BIOLOGY OF DOTHIDELLA ULEI
I. IN VITRO PRODUCTION OF TOXIN
II. DIFFERENTIAL CLONES OF HEVEA FOR
IDENTIFYING RACES OF THE FUNGUS

By
JOHN WESLEY MILLER

A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF
THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

April, 1965

ACKNOWLEDGMENTS

I am deeply indebted to the Firestone Plantations Co., B. H. Larabee, President, for providing the fellowship which made this research possible. Acknowledgment is made to Dr. K. G. McIndoe, Firestone Plantations Co., for his cooperation and advice during this study and for his helpful review of the manuscript.

I wish to express my sincere gratitude to Dr. D. A. Roberts, Chairman of the Supervisory Committee, for encouragement and guidance throughout the course of this research and in preparation of the manuscript. Acknowledgment is also made to Dr. P. Decker, Dr. V. G. Perry, and Dr. D. S. Anthony for their critical review of the manuscript. I am indebted to Dr. R. E. Stall who suggested an injection technique for bioassay of the toxin produced by Dothidella ulei.

FOREWORD

The research reported in this dissertation concerned two distinct facets of the biology of Dothidella ulei. Therefore, each phase was written in the form of a separate manuscript in the style used for publication in "Phytopathology," the journal of the American Phytopathological Society. The "Style Manual for Biological Journals" was followed for use of abbreviations, numerals, etc.

TABLE OF CONTENTS		Page
ACKNOWLEDGMENTS		ii
FOREWORD		iii
LIST OF TABLES		v
PART I. <u>IN VITRO</u> PRODUCTION OF TOXIN		
Introduction		1
Materials and Methods		2
Results		5
Discussion		6
PART II. DIFFERENTIAL CLONES OF <u>HEVEA</u> FOR IDENTIFYING RACES OF THE FUNGUS		
Introduction		9
Materials and Methods		10
Results		11
Discussion		15
LITERATURE CITED		18
BIOGRAPHICAL SKETCH		20

LIST OF TABLES

Table	Page
1. Number of times that <u>Hevea</u> clones gave indicated reactions to 3 races of <u>Dothidella ulei</u>	12
2. Differential <u>Hevea</u> clones and their reactions ^a to 4 races of <u>Dothidella ulei</u>	14

PART I

IN VITRO PRODUCTION OF TOXIN

Introduction

DeBary (1) was among the first to show the pathological effect of a fungal culture filtrate against plant tissue. Many toxic substances produced by various micro-organisms have since been discovered; these include the host-specific (16) pathotoxins (19) Victorin (14) and the Periconia toxin (17), the vivotoxins (5, 19) Fusaric acid (7) and the wildfire toxin (21), and the phytotoxins (19) Alternaric acid (15), Colletotxin (8), Victoxinine (18), and Lycomarasmin (20). The literature on this subject has been reviewed (4, 5, 16, 19).

Penetration of para rubber tree leaves, Hevea spp., by Dothi-
della ulei P. Henn., causal agent of South American Leaf Blight, occurs within 24 hr after inoculation (3). A depressed area in the leaf forms approximately 4 days later, and is the first symptom of disease (3). The thin, chloranemic spots enlarge to diameters of 5-8 mm; sporulation of the fungus begins 8-14 days after inoculation. The lesions are later delimited by a thin, black margin and the centers usually drop out, producing a "shot-hole" effect (9). This suggests that a toxin is produced by the fungus, since the histogenic demarcation that causes the "shot-hole" symptom is said to be a specific defense reaction against toxins produced by the parasite or by the host as a result of infection (6). The purpose of the research reported here was to determine

whether D. ulei produces a toxin capable of inciting symptoms in Hevea leaves similar to those diagnostic of the disease caused by the fungus itself.

Materials and Methods

Dothidella ulei was grown in culture on agar slants and in a liquid medium. The ingredients, per liter, in the agar medium were: 39 g of "Difco" potato-dextrose agar, 5 g of peptone, 5 g of phytone (a papain digest of Soya bean meal), 5 g of malt extract, 0.5 g of yeast extract and enough deionized water to make 1,000 ml (11). Samples of approximately 10 ml of the culture medium were placed in test tubes, autoclaved for 30 min at 15 psi, and were allowed to harden as agar slants. Isolations of the fungus were made from infected leaves by scraping off conidia and inserting them into the agar medium. Blazquez (2) observed that germ tubes from conidia germinating on the surface of an agar medium grew aeri ally; growth of the fungus ceased if the aerial germ tube touched the agar before branches near its base had grown enough to establish a colony. Langdon (10) found that germ tubes from conidia germinated below the surface of agar grew directly into the surrounding medium with no apparent difficulty and colonies were established with a much higher degree of success than with surface-germinated spores. Consequently, the method of sub-surface germination was used for isolating the fungus from leaves.

Colonies that developed in pure cultures were allowed to grow for 2-3 months, and the elevated, stromatic bodies were picked off the agar. These were ground for 1 min in the micro-attachment of the Servall Omni-

mixer while being kept cold in an ice water bath. The resulting suspension was centrifuged at about 3500 g, and was filtered by suction through a Seitz filter pad with a pore diameter of 0.1 μ . The resulting fungal-free filtrate was tested later for toxicity to Hevea leaves.

To determine whether D. ulei produces an extracellular toxin, the fungus was grown in a liquid culture medium similar to the agar medium, except that the commercial potato-dextrose agar was replaced by 20 g of dextrose and by the broth from 200 g of potatoes cooked for 40 min in 500 ml water. Samples of 250 and 500 ml were placed in 500- and 1,000-ml flasks, respectively, and were autoclaved for 30 min at 15 psi. In some experiments, a bacterial contaminant that could withstand autoclaving grew profusely in the rich medium. Sterilization then was accomplished by autoclaving for 30 min at 15 psi, 3-5 times at daily intervals or by autoclaving once for 2 hr at 15 psi. These treatments caused the medium to darken, but had no apparent effects on subsequent growth of the fungus.

The liquid culture medium in each flask was infested with sporulating colonies of D. ulei and was kept at 20° C for 7 weeks to 6 months without shaking. Following the growth period, the cultures were filtered aseptically by suction through a Seitz filter pad with pore diameter of 0.1 μ . This provided a fungal-free culture filtrate; uninfested medium of the same age was filtered in the same manner and served as a control. Concentration, when desired, was accomplished by either of 2 methods. The filtrates were placed in dialysis tubing and hung either in an incubator equipped with a fan and with temperature controlled at approxi-

mately 5° C or in front of a fan in a room with the temperature controlled at 20° C. The latter method was the more efficient, as concentrations of more than 20-fold were achieved in 3-4 days and fungal contamination on the outside of the dialysis tubing was avoided. Contamination occasionally occurred on the outside of the bags in the incubator. This was controlled by wiping the bags with a cloth saturated with 10% commercial Clorox.

Several methods of bioassay for toxic activity in mycelial extracts and culture filtrates were tried. No symptoms developed in leaves floated on water and on which drops of test solutions were placed, even though some leaflet tissues under the drops were wounded with a pin to provide entrance into the leaf. Test solutions also failed to induce symptoms after vacuum infiltrations of leaf disks.

Symptoms of toxic activity were observed, however, when the cut ends of petioles of detached leaves were submerged in test solutions. Tip and marginal curl of the leaflets, followed by drying and blackening, developed in leaves placed in fungal culture filtrates or mycelial extracts. Symptoms frequently developed in control leaves placed in sterile culture medium, although they were usually milder and were always slower in appearing. It was later observed that mechanical injury also caused leaflet curl, drying, and blackening.

Distinct symptoms developed in leaves of greenhouse-grown Hevea seedlings after mycelial extracts or culture filtrates had been injected into their petioles or stems. Seedlings of clones PB 86, AV 1126, AV 1581, and Tjirandji 1, all susceptible to all known races of the

fungus, were used. Injections with a syringe and needle (size 26 or 27) were made either into the stem just below the developing leaf flush or into the petioles of leaves in Stage II or early Stage III (3). Stage II in the growth of Hevea leaves occurs when the leaflets are folded dorsally and are 1-2 cm long. Stage III occurs when the leaflets are unfolding and are 2-10 cm long. In early Stage III the leaflets are 2-4 cm long. No symptoms were observed to develop when treatments were begun on leaves past this stage of development. Injections were made once or twice daily for 7-10 days. A total of 0.5-0.75 ml of solution was injected into each treated petiole or stem. Results reported below were obtained from experiments in which the injection method of testing had been used.

Results

Chloranemic spots with thin, transparent centers developed in susceptible leaflets 2-9 days after the first injection of mycelial extract or culture filtrate. The tissue surrounding the lesions soon became savoyed, and affected leaflets became distorted because their normal expansion was retarded near the lesions. These symptoms induced by fungal-free mycelial extracts or by sterile culture filtrates were strikingly similar to those induced by the fungus itself (3, 9). Symptoms developed in 2-6 days after the first injection when the stem below the developing leaf flush or when leaflet petioles were injected with the fungal-free mycelial extract, whereas symptoms appeared in 4-9 days after the first injection with sterile culture filtrate. The variation in time of symptom appearance was apparently related to the source of toxic

material, and may be due to differences in concentration in the different materials.

The culture medium or deionized water occasionally caused small flecks to form in leaflets when their petioles had been treated. There was, however, neither chloranemia nor any adverse effect on the expansion of leaflets. Leaf growth ceased when water or the culture medium or test materials were injected into petioles before leaves had reached Stage II of their development. Growth cessation was occasionally observed in Stage II leaves, but never in Stage III leaves. Affected leaves became anthocyanescent, distorted, and desiccated before they died some 7-10 days after the first appearance of symptoms.

In 3 experiments, the culture filtrate (concentrated 10X) was either boiled for 1 hr or dialyzed against deionized water for 96 hr before injection of susceptible Hevea seedlings. In 2 experiments with boiled material and in one with dialyzed material, neither chloranemia nor leaf distortion occurred in leaflets that developed on treated petioles. Thus, the symptom-inducing principle that occurs in fungal-free culture filtrates appears to be heat labile and dialyzable.

Discussion

Wheeler and Luke (19) proposed the term "pathotoxin" for those toxins that play an important causal role in plant diseases. Criteria for determining whether or not a toxin belongs in this category are: "(a) the toxin, applied at concentrations which could be reasonably expected in or around the diseased plant, produces in a susceptible host all the symptoms characteristic of the disease; (b) the pathogen and

the toxin exhibit similar suscept specificity; (c) the ability of the pathogen to produce the toxin varies directly with its ability to cause disease; (d) a single toxin is involved."

In the work reported here, symptoms produced by less than 1 ml of the crude fungal-free filtrate or the crude mycelial extract appeared to be the same as those induced by D. ulei itself. Fungal-free materials thus far tested, however, have not caused the symptom of "shot-hole." Thin, chloranemic spots and leaflet distortion occurred, regardless of whether plants were treated with the toxic materials or inoculated with conidia of the fungus. Since these results satisfy the modified (19, p. 233) first, and most important, criterion of a pathotoxin, the host-specific toxin of Pringle and Scheffer (16), the toxin produced by D. ulei is temporarily classified as such. It definitely is not a phytotoxin, because it produces almost all of the symptoms incited by the fungus, whereas a phytotoxin produces a few or none of the symptoms incited by the pathogen (19). Admittedly, future research may show that the toxin from D. ulei does not fit all of the criteria of a pathotoxin and it might have to be classed then as a vivotoxin (5, 19).

Best evidence for in vitro toxin production by D. ulei is the fact that the fungal-free mycelial extracts or culture filtrates induce symptoms indistinguishable from those incited by the fungus itself; this evidence is strongly supported, however, by the fact that boiled or dialyzed culture filtrates had no toxic effects on Hevea leaves. Moreover, only Stage II and early Stage III leaves were susceptible to the toxin, and leaflets in these stages of development were also most susceptible to attack by the fungus.

The high potency of mycelial extracts from colonies grown on a solid medium indicates that the toxin diffuses but slowly into agar. The toxin evidently is highly soluble in a liquid culture medium, however, because extracts prepared from the fungus grown in liquid culture showed no toxic activity by the injection method of bioassay.

If the toxin from D. ulei does indeed prove to be a pathotoxin, a valuable program of testing Hevea clones for resistance could be developed; it might be possible to make such tests with the toxic principle in the absence of the fungus itself. Thus, Hevea clones would not have to be sent to infested areas (Central and South America) for resistance testing and then returned to areas free of Dothidella (Africa and the Far East) for further development. The time needed to develop resistant, high-yielding clones for commercial use would thereby be reduced. These benefits would represent great economic savings for the commercial rubber industry. Most important, however, would be reduction of the danger of the fungus being carried as a contaminant to the uninfested regions, where establishment of the fungus could result in severe damage to the plantation rubber industry.

It should be pointed out, however, that many problems involved in using the toxic material in resistance testing would have to be solved. Among these are how to obtain sufficient material for injection of thousands of seedlings, how to purify the toxin and under what conditions the toxic material retains its potency. Obviously, it would take years of research to solve these and, possibly, other problems inherent in such a project.

PART II

DIFFERENTIAL CLONES OF HEVEA FOR IDENTIFYING RACES OF THE FUNGUS

Introduction

Pathological specialization in Dothidella ulei has been suspected since 1946. Langford (13) observed near Belterra, Brazil, that Ford clones (F 409 and F 1619) of Hevea brasiliensis, that were previously rated as resistant to the disease caused by D. ulei, were attacked by a local strain of the fungus. In 1960, Langford (13) noted that progeny of the resistant clone F 4542 (H. benthamiana) were infected by a strain of the fungus in Costa Rica. This observation was confirmed experimentally by Langdon (12), who was the first to obtain direct evidence that pathological races of D. ulei exist. Working with isolates from Guatemala and Costa Rica and under greenhouse conditions, he made pathogenicity studies on clones with and without F 4542 parentage. The isolate from Costa Rica attacked and sporulated heavily on all F 4542 clones tested and was designated Race 2. The Guatemalan isolate attacked no F 4542 clones, except IAN 717, and was designated Race 1.

Since it had been confirmed that at least 2 races of D. ulei exist, the need for establishing a set of differential host clones for race identification became apparent (12). Also needed is further testing of established races and testing of new isolates of the fungus against different clones that represent various sources of resistance.

The objectives of the research reported here were to determine which clones could be used to differentiate among known existing races and to identify additional races.

Materials and Methods

Race 1 of Dothidella ulei from central Guatemala had been established in the greenhouse by Langdon (12). Race 2 was not used. Two new isolates were sent by Dr. K. G. McIndoe from Navajoa, Guatemala, a third was obtained from Mr. H. Echeverri, Goodyear Speedway Estates, Costa Rica, and a fourth was received from the Instituto Agronomico do Norte, Belem, Para, Brazil. All were established and maintained on seedlings of the family Tjir. 1 X Tjir. 16 growing in the greenhouse. They were also kept in culture on agar slants containing, per liter, 39 g of "Difco" potato-dextrose agar, 5 g of peptone, 5 g of phytone (a papaic digest of soya bean meal), 5 g of malt extract, and 0.5 g of yeast extract (11). In order to maintain a virulent culture of the races of D. ulei (10), isolations from infected leaves were made about every 6 months, because pathogenicity decreased after 6 months and cultures became avirulent at the end of approximately 12 months.

Inoculations of Hevea plants were made by picking conidia from sporulating lesions with a wet camelhair brush and placing the conidia onto the abaxial surface of leaflets in Stage III (3) of their development. The leaf flush was then covered with a moistened plastic bag, which was left in place 16-24 hr. On seedlings used to maintain inoculum, the entire leaf flush was inoculated with a single race or isolate. No mixing of the races occurred and on only 2 occasions did seedlings

that had not been inoculated by hand become infected. On test clones a separate pair of leaves was inoculated with each race or isolate to be tested, each inoculated leaf was labelled, and the entire leaf flush was bagged overnight. This permitted testing of the different isolates under very similar conditions.

Disease severity ratings on test clones were made 2-4 weeks after inoculation, using the Firestone Plantations Co. system, modified for greenhouse use (12). Ratings under this system were divided into 4 classes: resistant (R), flecks or very small non-sporulating lesions; highly resistant (HR), small non-sporulating lesions; moderately resistant (MR), larger lesions with light or no sporulation; susceptible (S), large lesions with heavy sporulation. In this research conducted under conditions where air temperatures were held at 70° F or higher and where humidity was maintained at high levels by an intermittent mist system, light sporulation was considered necessary for a rating of MR.

Results

Further testing with Race 1 and with new isolates of the fungus was carried out, with the results summarized in Table 1. The 2 isolates from Navajoa, Guatemala, and the isolate from Costa Rica proved similar and were designated as Race 3. All data on Race 2 were obtained from research done by Langdon (10). Clone IAN 717, a clone having F 4542 in its parentage, was rated as susceptible to Race 2 and Race 3, but was highly resistant or moderately resistant to Race 1. All other clones with F 4542 parentage, however, were rated as highly resistant to Race 3, but were moderately resistant (with sporulation) or suscepti-

Table 1. Number of times that Hevea clones gave indicated reactions to 3 races of Dothidella ulei

Clone	Parentage ^a	Race 1				Race 3				Race 4			
		R	HR	MR	S	R	HR	MR	S	RR	HR	MR	S
IAN 710	PB 86 X F 409				29				30				1 1
IAN 713	PB 86 X F 409				26				26				1
IAN 717	PB 86 X F 4542				11				2 10		4		
IAN 873	PB 86 X FA 1717				15	4	1		11	6	1		5 1
FX 25	F 351 X AV 49				20				17				3
FX 232	F 351 X PB 186				4				3				
FX 637	F 4542 X Tjir. 1	1	4						4			1	
FX 664	F 4542 X Tjir. 1	3	12						14				2
FX 2831	F 4542 X Tjir. 1				7				6				2
FX 3810	F 4542 X AV 363	5	6					2	7				
FX 3925	F 4542 X AV 363				22				25				3
MDF 72	Seedling Selection				1				1				
MDF 138	Seedling Selection				4				2				
MDF 158	Seedling Selection				8				9				1
MDF 180	Seedling Selection				22				23			1	4
MDF 232	Seedling Selection				10				10				1
MDF 350	Seedling Selection				11				6				
MDF 363	Seedling Selection				3				4				1
MDX 13	AV 308 X MDF								2				1 1
P 122	Forest Selection				4				9				1

^aMDF (Madre de Dios Firestone) clones were selected in Guatemala from seedlings arising from seedlings obtained from the Madre de Dios region of Peru. P 122 was obtained as budwood from a native tree of the Madre de Dios region of Peru.

ble to Race 2; this clearly distinguished Race 2 from Race 3.

The isolate from Brazil was designated as Race 4. Ratings of susceptible in IAN 710 and IAN 713, clones of F 409 parentage, indicated that this was the same or a similar isolate to the one from Brazil previously noted by Langford (13). These clones were highly resistant to Races 1, 2, and 3, and can, therefore, be used to distinguish between any of these races and Race 4. Also, FX 25, MDF 180, MDX 13, and P 122 were rated as moderately resistant (with sporulation) to Race 4. These clones are from a variety of parentages (Table 1) and demonstrate the ability of Race 4 to overcome several sources of resistance. Clones derived from F 4542, however, generally showed a high degree of resistance to this race.

Based on reactions of the different clones to various races of the fungus already identified, a set of differentials was established (Table 2). IAN 717, with F 4542 as one of its parents, was rated as susceptible to Races 2 and 3. This differentiates these races from Race 1, to which IAN 717 was rated as highly resistant to moderately resistant. FX 3925, another F 4542 selection, differentiates Race 3 from Race 2, because this clone is resistant to the former race and susceptible to Race 2. IAN 710 and IAN 713, both F 409 progeny, and MDF 180, a Madre de Dios selection of Firestone, separates Race 4 from all other races, since this race was able to sporulate on these clones, whereas Races 1 and 3 could not. The clones derived from F 409 are also highly resistant to Race 2 (10), but this race has not been tested on MDF 180.

IAN 873, a clone selected from a cross of PB 86 X FA 1717, which

Table 2. Differential Hevea clones and their reactions^a to 4 races of Dothidella ulei

Clone	Race 1	Race 2	Race 3	Race 4
IAN 717	HR	S	S	HR
FX 3925	HR	S	HR	HR
IAN 710 or 713	HR	HR	HR	S
MDF 180	HR	-- ^b	HR	MR
P 122	R	R	R	MR

^aR, resistant; HR, highly resistant; MR, moderately resistant; S, susceptible.

^bRace 2 not tested against MDF 180.

had previously rated as highly resistant to Races 1, 2, and 3, suddenly began to exhibit sporulation and to show ratings of moderately resistant or susceptible to Races 1, 3, and 4. This change occurred in November, 1964, and was observed on several individuals of this clone in the greenhouse. The change was not due to changes in the races because all other clones tested since then showed their normal reactions to Races 1 and 3. Apparently IAN 873 has resistance that is more sensitive than that of related clones to subtle changes in the environment. In any event, its reaction to Races 1 and 3 is unpredictable. It was not exposed to Race 2 after this variability in its response to other races was noted. Also, it had not been inoculated with Race 4 prior to the time of its apparent change.

Discussion

Multiple race testing of Dothidella ulei was continued and expanded (Table 1), with the identification of 2 additional races of the fungus. A set of 5 differential clones for race identification was also established (Table 2). Four races of the fungus have now been identified. Some of these known races are able to break down sources of resistance derived from F 4542, F 409, and/or MDF clones, when these sources are used singly.

With 4 ratings on each of the 5 differential clones, it is theoretically possible to identify 5^4 , or 625, races of D. ulei. Undoubtedly, there are other races, as yet undiscovered, that attack clones of different genetic makeup than those presently used as differentials (Table 2), and new clones to detect these races will need to be added. Moreover,

there are only 2 groups of distinctive ratings, resistant (R and HR) and susceptible (MR and S). The resistant group shows no sporulation, whereas the susceptible group shows light to heavy sporulation. This allows for ready identification of only 5^2 , or 25 races of the fungus. Low humidity conditions, which often occur in the field, inhibit sporulation on clones that would be rated as MR with light sporulation. This absence of sporulation would result in the erroneous rating, HR. Under the conditions of high humidity in the greenhouse used for this research, sporulation occurred on all susceptible (MR and S) clones, but never occurred on resistant clones. Such consistencies make the ratings reported here appear reliable.

In one instance, Race 4 failed to sporulate on one individual of the clone MDF 180 (Table 1). The mist system was out of order at the time of this inoculation, and the relative humidity in the greenhouse was lower than usual. Later, when high humidity was maintained, Race 4 sporulated on the first and one other individual of clone MDF 180. These results are interpreted to mean that this clone is actually susceptible (MR) to Race 4. They also emphasize the necessity for maintaining high humidity around test plants.

The results reported in this research strengthen Langdon's (12) argument for the need of using a race identification program and multiple race resistance testing in conjunction with multiple source breeding for resistance. The use of only single sources of resistance limits the areas of usefulness of a particular clone to locations where the dominant races are those to which that clone is resistant. Such a practice

also increases the chances that races which can attack clones with this type of resistance will build up and perhaps destroy the commercial planting. The use of multiple sources of resistance would probably increase the geographical range and life of a particular clone developed in this manner.

LITERATURE CITED

1. Bary, A. de. 1886. Ueber einige Sclerotinien und Sclerotienkrankheiten. Botan. Ztg. 44: 409-426.
2. Blazquez, C. H., and J. H. Owen. 1957. Physiological studies of Dothidella ulei. Phytopathology 47: 727-732.
3. Blazquez, C. H., and J. H. Owen. 1963. Histological studies of Dothidella ulei on susceptible and resistant clones. Phytopathology 53: 58-63.
4. Braun, A. C., and R. B. Pringle. 1958. Pathogen factors in the physiology of disease. Toxins and other metabolites, p. 88-99. In C. S. Holton, et al. (eds.), Plant pathology, problems and progress, 1908-1958. Univ. Wisconsin Press, Madison.
5. Dimond, A. E., and P. E. Waggoner. 1953. On the nature and role of vivotoxins in plant disease. Phytopathology 43: 229-235.
6. Gaumann, E. 1946. Types of defensive reactions in plants. Phytopathology 36: 624-633.
7. Gaumann, E. 1957. Fusaric acid as a wilt toxin. Phytopathology 47: 342-357.
8. Goodman, R. N. 1959. Observations on the production, physiological activity, and chemical nature of Colletotrin, a toxin from Colletotrichum fuscum Laub. Phytopathol. Z. 37: 187-194.
9. Hilton, R. N. 1955. South American leaf blight. J. Rubber Research Inst. Malaya 14: 287-354.
10. Langdon, K. R. 1963. Culture and pathogenicity of Dothidella ulei. Ph.D. Thesis. Univ. of Florida, Gainesville. 36 p.

11. Langdon, K. R. 1963. Personal Communication.
12. Langdon, K. R. 1965. Relative resistance or susceptibility of several clones of Hevea brasiliensis and H. brasiliensis X H. benthamiana to two races of Dothidella ulei. Plant Dis. Repr. 49: 12-14.
13. Langford, M. H. 1960. A new strain of leaf blight on rubber trees in Costa Rica. (Mimeo.) Report to AID, Washington, D. C. 4 p.
14. Meehan, Frances L., and H. C. Murphy. 1947. Differential phytotoxicity of metabolic by-products of Helminthosporium victoriae. Science 106: 270-271.
15. Pound, G. A., and M. A. Stahmann. 1951. The production of a toxic material by Alternaria solani and its relation to the early blight disease of tomato. Phytopathology 41: 1104-1114.
16. Pringle, R. B., and R. P. Scheffer. 1964. Host-specific plant toxins. Ann. Rev. Phytopathol. 2: 133-156.
17. Scheffer, R. P., and R. B. Pringle. 1961. A selective toxin by Periconia circinata. Nature 191: 912-913.
18. Scheffer, R. P., and R. B. Pringle. 1963. Toxicity of victoxinine. Phytopathology 53: 558-561.
19. Wheeler, H., and H. H. Luke. 1963. Microbial toxins in plant disease. Ann. Rev. Microbiol. 17: 223-242.
20. Woolley, D. W. 1946. Strepogenin activity of serylglycylglutamic acid. J. Biol. Chem. 166: 783-784.
21. Woolley, D. W., R. B. Pringle, and A. C. Braum. 1952. Isolation of the phytopathogenic toxin of Pseudomonas tabaci, an antagonist of methionine. J. Biol. Chem. 197: 409-417.

BIOGRAPHICAL SKETCH

John Wesley Miller was born February 23, 1937, at Dilley, Texas. He graduated from Dilley High School in May, 1955. He attended Texas University, Austin, Texas, from September, 1955, until May, 1958, majoring in Botany. He entered the Agricultural and Mechanical College of Texas, College Station, Texas, in September, 1958 and received the Bachelor of Science degree with a major in Plant and Soil Science in May, 1960. He began graduate work immediately at the same institution and received the Master of Science degree with a major in Plant Pathology. He entered the University of Florida in September, 1962, and was on a research assistantship until July, 1963, when he was awarded a research fellowship provided by Firestone Plantation Co. He pursued his work in Plant Pathology toward the degree of Doctor of Philosophy to be granted in April, 1965. He is a member of the American Phytopathological Society and Alpha Zeta Honorary Fraternity.

This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

April, 1965

Dean, College of Agriculture

Dean, Graduate School

Supervisory Committee:

Chairman