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SEMI-ANNUAL PROGRESS REPORT

JANUARY 1-JULY 31, 1989

BIOCONTROL OF BEAN ASHY STEM BLIGHT BY IMPROVED  
RHIZOBIUM BIOTECHNOLOGY

SECRETARIA DE ESTADO DE AGRICULTURA (DOMINICAN REPUBLIC)  
UNIVERSITY OF PUERTO RICO  
AID-PSTC PROJECT

SUBMITTED TO

AID-OFFICE OF AGRICULTURE BUREAU FOR  
SCIENCE AND TECHNOLOGY

BY

DEPARTMENTS OF AGRONOMY AND SOILS AND CROP PROTECTION  
COLLEGE OF AGRICULTURAL SCIENCES  
UNIVERSITY OF PUERTO RICO  
MAYAGUEZ CAMPUS

Secretaría de Estado de Agricultura (Dominican Republic)  
University of Puerto Rico-RUM/AID-PSTC Project

Biocontrol of Bean Ashy Stem Blight by Improved Rhizobium  
Biotechnology

#### SUMMARY

Twenty three indigenous Rhizobium strains collected and characterized in the Dominican Republic, were tested in vitro to measure their antagonistic reaction toward *Macrophomina phaseolina* virulent isolate PRMp2. KIM 5 strain was included as inhibitory control check. Rhizobium RD-019 strain was identified as a potential biocontrol agent of PRMp2 by Streak Plate, Double-Layer, and Modified Spent Methods. The performance of KIM 5 was similar to that obtained with UPR Collection Screening Tests. Surveys of *Macrophomina* incidence at the Dominican Republic (San Juan de la Maguana region) indicate that the incidence ranged from 2% to 82%. Pompadour B bean variety was resistant to *M. phaseolina* (RDMp2) isolated from the Arroyo Loro Agricultural Experiment Station fields at San Juan de la Maguana.

Secretaria de Estado de Agricultura (Dominican Republic)/  
Univ. of Puerto Rico/AID-PSTC Project

Biocontrol of Bean Ashy Stem Blight by Improved  
Rhizobium Biotechnology

Period Covered: January 1 July 31, 1989

Scientific Personnel Univ. of P.R. :            -Rodrigo Echávez-Badel  
  -Myrna Alameda  
  -Eduardo C. Schröder  
  (on sabbatical)

Scientific Personnel SEA Dominican Rep.:    -Yovanny Velázquez  
  -Alfonsina Sánchez

OBJECTIVES:

The principal objectives of this project are: a) to identify and select Rhizobium strains that inhibit root rot producing fungi in vitro and in vivo; b) to evaluate biological control effect of Rhizobium inoculation on bean genotypes susceptible to Ashy Stem Blight (ASB).

RESEARCH IN PROGRESS:

I. Puerto Rico Institution:

Rhizobium-Macrophomina phaseolina Laboratory Tests:

Twenty three indigenous Rhizobium (RD) collected and characterized in the Dominican Republic, were tested in vitro to measure their antagonistic reaction toward M.phaseolina virulent isolate (PR Mp2). The methods used in these tests were similar than those reported in the August-December 1988 Semi-Annual Report. Best antagonistic strain KIM 5 was included as inhibitory control check. It is very important to note that a small group of R.phaseoli from the UPR Collection had a stimulatory effect on the fungus growth.

Results from the Streak Plate Test indicate that the strain RD-019 is a potential as biological agent of PR Mp2. However, it was not significantly different ( $P=0.01$ ) for radial growth rates compared with the control check (fungus alone). The performance of KIM 5 was similar to that obtained with UPR Collection Screening Tests (Table 1). Highly significant differences ( $P=0.01$ ) in mycelial weights between control (fungus alone) and twelve RD strains, including RD-019, were obtained by the Modified Spent Culture Method (Table 2).

Six RD strains were found to inhibit the fungus growth at the 96 hr period incubation by the Double-Layer Method (Table 1).

TABLE 1. IN VITRO EVALUATION FOR THE BIOCCNTROL OF  
MACROPHOMINA PHASEOLINA WITH RHIZOBIUM SP. BY STREAK  
PLATE AND DOUBLE LAYER METHODS

TREATMENTS	STREAK PLATES		DOUBLE LAYER	
	RADIAL GROWTH (MM) <sup>1</sup>		RADIAL GROWTH (MM) <sup>1</sup>	
	48HRS <sup>2</sup>	120HRS <sup>2</sup>	48HRS <sup>2</sup>	96HRS <sup>2</sup>
RD-003	22.50NS	44.25NS	10.00**	50.00**
RD-005	23.00NS	44.50NS	9.75**	66.00*
RD-006	22.25NS	44.75NS	29.00**	64.25**
RD-007	22.50NS	44.50NS	9.00**	63.75**
RD-008	22.75NS	44.75NS	8.75**	52.25**
RD-009	21.50NS	44.25NS	9.25**	13.00**
RD-011	22.50NS	44.50NS	9.00**	61.00**
RD-012	21.75NS	45.00NS	26.00**	62.75**
RD-013	22.50NS	44.25NS	24.25**	67.75*
RD-014	21.25NS	46.00NS	13.50**	54.50**
RD-015	22.00NS	44.65NS	17.25**	56.50**
RD-016	22.25NS	45.00NS	20.75**	63.25**
RD-017	20.50NS	44.25NS	20.75**	67.25*
RD-018	22.25NS	44.25NS	25.00**	34.50**
RD-019	20.75NS	43.25NS	17.50**	67.00**
RD-020	22.00NS	44.75NS	17.00**	48.00**
RD-021	22.25NS	44.75NS	9.75**	52.50**
RD-022	22.50NS	44.00NS	9.25**	58.50**
RD-023	22.50NS	44.25NS	8.67**	55.00**
RD-024	22.00NS	44.75NS	17.60**	64.40**
RD-025	21.00NS	45.00NS	24.00**	55.00**
RD-026	22.75NS	44.00NS	16.50**	63.00**
RD-027	21.75NS	44.00NS	20.00**	61.75**
CONTROL <sup>3</sup>	22.50	44.00	40.50	76.75
KIM 5	6.75**	9.75	10.00	10.25
DUNNET'S (0.05)	1.80	3.30	6.69	8.94
DUNNET'S (0.01)	2.62	4.12	8.36	11.17

<sup>1</sup> MEAN 4 REPETITIONS

<sup>2</sup> INCUBATION PERIOD

<sup>3</sup> FUNGUS ALONE

\*, \*\* SIGNIFICANCE DIFFERENCE ( $\alpha = 0.05$  AND  $\alpha = 0.01$ )  
BETWEEN CONTROL AND TREATMENTS

TABLE 2. IN VITRO EVALUATION FOR THE BIOCONTROL OF MACROPHOMINA PHASEOLINA WITH RHIZOBIUM SP. BY THE MODIFIED SPENT CULTURE

TREATMENTS	MICELIAL DRY WEIGHT (MG)
KIM-5	2.3**
USDA 76	2.1**
RD-003	7.8NS
RD-005	10.9NS
RD-006	8.4NS
RD-007	8.2NS
RD-008	8.8NS
RD-009	9.0NS
RD-011	4.6**
RD-012	8.8NS
RD-013	5.6**
RD-014	3.7**
RD-015	5.9**
RD-016	6.0**
RD-017	5.2**
RD-018	4.0**
RD-019	3.8**
RD-020	4.0**
RD-021	4.3**
RD-022	11.0NS
RD-023	9.0NS
RD-024	4.0**
RD-025	2.0**
RD-026	10.8NS
RD-027	16.3NS
CONTROL <sup>1</sup>	11.9
DUNNET'S (P<0.05)	4.19
DUNNET'S (P<0.01)	5.27

<sup>1</sup> MEAN 4 REPETITIONS

<sup>2</sup> FUNGUS ALONE

\*\* SIGNIFICANT DIFFERENCE  $\alpha = 0.01$

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### Alfalfa-Rhizobium-Macrophomina Bioassay:

Selected Rhizobium strains from in vitro tests were used in a preliminary evaluation of Rhizobium antifungal activity in vivo using alfalfa (Medicago sativa) plants grown in Jensen medium. Florida 77 alfalfa variety (susceptible to PR Mp2) was evaluated in the following treatments:

- Control (Alfalfa alone)
- Alfalfa + Rhizobium
- Alfalfa + Macrophomina
- Alfalfa + Macrophomina + Rhizobium

A Random Complete Design (RCD) with 5 replications was used in this test. Data are being analyzed for a complete report of this test.

### II. Secretaria de Estado de Agricultura (SEA) of the Dominican Republic:

A group of indigenous Rhizobium strains were isolated from San Juan de la Maguana bean producing region. These strains were characterized at the Soil Microbiology Laboratory of CESDA, San Cristobal (Appendix 1). Twenty three strains from DR Collection have been screened in vitro as mentioned in item I.

Surveys of Macrophomina incidence were conducted at San Juan de la Maguana during winter (September-December) and autumn (January-March) seasons. The incidence of M.phaseolina ranged from 2%, at the Herradura area, to 82%, at the Arroyo Loro Agricultural Experiment Station. Three bean farms were selected as potential sites to conduct future biocontrol experiments.

The Arroyo Loro isolate of M.phaseolina (RDMp2) was used in a pathogenicity test in order to evaluate a group of 17 red mottled bean varieties (Pompadour types). Results of this test indicate that Pompadour B was resistant to RDMp2 (Appendix 2).

The disease incidence of RDMp2 is highest in wet fields of the Arroyo Loro Station, which might indicate that plants were probably being infected with another race of M.phaseolina. Ashy Stem Blight severe symptoms are observed in Puerto Rico when Pompadour adult plants (stages R5-R8) are under stress from moisture deficit. Further studies are planned to confirm these observations.

In August Ing. Alfonsina Sánchez (our Plant Pathologist counterpart in DR) will take a leave of absence from the Secretaria de Estado de Agricultura to begin studies at the University of Puerto Rico, Mayaguez Campus, towards a M.S.degree in Crop Protection. Ing. Patricio de la Cruz from CESDA, has been appointed in the project in substitution of Ms.Sánchez. Ing.

Floilán Perdomo expects to complete a M.S. degree during the upcoming year. His thesis research deals with the biocontrol of *M. phaseolina* by *Rhizobium* strains under laboratory conditions. At the UPR the project collaborates with the Bean Breeding Project (Bean/Cowpea CRSP), and with Dr. Linda Blum project of biocontrol from the University of Virginia.

During the XXIX Annual Meeting of the American Phytopathological (A.P.S.)-Caribbean Division at CIAT, Colombia, a paper was contributed by the project staff: "Biological Control of *Macrophomina phaseolina* By *Rhizobium* Strains in Laboratory Tests".

Publications:

Perdomo, F., R. Echávez-Badel, M. Alameda, and E.C. Schröder. 1989. In vitro *Rhizobium* Strains Evaluation for Biocontrol of *Macrophomina phaseolina*. Ann. Rep. B.I.C. 32:103-104.

Perdomo, F., R. Echávez Badel, M. Alameda, and E.C. Schröder. 1989. Biological Control of *Macrophomina phaseolina* by *Rhizobium* Strains in Laboratory Tests. Proceedings of the First International Congress of APS-CD/ALF/ASCOLFI. p 33. CIAT, Colombia. Abstract submitted to Phytopathology.

Appendix I

Abbreviations (Symbols)

Growth

A - ++++

M - +++

MA- ++

P - +

Texture

E - Elastic

C - Creamy

G - Gummy

Consistency

G - Gelatinous

S - Dry

A - Watery

pH

A - Acid produced

N - Non-acid produced

NA- Late acid production

C - Alkaline

Color and Shape

R - Round

P - Flat

C - Conical

STRAIN	SITE	DATE	GROWTH(Hours)					pH				TEXTURE	COLONY SHAPE	CONSISTENCY
			24	48	72	96	120	24	48	72	96			
RD-003	Lucero (Jinova)	2/24/89	MA	MA	MA	MA	MA	A	A	A	A	C	R	S
RD-004	Lucero (Jinova)	2/24/89	M	M	M	P	A	A	A	A	A	C	R	S
RD-005	Lucero (Jinova)	"	M	M	M	MA	MA	A	A	A	A	E	R	S
RD-006	Sucesión Ramírez	"	M	M	M	MA	MA	A	A	A	A	E	R	G
RD-007	Lucero (Jinova)	"	P	M	M	A	MA	A	A	A	A	E	R	G
RD-008	Suc. Ramírez	"	-	A	A	A	A	NA	A	A	A	E	R	G
RD-009	Lucero (Jinova)	"	M	A	A	A	A	A	A	A	A	E	R	G
RD-0010	"	"	A	MA	MA	MA	MA	NA	NA	NA	A	E	R	G
RD-0011	"	"	M	MA	MA	MA	MA	A	A	A	A	E	R	G
RD-0012	Juan Herrera	2/28/89	P	A	A	A	A	NA	A	A	A	E	R	G
RD-0013	Juan Herrera	2/28/89	M	MA	MA	MA	MA	A	A	A	A	E	R	G
RD-0014	Lucero (Jinova)	"	A	A	A	A	A	A	A	A	A	E	R	G
RD-0015	Lucero (Jinova)	"	A	A	A	A	MA	A	A	A	A	E	R	G
RD-0016	Lucero (Jinova)	3/3/89	M	A	A	A	MA	NA	A	A	A	E	R	G
RD-0017	La Barranca	3/3/89	A	MA	MA	MA	MA	A	A	A	A	E	R	G
RD-0018	Lucero (Jinova)	3/3/89	A	A	A	A	A	A	A	A	A	E	R	G
RD-0019	"	3/6/89	P	P	MA	MA	MA	A	A	A	A	E	R	G
RD-0020	"	3/6/89	M	P	A	A	A	A	A	A	A	C	R	S
RD-0021	La Barranca	"	A	P	P	MA	MA	A	A	A	A	E	R	G
RD-0022	La Barranca	"	A	P	P	A	A	NA	NA	A	A	C	R	S
RD-0023	Lucero (Jinova)	"	M	M	P	A	A	A	A	A	A	E	R	G
RD-0024	Lucero (Jinova)	"	P	P	M	A	A	NA	NA	NA	A	E	R	G
RD-0025	"	"	P	P	M	M	M	A	A	A	A	E	R	G
RD-0026	"	3/10/89	P	P	M	M	M	NA	A	A	A	E	R	G
RD-0027	"	3/10/89	P	P	M	A	A	NA	A	A	A	E	R	G

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APPENDIX 2

TABLE: Disease reaction of 17 red mottled bean varieties to Ashy Stem Blight, *Macrophomina phaseolina* (RDMP2), at the Arroyo Loro Agricultural Experiment Station.

Entry No.	Identity	Disease severity <sup>1</sup>
1	Pompadour AE	2 <sup>2</sup>
2	Pompadour Q	4
3	Pompadour H	4
4	Pompadour XB	4
5	Pompadour B	1
6	Pomp. Rocío	4
7	Pomp. Jorgillo	4
8	Pompadour T	4
9	Pompadour S	3
10	Pompadour K	4
11	Pompadour V	4
12	Pompadour M	4
13	Pompadour AC	3
14	Pompadour E	2
15	Pompadour P	2
16	Pompadour U	2
17	Pomp. PC-50	3

<sup>1</sup> Based on a scale from 0 to 4, with 0 showing no symptoms and 4= severe symptoms.

<sup>2</sup> Mean of 2 replications.

APPENDIX 2

TABLE: Incidence of *M.phaseolina* in different areas of San Juan de la Maguana region (Dominican Republic).

Area	Farmer	Incidence (%)
Juan Herrera		14.1
Jínova	Paulino García	57.0
Jínova	Augusto Cepeda	68.3*
Punta Caña		10.5
Cuenda		7.0
La Culata		3.5
Chalona		8.0
Vallejuelo	Rafael Montero	51.0
Arroyo Loro	Agr.Exp.Station	82.0*
Buenvista		4.0
La Herradura		2.0
Barranca	Tulio Lapaix	64.0*

\* % of incidence during the autumn season. Bean fields selected by SEA technicians to conduct future biocontrol experiments.

ANNUAL REPORT OF THE  
BEAN IMPROVEMENT  
COOPERATIVE



A VOLUNTARY AND INFORMAL ORGANIZATION  
TO EFFECT THE EXCHANGE OF INFORMATION AND MATERIALS

**VOLUME 32**  
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In vitro Rhizobium STRAINS EVALUATION FOR BIOCONTROL  
OF Macrohomina phaseolina<sup>1</sup>

Floilán Perdomo, Rodrigo Echávez-Badel, Myrna Alameda, and Eduardo C. Schroder. Depts. of Agronomy & Soils and Crop Protection, Univ. of Puerto Rico, Mayaguez Campus, Mayaguez, P.R. 00709.

In recent years, ashy stem blight (Macrohomina phaseolina) has caused losses in experimental plantings in Puerto Rico and in the Dominican Republic, particularly when plants are under stress from moisture deficit (1,3). Severe symptoms are observed in the field when bean plants are in the seedling stage, and frequently in adult plants. Biological control of pathogens can be accomplished through host plant resistance, cultural practices antagonistic microorganisms. Rhizobium is abundant in the rhizosphere, provides N to legumes, produces plant growth factors and has been shown to reduce root rot diseases in some cases (2,4). The objective of this study was to identify and select Rhizobium strains that inhibit M. phaseolina growth in bioassay tests.

Three in vitro methods were used to measure the antibiosis of Rhizobium strains toward M. phaseolina virulent isolate PR Mp2. The inoculum was grown on potato dextrose agar (PDA) and incubated at 28°C for five days. Treatments were replicated 4 times and results statistically analyzed by analysis of variance. Differences between each treatment and control (fungus alone) were obtained by the Dunnet's test.

In the streak plate method Rhizobium strains were streaked on one side of Yeast Extract Mannitol Agar (YMA) and incubated for 48 hrs at 28°C. Disks of active growing PR Mp2 were cut and placed 5 cm from bacterial streak on each plate. After incubation for 48 and 120 hrs, radial growth was measured. Significant differences in radial growth rates between control and KIM-5 strain were obtained (table 1).

In the double-layer plate method, Rhizobium strains were grown on Yeast Extract Mannitol broth (YEM) for 3-4 days. Bacterial culture was centrifuged. Cells obtained were suspended in 1 ml of YEM broth. Tempered YMA was added to 1 ml of cell suspension on a Petri dish and distributed throughout the agar by swirling gently the plate. A second layer of YEM was poured over the first layer previously solidified. Plates were incubated at 28°C for 24 hrs. Disks of PR Mp2 were plated and incubated at 28°C for 48 and 96 hrs. Radial growth rates were considered in this test. Nine strains, including KIM-5, were found to inhibit the fungus growth (table 1).

The spent culture, slightly modified for us, was the third method used in this study. Disks of M. phaseolina mycelia were transferred to YEM cultures of Rhizobium strains previously grown on Erlenmeyer flasks at 28°C during 4 to 7 days. Cultures were incubated in a shaker for 5 days at room temperature. Mycelia were collected, dried at 60°C for 4 days, and weighted. Results indicated significant differences in fungal biomass between control and fifteen strains, including KIM-5. The most effective and more consistent antagonist was KIM-5. However, its mechanism of biocontrol has not been identified conclusively. It appears that antibiosis and nutrient competition may play important roles. Further studies on mechanism of action are contemplated.

<sup>1</sup> This research is supported by AID-PSTC Grant No. DPE-1159-G-SS-8012-00.

## LITERATURE CITED

1. Anonimous. 1986. Improvement of bean production in the Dominican Republic through breeding for multiple disease resistancé. Progress Report UN/UPR/Dominican Republic Bean/Cowpea CRSP Project. 4pp.
2. Buonassisi, A.J., R.J. Copeman., H.S. Pepin., and G.W. Eaton.1986. Effect of Rhizobium spp. on Fusarium solani f.sp.phaseoli. Can. J. Plant Pathology 8:140-146.
3. Echfraz-Badal. R. and J.S. Beaver.1987. Dry bean genotypes and Macrophomina phaseolina (Tassi) Goid in inoculated and non-inoculated field plots.J. Agr. Univ. P.R. 71(4):385-390.
4. Zaki, M.J ,and A. Ghaffar.1986. Effect of Rhizobium spp. on Macrophomina phaseolina. Phytopathology 76:1134 (Abstr.622).

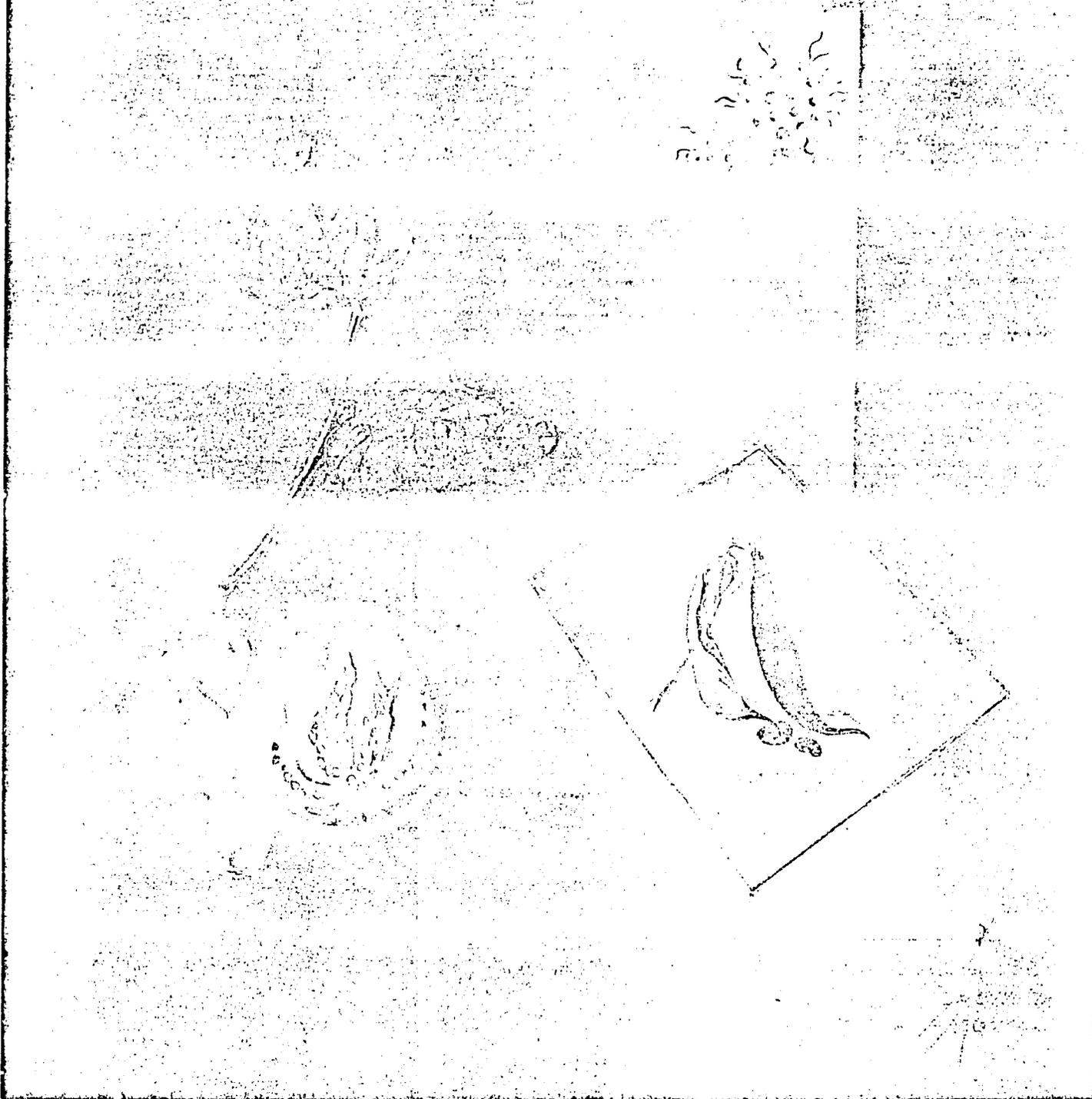
Table 1. In vitro Rhizobium strains KIM-5, USDA 76; USDA 110 and UPRM 6000 among 32 strains evaluated as biological control of M. phaseolina.

Treatments	Streak plate Radial growth <sup>1</sup>		Doble-layer Radial growth <sup>1</sup>		Fungal biomass <sup>1</sup> (mg)
	48 hr <sup>2</sup>	120 <sup>2</sup>	48 hr <sup>2</sup>	96 hr <sup>2</sup>	
KIM-5	2.4*	9.8*	10.3*	10.0*	5.7*
USDA 76	22.8	43.3	23.5*	46.5*	2.1*
USDA 110	22.8	36.0*	10.0*	21.0*	7.5*
UPRM 6000	23.0	44.0	25.2*	63.0*	14.3
CONTROL (Fungus alone)	22.5	44.0	40.5	76.8	15.5
Dunnet's test	2.4	4.0	6.5	8.9	5.3

<sup>1</sup> Means of 4 replications

<sup>2</sup> Incubation periods

\* Significant difference ( $\alpha=0.05$ ) between control and treatment.



**RESUMENES / ABSTRACTS**

**X CONGRESO ASCOLFI**

**V REUNION ALF**

**XXIX REUNION APS-CD**

**CALI, CIAT, 10-14 JULIO 1989**

BIOLOGICAL CONTROL OF Macrophomina phaseolina BY Rhizobium STRAINS IN LABORATORY TESTS. F. Pardo, R.E. Chávez-Badal, K. Alameda, and E.C. Schroder. Departments of Agronomy and Soils and Crop Protection. University of Puerto Rico, Mayaguez Campus, Mayaguez, Puerto Rico 00709.

Thirty two Rhizobium strains were evaluated in vitro for their potential as biological control agents of Macrophomina phaseolina (PR Mp2 isolate). Evaluations identified several Rhizobium strains with the ability to inhibit the radial growth of PR Mp2 virulent isolate. The most effective and more consistent antagonist was KIM 5, which inhibited the fungus growth using the streak plate, the double layer, and the modified spent culture methods. This level of inhibition was significantly different ( $P=0.05$ ) compared with the control. The mechanism of KIM 5 as biocontrol agent has not been identified conclusively. However, it appears that antibiosis and nutrient competition may play important roles. Further studies on mechanisms of action are contemplated. This research was supported in part by the Program in Science and Technology Cooperation (PSTC) of the US Agency for International Development (AID).

✓ ESTUDIO MACRO Y MICROSCOPICO DEL EFECTO DE Verticillium lecanii (Zimm.) Viegas SOBRE Hemileia vastatrix Berk. y Br., AGENTE CAUSAL DE LA ROYA DEL CAFEIO. P.E. Vélez. Centro Nacional de Investigaciones de Café "CENICAFE", Chinchiná, Caldas, Colombia.

Crecimiento micelial y extracto metabólico de V. lecanii cultivado en caldo papa dextrosa (CPD) afectan la evolución de la lesión y la germinación de uredosporas de H. vastatrix. Al asperjar cultivo licuado (micelio y conidias) de V. lecanii sobre lesiones establecidas de H. vastatrix, se observa cubrimiento e invasión de la pústula por el micelio blanco del hongo con pérdida completa de ésta. El extracto metabólico de V. lecanii asperjado a lesiones establecidas de H. vastatrix, origina una depresión central en la pústula, la cual se acentúa a través del tiempo. Microscópicamente el efecto se manifiesta con inhibición total de la germinación y algunos cambios aparentes en las uredosporas. Histológicamente las hojas asperjadas con el cultivo de V. lecanii muestran al micelio del hongo formando redes alrededor de las uredosporas de roya, en el sitio de penetración al estoma. Cultivo y extracto metabólico de V. lecanii asperjado en hojas sanas, no provocan ningún cambio visible en la hoja. El seguimiento efectuado, evidencia el efecto curativo de V. lecanii y su inocuidad en plantas sanas; factores fundamentales en un microorganismo usado en Control Biológico.

# FINANCIAL STATUS REPORT

(Follow instructions on the back)

1. FEDERAL AGENCY AND ORGANIZATIONAL ELEMENT TO WHICH REPORT IS SUBMITTED AGENCY FOR INTERNATIONAL DEVELOPMENT		2. FEDERAL GRANT OR OTHER IDENTIFYING NUMBER DPE-1159-G-55-8012-00		OMB Approved No. 80-RO180	PAGE 1	OF 1
3. RECIPIENT ORGANIZATION (Name and complete address, including ZIP code) UNIVERSITY OF PUERTO RICO MAYAGUEZ CAMPUS FINANCE DEPARTMENT MAYAGUEZ, PUERTO RICO 00708		4. EMPLOYER IDENTIFICATION NUMBER 66-0433761	5. RECIPIENT ACCOUNT NUMBER OR IDENTIFYING NUMBER 00-206-36-35-FRLC-72-001429	6. FINAL REPORT <input type="checkbox"/> YES <input checked="" type="checkbox"/> NO		7. BASIS <input type="checkbox"/> CASH <input type="checkbox"/> ACCRUAL
8. PROJECT/GRANT PERIOD (See instructions)		9. PERIOD COVERED BY THIS REPORT				
FROM (Month, day, year) 08-01-88		TO (Month, day, year) 07-31-91		FROM (Month, day, year) 01-01-89		TO (Month, day, year) 06-30-89

10. PROGRAMS/FUNCTIONS/ACTIVITIES ▶	STATUS OF FUNDS							TOTAL (g)
	(a) SALARIES	(b) FRINGE BENEFITS	(c) TRAVELS	(d) EQUIPMENTS	(e) MAT. & SUPPLIES	(f) OTHERS		
a. Net outlays previously reported	\$ 1,811.00	\$ 54.78	\$ 506.54	\$ -0-	\$ 178.77	\$ 3,300.00	\$ 5,851.09	
b. Total outlays this report period	9,066.18	747.40	656.64	3,432.00	2,124.99	800.00	16,827.21	
c. Less: Program income credits	-0-	-0-	-0-	-0-	-0-	-0-	-0-	
d. Net outlays this report period (Line b minus line c)	9,066.18	747.40	656.64	3,432.00	2,124.99	800.00	16,827.21	
e. Net outlays to date (Line a plus line d)	10,877.18	802.18	1,163.18	3,432.00	2,303.76	4,100.00	22,678.30	
f. Less: Non-Federal share of outlays	-0-	-0-	-0-	-0-	-0-	-0-	-0-	
g. Total Federal share of outlays (Line e minus line f)	10,877.18	802.18	1,163.18	3,432.00	2,303.76	4,100.00	22,678.30	
h. Total unliquidated obligations	243.00	90.44	-0-	-0-	43.79	1,200.00	1,577.23	
i. Less: Non-Federal share of unliquidated obligations shown on line h	-0-	-0-	-0-	-0-	-0-	-0-	-0-	
j. Federal share of unliquidated obligations	243.00	90.44	-0-	-0-	43.79	1,200.00	1,577.23	
k. Total Federal share of outlays and unliquidated obligations	11,120.18	892.62	1,163.18	3,432.00	2,347.55	5,300.00	24,255.53	
l. Total cumulative amount of Federal funds authorized	77,800.00	8,139.00	13,200.00	4,300.00	13,961.00	11,800.00	129,200.00	
m. Unobligated balance of Federal funds	66,679.82	7,246.38	12,036.82	868.00	11,613.45	6,500.00	104,944.47	

11. INDIRECT EXPENSE	a. TYPE OF RATE (Place "X" in appropriate box)		13. CERTIFICATION		SIGNATURE OF AUTHORIZED CERTIFYING OFFICIAL <i>Carlos Irizarry</i>	DATE REPORT SUBMITTED 7-28-89
	<input type="checkbox"/> PROVISIONAL	<input type="checkbox"/> PREDETERMINCO	<input type="checkbox"/> FINAL	<input type="checkbox"/> FIXED		
b. RATE	c. BASE	d. TOTAL AMOUNT	e. FEDERAL SHARE	TYPED OR PRINTED NAME AND TITLE CARLOS IRIZARRY - ACCOUNTANT		TELEPHONE (Area code, number and extension) 832-4968 EXT. 3095, 3150

12. REMARKS: Attach any explanations deemed necessary or information required by Federal sponsoring agency in compliance with governing legislation.