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BIOCONTROL OF CASSAVA PATHOGENS: A USEFUL APPROACH

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The biocontrol of diseases of different crops has been reported as a feasible measure (Baker and Cook, 1974). Investigations on the subject are increasing and are being carefully taken into consideration in many plant pathology programs around the world.

Research on the biocontrol of cassava pathogens was initiated at CIAT in 1975 (CIAT, 1976). Preliminary results are very encouraging, suggesting a useful, practical tool for controlling several pathological problems of cassava. This paper summarizes the research results obtained during the last twelve years on cassava pathogens, with emphasis on the use of fluorescent pseudomonads as biocontrol agents in different cassava production systems.

A. Biocontrol of cassava rusts

There are six species of *Uromyces* infecting cassava and other Euphorbiaceae species (Laberry *et al*., 1984). Their damage is generally considered moderate and of low economic importance (Laberry, 1976), with the exception of a few outbreaks reported in new cassava-growing areas of Brasil (Muniz, 1981). A survey on endemic areas in 1975 indicated

that around 95% of the rust pustules examined were infected by the mycoparasite *Darluca filum*. Surveys during rust outbreaks in nonendemic areas indicated that the percentage of parasitized pustules ranged from 0 to 21% of total examined pustules/plant. *D. filum* was probably a factor in maintaining *Uromyces* spp. at low inoculum potential levels, reducing disease severity and economic losses.

The mycoparasite is an imperfect fungus that belongs to the order Sphaeropsidales. Pycnidia are black, spherical, ostiolate, superficial and located in rust sori. Conidia are hyaline, two-celled, ellipsoid and tipped with mucous or bristlelike appendages at the ends (Barnett and Hunter, 1972).

The mycoparasite infects rust uredospores, decreasing the inoculum potential of the parasite. It grows on common laboratory media and utilizes a variety of carbon and nitrogen sources. Isolates are highly variable, but there seems to be no nutritional specificity that would account for the exclusive association of this parasite with rust fungi (Barnett and Binder, 1973).

In addition to parasitism by *D. filum*, cassava rust uredospores are eaten by several species (larvae stage) of *Cecidomyiidae* and *Coccinellidae*.

These insect species are commonly found on sori of cassava rust in affected areas. Unfortunately, research on relationships between rust pathogens and these insect species is unknown, but further investigation is merited.

B. Biocontrol of other cassava pathogens with beneficial fluorescent pseudomonads

1. The bacteria

Among beneficial bacteria, fluorescent pseudomonads (*Pseudomonas putida* and *P. fluorescens*) are the most promising for use in biocontrol programs due to their nutritional diversity, ability to grow under a wide range of environmental conditions, and ability to colonize the rhizosphere of many plant species (Kloepper *et al*., 1980).

They can be easily isolated from soil or the plant rhizosphere of many crop species by using King's B (KB) medium incubated at $27^{o}C$ for 24-36h. Bacterial isolates showing fluorescence on KB under ultraviolet light can be purified from single colonies after serial dilutions seeded on KB medium (Hernandez *et al*, 1986).

2. Survival

High bacterial populations were found in the rhizosphere of cassava plants or rooted shoot tips two months after inoculation. There were variations in bacterial populations among clones and between strains; but a clone X strain interaction was not found (Rosas, 1986), which indicates lack of host specificity with the strains tested. It has been reported that survival of fluorescent bacteria is probably dependent upon nutrient availability, space and cellular migration to new sites, or a combination of these factors (Brown, 1974; Burr *et al*, 1978). Similarly, quality of exudates (e.g. production and quantity of specific amino acids or toxic compounds) may influence the survival of these bacterial species in the rhizosphere.

3. The effect on cassava plantlets under glasshouse conditions

Isolates of beneficial fluorescent pseudomonads have been characterized according to their: (I) inhibitory effect, *in vitro* on both bacterial and/or fungal pathogens; and (II) according to their ability to promote root system growth of plantlets as related to pathogen inhibition *in vitro*. With method I four groups were identified: (a) isolates with no or very mild inhibition of pathogenic bacteria and fungi; (b)

isolates that strongly inhibit pathogenic bacteria, but no or very mild inhibition of fungi; (c) isolates causing very strong inhibition of fungi, but no or very mild inhibition of bacteria; and (d) isolates inducing strong inhibition of both fungi and bacteria (CIAT, 1986). Generally, there were more isolates able to inhibit pathogenic bacteria than fungi. Among all isolates collected, those of _P. fluorescens_ showed broader _in vitro_ inhibition of the bacterial and fungal species tested than _P. putida_ (CIAT, 1985).

Isolates characterized by method II were also classified into four groups: (a) those that did not inhibit cassava pathogens _in vitro_, or increase root growth of cassava plantlets; (b) strains showing a high inhibitory effect _in vitro_ of cassava pathogens (both fungi and bacteria; some strains of group d in method I), but did not promote an increase in foliage or root system of inoculated clones. As reported for other crop species (Leisinger and Margroff, 1979), secondary metabolities may have phytotoxic or antibiotic activity; (c) strains that did not inhibit cassava pathogens, but increased both the foliage and root system of inoculated clones. It is possible that antagonistic interactions with pathogens (nutrient competition or antibiotic effects) resulted in the

exclusion of pathogens from the rhizosphere. Healthy roots promote better total plant growth; (d) strains of this group induced a strong inhibitory effect on cassava pathogens and a significant increase in the aerial parts and roots of inoculated plantlets (Fig. 1) (CIAT, 1976). It has been reported for other crop species that some strains of fluorescent pseudomonads produce growth regulators (Eklund, 1970).

4. Practical applications

a. Biocontrol of foliar pathogens

Strains of method I group b, which induced the highest *in vitro* inhibition of *Xanthomonas campestris* pv. *manihotis* (causal agent of cassava bacterial blight, CBB), were used to spray plots of susceptible, intermediate resistant and resistant clones planted in an area where CBB is epidemic. Results of these treatments are shown in Tables 1 and 2. Both the number of angular leaf spots/leaf and the number of blighted leaves/plant were significantly reduced by foliar applications of a strain of *P. putida* (Table 1). Yield of the susceptible clone (M Col 22) also increased 2.7 times (Table 2) but did not increase in either the intermediate resistant or resistant clones (CIAT, 1985; Lozano, 1987).

The control of other foliar pathogens of cassava by spray applications of specific strains of beneficial fluorescent pseudomonads has not been reported, but is possible. Practical and economic problems exist, such as base inoculum production, inoculum storage and preparation under aseptic conditions and costs for spray applications. Further research is required to test the practical feasibility of controlling foliar pathogens of cassava.

b. Biocontrol of preharvest root rots

When soils infested with *Pythium* spp. or *Diplodia manihotis* were drenched with a bacterial suspension of *P. putida* (method I, group d) before planting cassava plantlets, satisfactory control of root rot was obtained (Hernandez *et al*, 1986). Strains of *P. fluorescens* (belonging to method I, group c) were also able to protect cuttings against *D. manihotis* (Table 3 and 4)(CIAT, 1985; Lozano, 1987). The protective effect was evident when cuttings were treated with the bacterial suspension before or after fungal inoculations. Protection was related to sprouting of buds and fungal establishment, as well as invasion through the tissues of the cuttings (Table 3). This type of protection was also evident for three clones

taken either from farmers' fields or from meristem-derived plants. The bacterial protection was nearly as efficient as that obtained with the best fungicidal treatment (Table 4) (CIAT, 1985).

Yield of fresh roots increased when plants were watered with a 10ml bacterial suspension of a beneficial strain of _P. fluorescens_ (method II, group d). Yields tended to increase with an increased number of waterings (Table 5). However, level of increase also varied according to clone (CIAT, 1985), suggesting differential clonal responses to bacterial strains used or differences in susceptibility of the pathogens to the beneficial bacteria.

These results open the possibility of controlling root rots of cassava with beneficial bacteria, replacing fungicidal treatments of cuttings. However, more extensive research on this subject is needed to define practical systems for biocontrol of root rots.

c. Biocontrol of postharvest root rots

In an experiment to test the potential of biocontrol of postharvest root rots, recently harvested roots were dip-treated in a bacterial suspension of _P. fluorescens_ and _P. putida_ (strains belonging to method II, group d)

and stored for three weeks in sealed plastic bags under ambient conditions (Table 6). Strains of _P. putida_ gave variable results, however, a strain of _P. fluorescens_ gave consistently good root rot control during the first two weeks of storage and moderate control after three weeks (Table 6) (CIAT, 1985; Lozano, 1987).

The eventual aim of using a biological rather than a chemical means for controlling postharvest microbial deterioration in cassava is to avoid toxic risks arising from some chemicals. However, these still require considerable adaptive and applied research in order to develop a practical method for on-farm use.

d. _Plant growth effects_

Dip-treated cuttings (with strains from method II, groups c and d) planted in pots with sterile soils under glasshouse conditions showed a significant (at 5% level) increase in number and weight of roots in relation to distilled water-treated controls (CIAT, 1986; Rosas, 1986). Similarly, shoot tips rooted in a suspension of beneficial bacteria (strains belonging to method II, group c) showed the following significant effects (at 5% levels) in relation to controls: (i) faster root initation (8 days vs. 10 days); (ii) higher

number and longer roots; and (iii) increased root system weight (Fig. 2) (Hernández and Lozano, unpublished data). The above results,especially those obtained with strains belonging to method II, groups c and d, strongly suggest the production of growth regulator(s) by strains of these bacterial species. Inoculations of plantlets [(obtained by the shoot rooting system in water (Cock et al. 1976)], under sterile conditions in Leonard jars (Bradley et al, 1985) confirmed previous findings (Table 7). Further research on the purification of such growth regulator(s) is in progress (Rosas, 1986; CIAT, 1986). The above results suggest the possibility of using strains of both P. putida and P. fluorescens as root promotors, especially to accelerate and increase the root systems of cassava plantlets in rapid multiplication systems. Applications for accelerating the rooting and sprouting of cuttings in commercial plantations requires further research.

C. General conclusions

Even though research on biocontrol of cassava pathogens is recent and preliminary, results suggest it is feasible. Spray application of D. filum in highly endemic rust-affected areas is now possible. Effective strains of this mycoparasite are available.

The use of strains of fluorescent pseudomonads effective in control of CBB needs more investigation on practical storage of beneficial strains and the distribution and multiplication of inoculum. Effective strains of this beneficial bacteria are available, and the methodology for their identification is known. Bacterization of cuttings is feasible in special situations, such as in planting material production fields, to control pathogens infesting the cuttings, and to protect them against pathogens in infested soils. This technology may not be useful in traditional cassava production systems because the treatment requires technical work and aseptic handling during the production of the inoculum. The use of beneficial bacterial suspensions to treat cassava roots before their storage in order to control microbial deterioration needs further research on the identification of effective strains and the development of treatment systems, giving levels of control similar to those obtained with thiabendazole (CIAT, 1983; 1984; 1985). Similarly, the use of growth-stimulating strains of fluorescent pseudomonads to treat both cuttings or plantlets before planting, for the promotion of root system growth, appears to be feasible in the near future.

The importance of the native microflora in protecting cassava against pathogens has been demonstrated recently by the following findings: (a) cassava plants derived from

meristem cultures are more susceptible to pathogens than plants grown from cuttings from the field (CIAT, 1983; Lozano *et al.*, 1984); and (b) plants grown from sterilized cuttings (by dip treatments with Na hypochlorite) of cassava clones with apparent resistance resulting from the effect of beneficial microflora are, in fact, genetically susceptible (CIAT, 1984; 1985). Native beneficial microflora, especially bacteria belonging to species different from fluorescent pseudomonads, were responsible for such protection. The interaction of these microorganisms with pathogens and the consequent effects of such interactions are unknown, but merit intensive research.

It can be seen from the foregoing that investigation on this topic will possibly open new fields that can be incorporated successfully in many cassava production systems. The integration of this control measure with others already known, especially applied to resistant clones, can undoutbtedly lead to the obtainment of better stable yields.

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Table 1. Control of cassava bacterial blight by foliar applications* of <u>Pseudomonas putida</u> (strain F-44 and F-88) on two susceptible clones during 1984 and 1985.

Clone	Scoring System	Year of control/strain number					
		1984		1985			
		F-44		F-44		F-88	
		Sprayed	Controls	Sprayed	Controls	Sprayed	Controls
M Col 22	Angular leaf spots/leaf**	1.0a	7.0b	2.5a	5.7b	2.5a	5.7b
	Number of blighted leaves/plant***	1.0a	6.0b	1.5a	5.0b	1.0a	4.0b
M Col 72	Angular leaf spots/leaf	-		4.3a	9.5b	4.2a	8.7b
	Number of blighted leaves/plant	-		2.1a	6.06b	2.3a	7.0b

* Plants received six foliar applications of a 1×10^{9} cells/ml suspension of the respective strain of <u>P putida</u> at 15-day intervals. Control plants were sprayed with distilled water. Numbers followed by the same letter are not significantly different at a 0.05 level of the Duncan Multiple Range Test.

** Average number of leaf spots/leaf of 15 leaves/plot. Plots contained 36 plants with three replications.

*** Average number of blighted leaves/plant of 20 leaves/plot. Plots contained 36 plants with three replications.

Table 2. Yield (t/ha) of a CBB-susceptible (M Col 22), intermediate resistant (CM 523-7), and resistant (M Ven-77) clones after foliar applications of *Pseudomonas putida* (strain F-44).

Clones	Sprayed plots	Control plots
M Col 22	6.8 a*	2.5 b
CM 523-7	14.7 a	14.0 a
M Ven 77	9.6 a	9.1 a

*Yield was recorded from three replicated plots of 30 plants each (12 harvested plants). Border plants were eliminated. Yields followed by the same letter(s) are not significantly different at the 0.05 level of the Duncan Multiple Range Test.

Table 3. Effect of Pseudomonas fluorescens (strain Pf. c5a) on sprouting of cuttings (clone M Col 1684) after inoculation with Diplodia manihotis (Dm.).

Treatments*	Sprouting** (% ofcuttings)	Cutting infection*** (% of invaded tissues)
10 min in Pf. c5a 20 min in Dm.	100 a	16 b
10 min in D H_2O 20 min in Dm.	10 c	97 a
18 h in Dm.a 20 min in D H_2O	40 b	85 a
20 min in Dm.	0 c	100 a
Controls****	100 a	0 c

* Dip treatment in suspensions of 1.1×10^9 cells/ml of P. flourescens (strain Pf. c5a) in distilled water (D-H_2O), followed by a dip treatment in a suspension of 5.8×10^4 pycniospores/ml of D. manihotis (Dm.) or viceversa.

** Data taken a month after planting in pots with sterile soils maintained in a glasshouse at 25°C (± 8°C), 70%RH and 12h photoperiod. Differences in sprouting of cuttings were found by the logit (X^2) system.

*** Data followed by the same letter are not significantly different at the 0.05 level of the Duncan Multiple Range Test.

**** Control consisted of cuttings dipped for 20 min in D-H^2O or in suspensions of strain Pf. c5a.

Table 4. Effect of <u>Pseudomonas fluorescens</u> (strain PF-88) on bud germination of cuttings of three clones collected from farmers' fields (FF) and from meristem-derived plants (MP)* and inoculated with <u>Diplodia manihotis</u> (Dm).

		Germination percentage in relation to treatments			
Clone	cuttings	20 min Dm	10 min PF-88/ 20 min Dm**	10 min fungicidal mixture/ 20 min Dm***	Controls
M Col 113	FF	80 (48)****	100 (29)	100 (5)	100 (4)
	MP	0 (100)	100 (29)	100 (26)	100 (17)
M Col 72	FF	60 (47)	90 (22)	100 (0)	100 (8)
	MP	10 (93)	70 (37)	80 (15)	100 (2)
M Col 1468	FF	20 (87)	100 (0)	100 (0)	100 (0)
	MP	10 (98)	100 (2)	100 (1)	100 (9)

* Meristem-derived plants (MP) were obtained originally from meristem cultures and propagated one cycle in the field to obtain stem cuttings.

** Dip treatments in suspensions of 5.8×10^4 pycniospores/ml of <u>D. manihotis</u> or 1×10^9 cells/ml of <u>P. flourescens</u> (PF-88).

*** Water suspension of Captan/BCM (3,000 ppm each).

****Data taken from 40 cuttings/clone/source treated. The average percentage of tissue showing fungal invasion after treatments is given in parentheses. Readings were taken a month after growing the cuttings in pots with sterile soils maintained under glasshouse conditions.

Table 5. Average yield of fresh roots of several clones planted in Carimagua, Media Luna and CIAT, in relation to treatments with Pseudomonas fluorescens (PF-88).

		Yield (ton/ha) in relation to bacterial treatments*			
Location	Clone	0	1	2	3
Carimagua	M Col 1914	12.1b**	14.5b	16.5a	17.6a
	M Col 1916	11.2b	14.5b	15.7a	17.7a
	M Pan 19	10.7a	10.1a	12.0a	12.3a
	M Ven 77	14.5b	14.5b	18.1a	21.6a
Media Luna	CM 342-170	9.0b	11.1ab	11.3ab	12.5a
	M Col 72	12.5b	16.0a	16.3a	18.3a
	Venezolana	10.3b	11.3b	12.3ab	13.3a
CIAT-Palmira	M Col 1468	38.1b	40.2b	44.5ab	49.3a
	M Col 72	42.1b	46.6ab	46.8ab	47.0a

* Bacterial treatments: 10 ml of a bacterial suspension (1.1 x 10^9 cells/ml) were poured at the base of each plant at: 1 = one month; 2 = one and two months, 3 = one, two and three months after planting the cuttings.

** Data taken from three replicates of 30 plants each/location/clone and treatment. Border plants were eliminated. Yield data followed by the same letter(s),compared across bacteria treatments, are not significantly different at the 0.05 level of the Duncan Multiple Range Test.

Table 6. Effect of six strains of <u>Pseudomonas putida</u> (Pp.), two of <u>P. fluorescens</u> (Pf.) and one of <u>Bacillus</u> (Bsp.) on postharvest microbial deterioration of cassava.

Bacterial strain number	Root deterioration (%) after storage*								
	CMC 40		HMC-1		CMC 40		M COL 22		
	1	2	1	2	2	3	1	2	3
Pp. f-56	0.6**	30.5	7.0	18.0					
Pp. f-44	8.8	10.0	7.3	23.0					
Bsp.	15.2	32.5	15.3	25.0					
Bsp. + Pp. f-56	6.6	23.5	7.8	28.5					
Bsp. + Pp. f-44	7.0	9.0	7.6	16.0					
Pf. c-5a					0.0	10.0	4.5	9.9	38.0
Pp. c-7a					20.4				
Pp. c-4b					7.8	14.8			
Pf. c-88					11.0	47.3			
Pp. c-5b							7.0	16.5	
Pp. c-7c							5.0	18.0	
Pp. c-7c							2.0	8.4	
Mertect	8.6	13.5	4.8	15.0	1.8	4.0	2.0	8.4	5.6
Control	10.3	34.5	13.8	74.5	32.5	77.0	18.0	64.0	100.0

* Readings taken after 1, 2 and/or 3 weeks of storage.

** Average score from 500 roots of approximately 0.5 Kg each. Score of 0 to 100 refers to percentage losses due to microbial deterioration (Wheatley, <u>et</u> al., <u>1984</u>).

Table 7. Effect of _P. fluorescens_ (strain 88) on the root system of cassava (Clone M Ven 77) two months after inoculation and incubation under sterile conditions.

Treatment	Root weight (g)	Increase (%)
P. fluorescens plus nitrogen	4.5*	164
Uninoculated control plus nitrogen	1.7	-

* Results of 10 plantlets which were immersed for 20 min in a bacterial suspension of 1 x 10^9 cfu/ml. Leonard jar contained sterile Morri's medium plus washed quartz.

Fig. 1. Effect of Pseudomonas putida (Strains F44 and F56) on Corticium rofsii and cassava cuttings (clone CM 523-7). Top picture: P. putida F44 inhibited fungal growth; P. putida F55 did not. Bottom picture: P. putida F44 controlled the pathogen and induced root system growth, while P. putida did not.

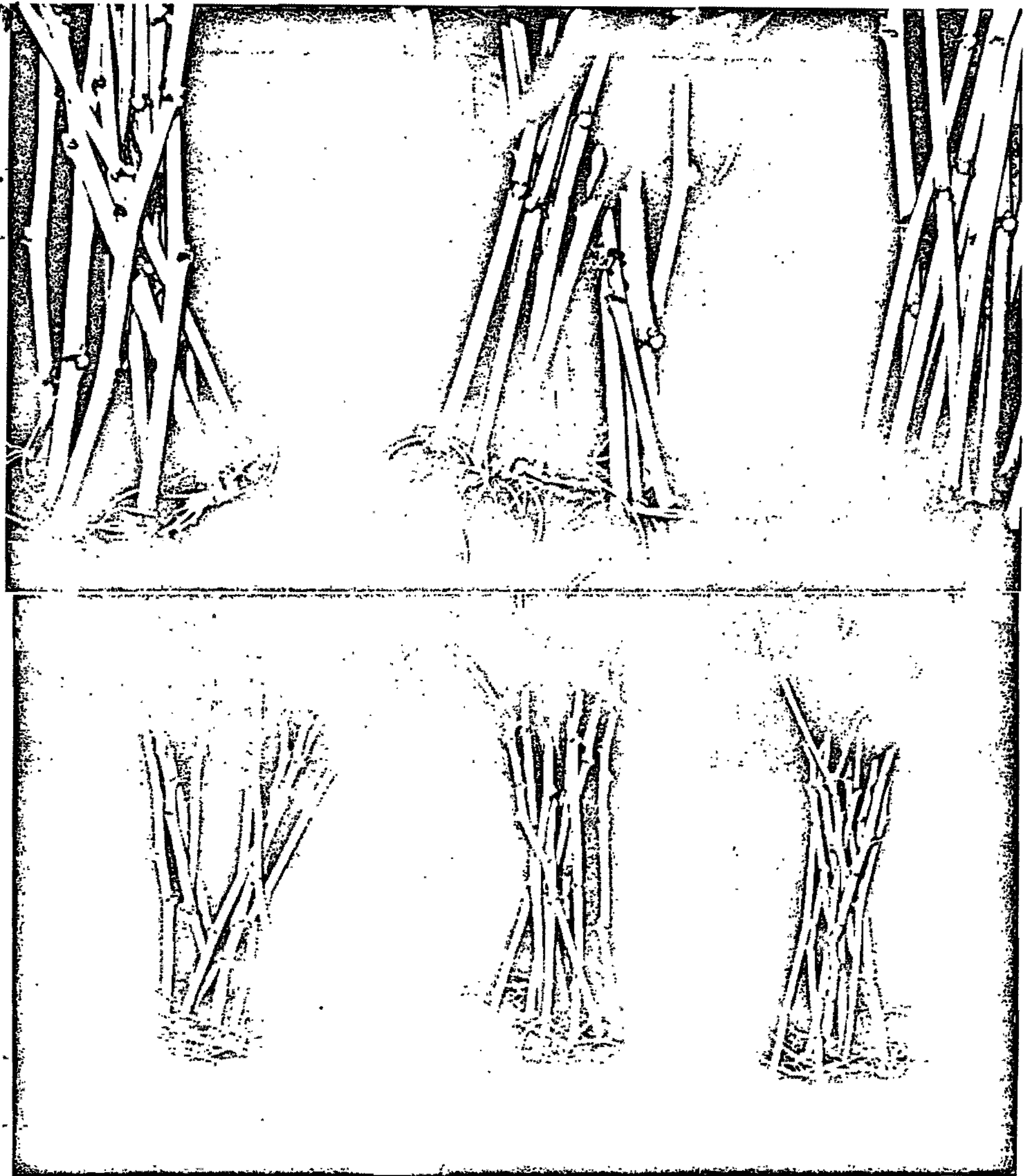

Fig. 2. Effect of *Pseudomonas putida* (Strain F44) on shoot tips of M Br 191 (top picture) and M Col 1684 (bottom picture) 18 days after rooting under continuous fluorescent light. Left: rooting in a 24h bacterial suspensions (10^8cfu/ml) in distilled water. The bacterial suspension was changed every 48h; Center: rooting in a crude extract from 24h bacterial suspensions diluted in distilled water at 1%; right: control rooted in sterile distilled water.