

87826

P. D.
3.5.83

THE RELATIONSHIP BETWEEN RESISTANCE TO
GLOEOSPORIUM LEAF DISEASE (Colletotrichum gloeosporioides)
AND
SOUTH AMERICAN LEAF BLIGHT (Microcyclus ulei) IN HEVEA

A thesis submitted to the Faculty of Applied Science,

University of Sri Jayawardenapura, Sri Lanka

for the

Degree of Master of Science

by

LANKANATH CHANDAN WIJETILAKA

87826

November, 1981

Library
Department of Biological Sciences
University of Sri Jayawardenapura
Nugegoda.

A B S T R A C T

1. Pre penetration studies revealed that there was no significant difference in conidia germination of C. gloeosporioides on susceptible and resistant Hevea leaves when compared within hours. However, the rate of germination was higher in susceptible clones. The rate of germtube elongation in resistant clones decreased after 12 h and the appressoria formation occurred earlier at 3 h after inoculation in resistant leaves.
2. The penetration of leaves of resistant clones was limited to epidermal and palisade layers. In susceptible clones the fungus colonised the mesophyll cell layers, after 48 h and acervuli development was detected after 72 h.
3. Conidia germination and germtube growth of C. gloeosporioides in the leachate of resistant Hevea clones was lower compared to their behaviour in leachate of susceptible clone. However, there was an increase of appressoria in leachate of resistant clones.
4. The germination of C. gloeosporioides conidia in chloroform extracts of leaf waxes of different Hevea clones showed that there was an inverse relationship between germtube length and SALB resistance. 4 compounds which were phenolic and unsaturated fatty acid in nature were isolated chromatographically. They were responsible for the inhibition of germtube length.

Bioassays on these compounds showed that there were quantitative differences in these compounds among the Hevea clones. An inhibition of germination and germtube length was observed with increased wax concentration.

5. Chromatographic studies of leaf methanol extracts showed 8 phenolic compounds. Of these 6 compounds, showed inhibition of germination and germtube elongation. Bioassays on these compounds, showed that the susceptible clones possessed quantitatively different amounts of phenols. 6 inhibitory compounds were observed in leaf methanol extracts of resistant clones and in susceptible clones there were 2 inhibitory compounds.

6. Among the naturally occurring phenols kaempferol and quercetin were present in considerable amounts in Hevea leaves. Some SALB susceptible Hevea leaves possessed higher amounts of quercetin.

7. The total phenol content and ortho-dihydroxy phenol content of Hevea leaves did not show any relationship to the SALB resistance. However, ortho-dihydroxy phenol content in bark of Hevea increased with SALB resistance.

8. Qualitatively there were no differences in the sugars and amino acids in leaves of Hevea clones tested.

9. Major and trace nutrient contents in Hevea did not show any relationship with SALB resistance.

10. The ortho-dihydroxy phenol content increased after 24 h, in inoculated resistant Hevea clones; inoculated susceptible leaves showed an increase of phenols after 48 h. The phenol content declined after 72 h and 96 h. The flavanol content of different Hevea leaves also showed a pattern similar to the ortho-dihydroxy phenol content.

11. Peroxidase increased with the maturity of all clones tested when examined at three different development stages, the 7-day-old copper-brown stage showed an inverse relationship with SALB resistance. Peroxidase activity was higher in SALB susceptible leaves than in the resistant leaves. An increased PA at 24 h after inoculation could be observed in resistant clones but susceptible clones showed increased PA after 72 h.

12. The glycoside content showed an inverse relationship to SALB resistance. However, in infected resistant leaves the liberation of HCN was higher after 24 h and 48 h than in the infected susceptible leaves.

13. Gibberellic acid showed 59% conidia germination even at 100 ppm concentration. An increased concentration of IAA significantly reduced the colony growth of resistant leaves when compared to susceptible leaves. The germination also increased with IAA concentration.

14. IAA increase the PA of inoculated resistant leaves, but susceptible leaves exhibited lowering of PA. Increased PA was not related to the reduction of colony growth.

15. Eventhough some of the reactions of Hevea leaves to M. ulei are not similar, it is possible that the glycoside content and PA can assist in early selection of SALB resistant clones.

C O N T E N T S

MEMORANDUM	i
ACKNOWLEDGEMENTS	ii
ABSTRACT	iii
ABBREVIATIONS	xiv
1. INTRODUCTION	1
1.1 Disease symptoms	5
1.2 The pathogen	8
1.3 Preventive measures for introduction of SALB	9
1.4 Chemical control	11
1.5 Breeding for resistance	12
2. REVIEW OF LITERATURE	17
2.1 Nomenclature	17
2.2 Physiological races and strains of the fungus	18
2.3 Leaf cuticle and disease resistance	19
2.4 Penetration by the fungus	20
2.5 Nutrients in plant resistance	21
2.6 Hypersensitivity	22
2.7 Phenols	23
2.8 Peroxidase	25
2.9 Hydrogen cyanide	28
2.10 Auxin in plant resistance	29
3. MATERIALS AND METHODS	31
3.1 Selection of clones	31
3.2 Selection of leaves	32

3.3	Inoculum preparation and maintenance	32
3.4	Preparation of standardised spore suspension	33
3.5	Inoculation	33
3.6	Pre and Post penetration studies	33
3.6.1	Pre-penetration studies	34
3.6.2	Assessment of growth	34
3.6.3	Post-penetration studies	34
3.7	Bioassays	35
3.8	Leachates	35
3.9	Leaf waxes	35
3.9.1	Quantitative determination and bioassays	35
3.9.2	The effect of different concentrations of leaf waxes on germination, germ tube length and appressoria formation of <u>C. gloeosporioides</u>	36
3.9.3	Thin layer chromatography (TLC) analysis of leaf waxes	36
3.10	Chromatographic studies on amino acids, sugars and phenols	37
3.10.1	Loading of chromatograms	37
3.10.2	Separation of sugars	38
3.10.3	Separation of amino acids	38
3.10.4	Separation of phenols	38
3.10.5	Elution of phenols	39
3.11	Identification of sugars, amino acids and phenols	40
3.11.1	Sugars	40
3.11.2	Amino acids	40
3.11.3	Phenols	41

3.12	Leaf extracts for spore germination studies	42
3.13	Determination of total phenols in petiole, leaf and bark	42
3.14	Determination of ortho dihydroxy phenols	43
3.14.1	Leaves	43
3.14.2	Bark	43
3.15	Extraction of quercetin and kaempferol	43
3.16	The effect of naturally occurring phenols on the <u>C. gloeosporioides</u> spore germination	44
3.17	Histochemical studies	44
3.17.1	Peroxidase activity	44
3.17.2	Polyphenols	44
3.18	Determination of macro and micro nutrients	45
3.18.1	N, P and K	45
3.18.2	Ca, Mg and other elements	46
3.19	Enzymological studies	46
3.19.1	Peroxidase activity	46
3.19.2	Glycoside content	47
I.	Distillation	47
II.	Application of chemicals	48
3.20	The change of o-dihydroxy phenols, flavanols, glycoside content and peroxidase activity of artificially inoculated leaves	48
3.20.1	Ortho dihydroxy phenols	48
3.20.2	Flavanol content	49
3.20.3	Glycoside content	50
3.20.4	Peroxidase activity	50

3.21	Auxins	51
3.21.1	The effect of auxins on the germination of <u>C. gloeosporioides</u> conidia	51
3.21.2	The effect of IAA on germination and colony growth of <u>C. gloeosporioides</u> conidia	51
3.21.3	The effect of IAA on peroxidase activity	52
4.	RESULTS	53
4.1	Pre and Post-penetration studies	53
4.1.1	Pre-penetration observations	53
4.1.2	Post-penetration studies	64
4.2	Leaf leachate	68
4.2.1	The germination and germtube length of <u>C. gloeosporioides</u> conidia in leaf leachate of different <u>Hevea</u> clones	68
4.2.2	Appressoria formation of conidia of <u>C. gloeosporioides</u> in leachate of different <u>Hevea</u> clones	68
4.3	Leaf waxes	68
4.3.1	Quantitative ditermination of leaf waxes of different <u>Hevea</u> clones	68
4.3.2	Effect of leaf waxes on germtube length of <u>C. gloeosporioides</u>	73
4.3.3	Thin layer chromatography (TLC) of leaf waxes	75
4.3.4	Effect of leaf waxes separated and eluted from thin layer chromatograms on germination and germtube length of conidia of <u>C. gloeosporioides</u>	77
4.3.5	The effect of different concentration of leaf waxes on conidia germination, appressoria formation and germtube length	77
4.4	Comparison of sugars of <u>Hevea</u> leaves	81

4.5	Comparison of amino acids of <u>Hevea</u> clones	81
4.6	Phenols	85
4.6.1	Total phenol content of leaves, bark and petiole extracts of different <u>Hevea</u> clones	85
4.6.1.1	Comparison of phenol content in leaves of <u>Hevea</u> clones	87
4.6.1.2	Comparison of total phenol content of petiole and bark of <u>Hevea</u> clones	87
4.6.2	Ortho-dihydroxy phenol content of <u>Hevea</u> leaves	88
4.6.3	Ortho-dihydroxy phenol content in bark of <u>Hevea</u>	88
4.6.4	Quercetin content in <u>Hevea</u> leaves	88
4.6.5	The effect of phenols extracted from leaves of SALB susceptible and resistant <u>Hevea</u> clones on conidia germination of <u>C. gloeosporioides</u>	92
4.6.6	The effect of naturally occurring phenols on the germination of <u>C. gloeosporioides</u> conidia	92
4.7	Extraction and chromatography of phenols	94
4.7.1	Bioassays on the <u>Hevea</u> leaf phenolics	96
4.7.2	Comparison of eluted compounds in water extracts of the resistant clone RRIC 117 and susceptible clone RRIC 114	101
4.7.3	Effect of phenols and their dilution on spore germination	106
4.7.4	Separation of the phenols extracted from petiole and bark tissues of different <u>Hevea</u> clones	107
4.7.4.1	Effect of petiole extracts on germtube length	109
4.7.4.2	Effect of bark extracts on germtube length	109

4.8	Histochemical studies	111
4.8.1	Peroxidase activity	111
4.8.2	Polyphenols	112
4.9	Nutrients in <u>Hevea</u>	112
4.9.1	Major nutrients present in leaves of <u>Hevea</u> in three development stages, petiole and bark	112
4.9.2	Trace elements in leaf, bark and petiole of <u>Hevea</u>	116
4.10	Peroxidase activity and glycoside content of healthy <u>Hevea</u> leaves	116
4.10.1	Peroxidase activity of leaves of different development stages	116
4.10.2	Peroxidase activity of leaves of different <u>Hevea</u> clones	122
4.10.3	Glycoside content of healthy leaves of <u>Hevea</u>	122
4.11	Change in ortho-dihydroxy phenol content, flavanol content, peroxidase activity and glycoside content of <u>Hevea</u> leaves during infection	125
4.11.1	Change in the ortho-dihydroxy phenol content of <u>Hevea</u> leaves during infection	125
4.11.2	Change of flavanol content during infection of <u>C. gloeosporioides</u> in leaves of <u>Hevea</u>	127
4.11.3	Peroxidase activity during infection of leaves of different <u>Hevea</u> clones	127
4.11.4	Change of glycoside content during infection of <u>C. gloeosporioides</u> in leaves of different <u>Hevea</u> clones	130

4.12	Auxins	132
4.12.1	The effect of auxins on the germination of conidia of <u>C. gloeosporioides</u>	132
4.12.2	The effect of exogenously supplied IAA on the germination of <u>C. gloeosporioides</u> conidia on leaves of <u>Hevea</u>	133
4.12.3	The effect of IAA on colony growth of <u>C. gloeosporioides</u> on leaves of <u>Hevea</u> cultivars	133
4.12.4	The effect of IAA on peroxidase activity of leaf discs of <u>Hevea</u> cultivars	134
5.	DISCUSSION	136
6.	REFERENCES	159