enviromental factors upon asexual reproduction of Choanephora cucurbitarum in culture. *Phytopath* 40:80-

- Baxter, J. W. 1956: *Cercospora* black stem of alfalfa. *Phytopath.* 46:398-400.
- Bremer and Petrak, 1947: Index of fungi 1940 - 1949 vol 1 Commonwealth mycological institute kew survey pp. 226
- Calpouzos, L. and G. H. Stallknecht, 1965: Sporulation of *Cercospora beticola* affected by an interaction between light and temperature. *Phytopath.* 55:1370-1371.
- Calpouzos, L and G. H. Stallknecht, 1967: Effects of light on sporulation of *Cercospora beticola*. *Phytopath* 57:679-681.
- Chupp, 1953: A monograph of the genus *cercospora*. Ithaca, New york, 1953 pp. 13, 269.
- Cooperman, C. J. and S. F. Jenkins. 1986: Conditions influencing growth and sporulation of *Cercospora asparagi* and cercospora blight development in asparagus. *Phytopath.* 76:617-619.
- Diachun, S. and W. D. Valleau. 1941: Conidial production in culture by *Cercospora nicotianae*. *Phytopath* 31:97-98.
- El-Gholt, N. E., S. A. Alfieri, W. H. Ridings and C.L. Schoulties. 1982: Growth and sporulation in vitro of

Cercospora apii, Cercospora archidicola, Cercospora kikuchii and other species of cercospora. Can. J. Bot. 60:862-868.

Elston, J. D Macdonald, C. Harkness. 1976: The effects of cercospora leaf disease on the growth of groundnuts in Nigeria. Samaru Research Bulletin. 261: 39-49.

Fawcett, H.S and L.J. Klotz. 1939: Four fungus parasite of sweet clover infecting seed. Phytopath. 29:661-662.

Garry A.S and E.G. Ruppel. 1971: Cercospora leaf spot as a predisposing factor in storage rot of sugar beet roots. Phytopath. 61:1485-1487.

Gatumbi, R. 1985: A new leaf spot disease on the ornamental plant Molucella laevis in Kenya (in press)

Gabona, S.M. and H.A. Melouk. 1981: Effect of temperature on sporulation and conidia germination Cercospora arachidicola. Phytioopath. 71:876.

Goode, M. J. and G. R. Brown 1970: Detection and characterization of Cercospora citrullina isolates that sporulate readily in culture. Phytopath. 60:1503-1503.

Grover S.T, D.H Smith and R.O Hammons. 1976: Resistance of peanut plant introduction to Cercospora arachidicola.

Plant Dis. Rep. 60:494-498.

- Harrison, A. L. 1969: Daconil and Benlate, two promising fungicides for peanut leaf spot control. *Phytopath.* 59:114.
- Hemingway, J. S. 1954: *Cercospora* leaf spots of groundnuts in Tanganyika. *E. Afr. Agri. J.* 19: 263-269.
- Hemingway, J. S. 1955: The prevalence of two species of *Cercospora* on groundnuts. *Trans. Brit. Mycol. Soc.* 38: 243-246.
- Hemingway, J. S. 1957: The resistance of groundnuts to *Cercospora* leaf spots. *Empire J. Exp. Agric.* 25: 61-68.
- Hilty, J. W., C. H. Hadden and J. T. Garden 1979: Response of maize hybrids and inbred lines to grey leaf spot disease and the effects on yield in Tennessee. *Plant Dis. Rep.* 63: 515-518.
- Horsfall, J. G. and R. W. Barratt. 1945. An improved grading system for measuring plant disease. *Phytopath.* 35:655.
- Anonymous, 1987: Exports statistics figures.
- Hughes, S. J. 1953: Conidiophores, conidia and

classification. Can. J. Bot. 31:577-659.

Jackson, L. F. 1980: Distribution and severity of peanut leaf spot in Florida. Phytopath. 71:324-328.

Jenkins, W. A. 1938: Two fungi causing leaf spot of peanuts. J. Agr. Research. 56:317-332.

Jensen, E. R, W. B. Lytton. 1965: Effect of temperature, relative humidity, precipitation on peanut leaf spot. Plant Dis. Rep. 49:975-978:

Jex-Blake, A. J. 1957: Gardening in East Africa. Longman press. 1957 PP. 56.

Kilpatrick, R. A. and H. W. Johnson, 1956: Sporulation of Cercospora species on carrot leaf decoction agar. Phytopath. 46:180-181.

Kingsland, C. G. 1963: Cercospora leaf blight of corn. A case history of a local epiphytotic in South Carolina. Plant Dis. Rep. 47: 724-725.

Larson, R. 1980: introduction to floriculture. Academic press. pp. 191.

Latch, C.G. and E.W. Hanson. 1962: Comparison of three stem disease of melitotus and their causal agents. Phytopath. 52:300-350.

- McCain, A.H. 1975: Fungicides for ornamental plants. Division of Agricultural Sciences. Univ. of California leaflet no. 2284.
- Meredith, D.S. 1967: Conidia release and dispersal in Cercospora beticola. Phytopath. 67:889-893.
- Miller, J.W. 1969: Cultural conditions affecting sporulation of Cercospora gossypina. Phytopath. 59:5111
- Nagel, C.M. 1934: Conidial production in species of cercospora in pure culture. Phytopath. 24:1101-1110
- Nattrass, R.M. 1961: Host lists of Kenya fungi and bacteria. Mycological papers No. 81:2-34.
- Nevill D.J. 1981: Components of resistance to Cercospora arachidicola and Cercosporadium personatum in groundnuts Ann. Appl. Biol. 99:77-86.
- Ondiek, J.J. 1973: Host list of kenya fungi and bacteria. E. Afr. Agri. For. J. 38: 1-25.
- Roane, C.W, R.L Harrison and C.F Genter. 1974: Observations of gray leaf spot of maize in virginia. Plant Dis. Rep. 58: 456-459.
- Rupe, J. C., M. R. Siegel and J. R. Hartman. 1982: Influence of enviroment and plant maturity on gray

- leaf spot of corn caused by Cercospora zea maydis.
Phytopath. 72:1587-1591.
- Sahim, K. 1987. Checklist of cut flowers to be grown from seeds pp. 85.
- Schneider, R. W. and J. W. Sinclair. 1975: Inhibition of conidial germination and germ tube growth of Cercospora canescens by cowpea leaf diffusates.
Phytopath. 64:1035-1039.
- Smith, D. H. 1971: A simple method for producing Cercospora arachidicola conidial inoculum. Phytopath. 61:1414.
- Smith, D. H. and R. H. littrell. 1980: Management of peanut foliar diseases with fungicides. Plant Dis. Rep. 64: 356-361.
- Sobers, E. K. 1969: The relationship of conidia morphology to colony characteristic in species of Cercospora.
Phytopath. 59: 118.
- Solel, Z. and G. Minz. 1971. Infection process of Cercospora beticola in sugarbeet in relation to susceptibility.
Phytopath. 61:463-466.
- Stavelly, J. R. and J. A. Nimmo. 1968: Relationship of plant and nutrition to growth and sporulation of Cercospora nicotianae. Phytopath. 58:1372-1376.

- Teri, J.M, H.D Thurston and C.J Lozano. 1980: Effect of brown leaf spot and cercospora leaf blight on cassava productivity. Trop. Agric. Trinidad 57: 239-242.
- Trione, J. E. and M. C. Leach. 1969: Light induced sporulation and sporogenic substances in fungi. Phytopath. 59:1077-1083.
- Vathakos, M. J. and H. J. Walters. 1979: Production of conidia by Cercospora kikuchii in culture. Phytopath 69:832-833.