Cooperative Project between the Centro Internacional de Agricultura Tropical (CIAT) and the Instituto Agronómico (IA), Campinas, Brazil

INACTIVATION OF PATHOGENIC ORGANISMS OF CASSAVA (MANIHOT ESCULENTA CRANTZ) BY HEAT AND OTHER TREATMENTS

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INTRODUCTION

In August, 1971, a cooperative project between the Centro Internacional de Agricultura Tropical (CIAT), Colombia, and the Instituto Agronomico (IA), Campinas, Brazil, was approved with the financial support of the International Development Research Council (IDRC) of Canada.

The general objectives of the project were to develop a safe, easy, economic and efficient method for producing disease-free cassava propagation material by using heat or other physical therapeutic agent or other method that may lead to the eradication of cassava pathogens from stem cuttings.

Although preliminary work was initiated in 1971-72, most of the work was done during 1973 when the cassava pathologist of CIAT took over total responsability of the project. This work was particularly concerned with the development of techniques for the production of bacteria-free planting material. Studies of the effect of physical therapeutic agents on cassava stem cuttings were also undertaken by using heat, microwaves and ultraviolet light (u.v.). Simultaneously,

the effects of these physical therapeutic agents were recorded to establish the possible inhibitory or inactivatory properties of such treatments in the host-parasite relationship as well as the tolerance in vitro of some other cassava pathogens present in Colombia. The following Part I deals with research done at CIAT as their contribution to the joint program. Part II of this report contains the work done at The Instituto Agronomico, Campinas, Brazil.

The investigation at CIAT was subdivided into two topics: A. Development of a technique for the production of bacteria-free planting stocks, and B. Studies of the effects of physical therapeutic agents of vegetative cassava material used for propagation.

A. A technique for the production of bacteria-free planting stock of cassava (Manihot esculenta Crantz)*.

1. Introduction

Cassava Bacterial Blight (CBB) is one of the most important diseases in Tropical America and West Africa, causing extensive losses to cassava. Symptoms of the disease include leaf spotting and blight of leaf tissues, wilt and die-back of young shoots with associated gum exudation, and necrosis of vascular strands. These symptoms appear four to eight days after infection, with young green tissues being the most susceptible to CBB. The pathogen penetrates the host via stomatal openings or wounded epidermis and invades systemically the vascular tissues.

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* Lozano J.C. and Wholey D.W. (1974) World Crops (in press).

The most important method of dissemination of CBB is by using infected planting material. By this means, transportation of planting material can disseminate the disease over long distances. The organism can survive dry seasons within the vascular system of infected stems and reinfect new plantings with onset of the wet season. It is therefore of great importance that CBB-free planting material is used to establish crops of cassava.

Dissemination of CBB within a plantation occurs through rainsplash, from diseased tissues or from infected tools. Dissemination from a focus of infection occurs rapidly under climatic conditions favourable to CBB (8).

Programmes for producing pathogen-free stock have been developed for food crops such as potatoes, strawberries, citrus and grapes (2, 5, 9, 11). The success of such programmes relies largely on initial selection and source of propagation stock, maintenance of stock free from reinfection, and systems of propagation and distribution which avoid reinfection before planting material reaches the grower. Success therefore depends on a complete understanding of the epidemiology of the disease.

Seed certification as a cooperative effort between producers and research bodies, or under governmental control has proved successful in the United States and Europe for potatoes (11). Continued economic production of this crop would be seriously handicapped without this system for producing pathogen-free planting material.

Propagation technique

Cassava is traditionally propagated from cuttings prepared from the leafless portion of the stem of mature plants. Research underway at CIAT (4) indicates that green shoot tips removed from plants in the field can be rooted using mist propagation which maintains high relative humidity by continuously or intermittently spraying cuttings with a fine mist. Adequate moisture is available for root development and the air surrounding the cuttings is maintained at high humidity which prevents excessive water loss from leaf surfaces.

For the propagation of green shoot cuttings, a mist chamber was designed. This chamber was constructed with an aluminum frame and transparent polyethylene film on a base of formica-faced board. The use of these materials enables frequent disinfectation of the chamber using surface sterilants and allows sufficient light to pass through to support photosynthesis. Doors on each side allow easy access for inspection of the cuttings during the rooting phase and facilitate removal of infected cuttings. Constant mist was generated by a standard electric humidifier.

Pea-sized gravel was found to be a suitable rooting medium, giving better results than sand or sand/gravel mixtures (Table 1). This supports general experience with mist propagation where findings indicate that the rooting medium should have excellent drainage and should not hold excessive amounts of water, thereby causing poor root aeration. A consistent rooting rate of over 80 percent was achieved with this medium.

TABLE 1.

Effect of rooting media on rooting of shoot tip cuttings under constant mist for 12-15 days at 25-30 °C.

Rooting medium	No. of cuttings	% Establishment
Sand	358	14.8
Sand/gravel	320	38.0
Gravel	383	93.5

The cuttings used in this propagation technique were 10-cm-long shoot tips (Figure 1). Care was taken to select the cuttings from shoots which were apparently free from CBB symptoms. The shoot tips were planted in sterile gravel in peat pots or waxed paper cups and placed in the mist chamber.

Roots were formed from the basal end of the cuttings during the second week in the mist chamber (Figure 1). After 12-15 days the rooted cuttings were transferred into plastic plant pots containing sterile sandy soil and maintained in a screenhouse for one month for further observation before transplanting to the field.

Disease testing

An experiment was performed to investigate whether the apparently healthy shoot tips were in reality CBB free. One-cm sections, removed from the base of 70 shoot tips taken from infected plants were examined using an isolation procedure (7) with Kelman's TZC medium (6). The shoot tips were placed in the mist chamber and observed for CBB symptoms.

The results of the isolation procedure were negative in all cases, showing the shoot tips to be CBB free. Similarly, no CBB symptoms were observed on the corresponding shoot tips in the mist chamber.

Although no infected shoots were found in this experiment, this does not indicate that all apparently healthy shoots will be completely CBB free; the contingency of diseased cuttings placed in the mist chamber must be anticipated.

As the environmental conditions in the mist chamber are ideal for CBB development (8), wilt symptoms appear within seven days if an infected shoot tip is placed inside the chamber. These infected cuttings should be eliminated immediately to prevent the rapid spread of the disease through out the chamber. Care should be taken not to confuse the normal loss of turgor with CBB wilt symptoms during the first three days after cutting.

Eradication of bacteria at CIAT

The CIAT germ plasm bank, collected from seven Latin American countries, became infected with CBB during 1971, when up to 80 percent of the cultivars showed disease symptoms. Following attempts to reduce the size of the disease outbreak by removal of the foliage (2) and by heat treatment of infected stakes, three plants of each cultivar were propagated using the above method, producing over 6,000 plants which were used to establish a CBB-free germ plasm bank. Strict security measures are enforced to avoid possible reinfection. No symptoms of CBB have been found at CIAT since May 1973, which marked the end of a vigorous program to eradicate the disease from the experimental station.

Seed Certification Program

Because of the success of the propagation method, CIAT intends to stimulate similar programs to eliminate CBB, not only on the farm scale, but also on regional and national levels.

It is proposed that the above method of producing CBB-free plants from shoot tips could provide a useful basis for a cassava seed certification program as outlined in Figure 2. CBB-free plants could be readily propagated from existing desirable varieties, even from those varieties which are infected with the disease. Research on rapid propagation methods (4) to reduce the time between establishment of a foundation stock and distribution of certified seed to farmers is underway. It is hoped that government bodies can be encouraged to establish increase blocks of promising varieties for distribution to farmers.

CIAT is in a position to offer advice in the organization of a program to reduce CBB-free planting material, or to supply small quantities of planting material of improved types of cassava when these are available.

Eradication of CBB from infected plantations

The following suggestions based on experience and results of investigations carried out at CIAT are presented as guidelines:

1. If certified CBB-free material is to be introduced to a farm, it is recommended that all plantations which may harbour CBB be eradicated, unless the distance between suspect and new planting is approximately one kilometer. Some varieties are tolerant and not immune to CBB (8). Although these resistant varieties may not show characteristic symptoms, they may

harbour the disease and thereby constitute a hazard.

2. To prevent the spread of disease from infected to non-infected plantations it is necessary to use non-infected tools and implements. Tools, boots and other potential carriers of the pathogen such as tractor wheels and implements should be surface sterilised using one of the substances commonly used for this purpose (10).

3. Surface litter from an infected cassava plantation can maintain a source of CBB in the soil, potentially capable of transferring the infection to new plantings. Therefore, planting clean material immediately after CBB infected cassava should be avoided. CBB is considered a poor competitor with other microorganisms in the soil (Lozano, unpublished). Therefore, the elimination of CBB from the soil may be possible through a fallow or crop rotation, releasing the land from cassava for at least six months. All infected cassava residues should be destroyed by burning.

4. It is recommended that large areas be maintained between clean and infected plantations because of danger from infection through windborne rainsplash, irrigation or drainage water or other methods previously referred to. Insects are reported as possible agents for the dissemination of CBB (1) and until further information is available they should be regarded as potential hazards.

Although this method was developed specifically for production of bacterial-free stocks it could be also applied for production of material free of other diseases such as cassava common mosaic virus of America, mycoplasma, cassava brown streak, cassava vein-mosaic and the superelonga

tion diseases, by selecting either healthy plants and shoots for propaga tion and screening for healthy rooted shoots and plantlets, after the rooting processes.

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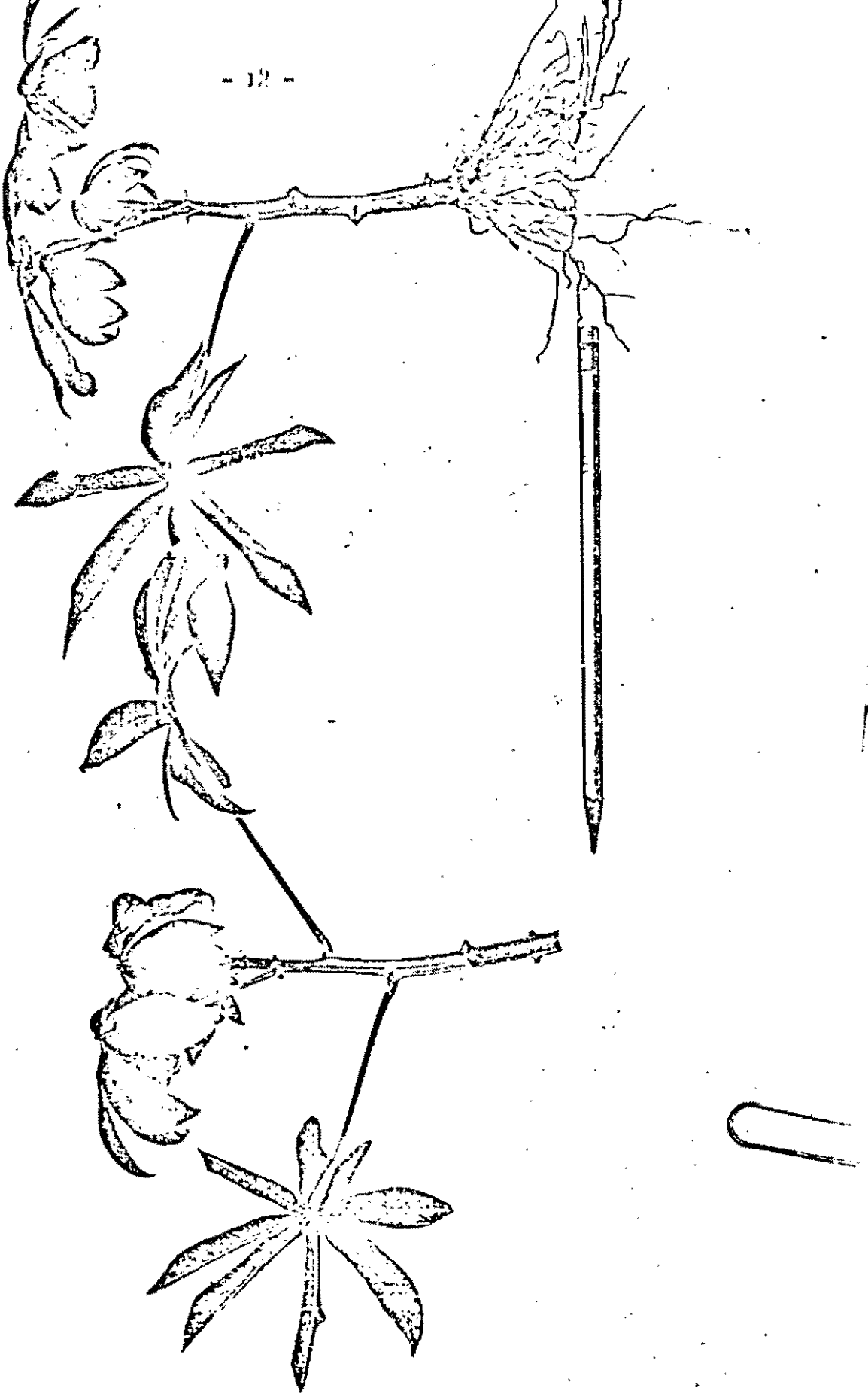

Figure 1. Shoot tip cuttings before and after the rooting process. Many roots are produced at the base of the cutting.

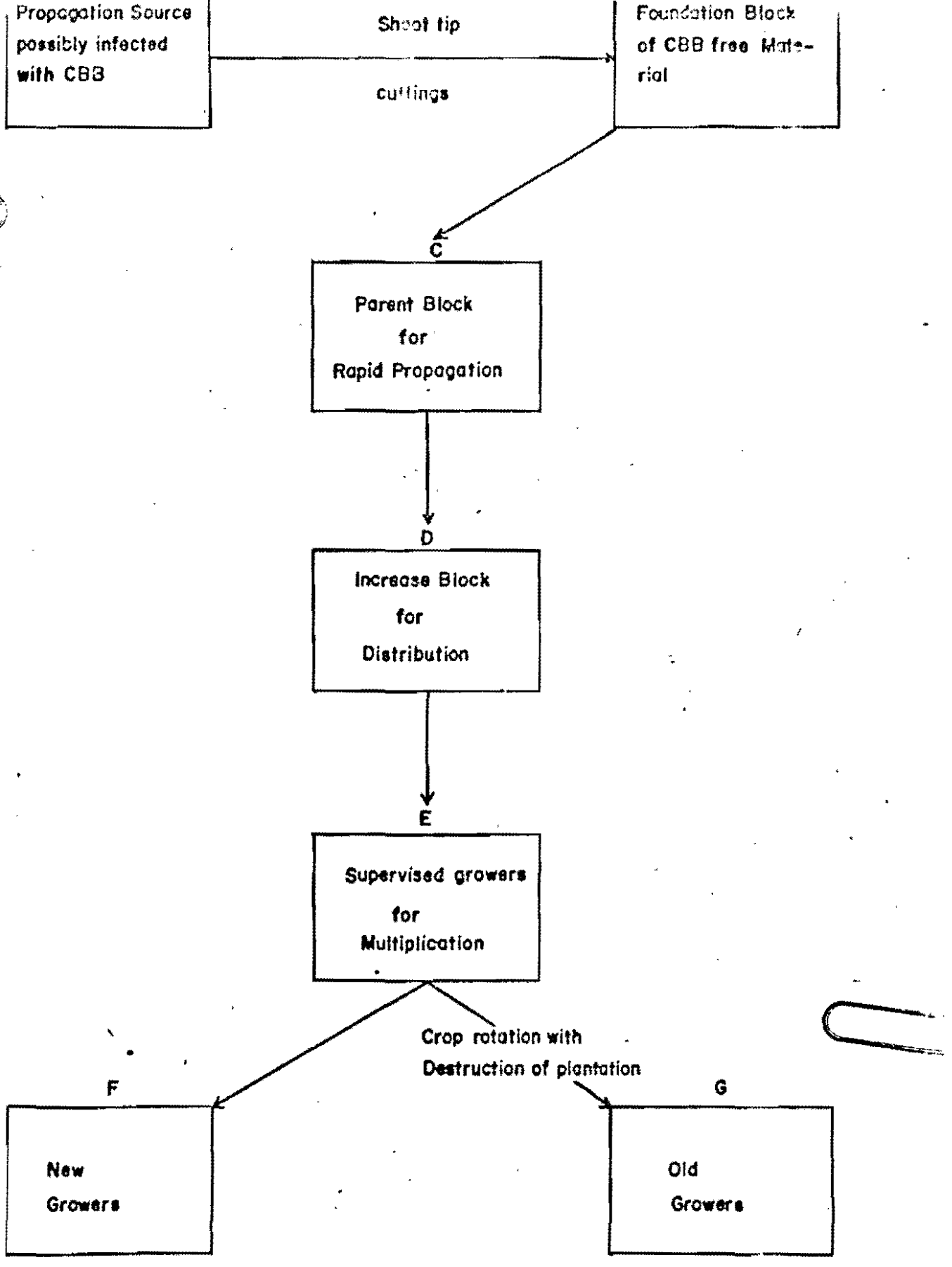

Figure 2. Suggested scheme for a CBB-free seed certification program.

B. Studies on the effects of physical therapeutic agents on cassava stem cuttings.

INTRODUCTION

Since commercial cassava is always propagated vegetatively, pathogenic causal agents such as bacteria, viruses, mycoplasma, fungi, nematodes and even insects, may be disseminated by moving planting material from one area to another.

Chemotherapeutants and physical therapeutical agents have been used effectively in other vegetatively propagated crops for the production of disease free-planting material. However, few reports relating to this subject in cassava are available, although it has been reported that several pathogens, such as cassava bacterium blight (CBB) (7, 9), the superelongation disease (8), the "superbrotamiento" (3, 6), and the common mosaic disease of America (3), are disseminated by the use of infected material for propagation.

The use of therapeutical agents for inactivation or inhibition of cassava common mosaic virus was reported by Costa and Normanha (4). However, the treatment (hot-water at 40-60C) did not have any effect on the common cassava mosaic virus, since its thermal death point threshold is 70C. In contrast, the African mosaic disease was apparently inactivated after hot-water treatment (35-39C) for prolonged periods of time (1). However, it was observed that germination was seriously reduced.

The primary objectives of this investigation were:

a. To evaluate the effects of hot-water, microwaves and ultraviolet light on germination cassava cuttings.

b. To determine the tolerance of the cuttings to each one of these treatments.

c. To establish the possible inhibitory or inactivatory effects of such treatments in the host-parasite relationships.

d. To determine the tolerance in vitro of some pathogens to these treatments.

MATERIALS AND METHODS

1. The effects of hot-water, microwaves and u.v. treatments on cutting germination were evaluated by using cuttings of three cultivars from the CIAT cassava collection.

The variants for each treatment were:

a. Varieties (cultivars):

CMC 84, and CMC 39; good natural germination (100%)

Llanera: poor natural germination (90%).

b. Type of vegetative material:

"old" cuttings, from the base of the stem.

"mature" cuttings, from the central part of the stem.

"immature" cuttings, from the uppermost part of leafless stem.

c. Range and time of exposure for treatment:

Water-heat treatment:

1. Temperature: Initial temperature: 44 - 47 - 53 - 56 - 58 - 60 - 65C

Critical temperatures: 50 - 51 - 52 - 53°C

2. Time: 0 - 10 - 20 - 30 - 40 - 50 - 60 minutes.

Ten cuttings (20 cms long; each with approximately 5 buds) per cultivar and type were immersed in hot-water, at each of the temperatures

for each time period , using a constant temperature water-bath. Each treated cutting was planted in a plastic bag filled with sterile sandy soil. Germination was evaluated by counting sprouted cuttings 25 days after planting. Each treatment was replicated 2 or 3 times, and untreated controls were included for comparison. The critical optimal point was determined from the experimental results using 80% germination as the lowest acceptable rate for commercial cassava production.

Microwave treatment

Ten cuttings per cultivar of varieties Llanera and CIC 39 of the three different types were treated for different periods of time by using a General Electric Microwave oven. Each exposure period was of 15, 30, 45, 60, 75, 90 and 105 seconds. All treatments were replicated 3 times. Treated cuttings were planted and evaluated as described above.

Ultraviolet light (u.v.)

This treatment was made to the same number of cuttings per cultivar and type, as described above. A Phillips u.v. lamp (short wave length) installed in the roof of an isolated chamber was used. Each cutting was exposed 30 cms from the u.v. source for the followings periods of exposure: 0.5, 1, 2, 2.5, 3, 3.5, 4, 4.5, 5, and 6 hrs. All treatments were replicated 3 times and germination data were obtained as described above.

2. To establish the possible inhibitory or inactivatory effects of above treatments in the host-parasite relationships of CBB, Botrydiplodia sp., Glomerella sp., and the causal agent of the superelongation disease* infected

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* The Commonwealth Mycological Institute who is working presently a the identification of the organism have provisionally defined it as a lower basidiomycete.

cuttings with these organisms were used. Recently infected cuttings were compared with cuttings infected for a long period of time. Ten infected cuttings per cultivar of varieties CMC 84 and Llanera were treated with hot-water, microwaves and u.v. at the highest levels of tolerance (optimal points) of the cultivars in each one of the treatments.

After each treatment, isolation of each of the pathogens was attempted using standard procedures. A positive isolation of a pathogen was considered to be a negative effect of the respective treatment.

3. To determine the sensitivity in vitro of some cassava pathogens to exposure to hot-water, microwaves and u.v. pure cultures of CBB, Botryodiplodia sp., Glomerella sp., and the causal agent of the superelongation disease were prepared for this study. CBB was suspended in sterile distilled water and its Optical Density adjusted to 0.7 (600 uw) (3×10^9 cells/ml). Three test tubes with 10 ml of this bacterial suspension was treated with hot water (water-bath technique) at 38, 40, 42, 44, 46, 48, 50, 52, 54, 58 and 60 °C for periods of 10, 20, 40, 50 and 60 minutes. Similar bacterial suspensions were treated with microwaves during 15, 30, 45, 60, 75, 90 and 105 seconds; and with u.v. during 1, 1.5, 2.5, 3, 3.5, 4 and 5 hrs, placed 30 cms from the source.

After each treatment a loopful of the treated bacterial suspension was streaked of Petri-dishes with Kelman's tetrazolium chloride (TZC) medium (5). Petri dishes with bacterial growth, after 48 hrs. of incubation, were considered as negative for sensitivity to a treatment.

Fungal growth on the surface of cassava cuttings was also treated with hot water, and steam at 40, 42, 44, 46, 48, 50, 52, 56, 58 and 60C for 10, 20, 30, 40, 50 and 60 minutes. Similarly microwave treatments were made during 15, 30, 45, 60, 75, 90, 105 seconds, and u.v. exposures during 1, 2, 2.5, 3, 4, 5 and 6 hours at 30 cms from the source. After each treatment, isolation of the respective organisms was attempted. No growth after 96 hours incubation was considered to be positive for sensitivity to a treatment-period.

RESULTS

1. Hot-water treatment of cuttings.

Tables 2, 3, 4 and 5 present results of the hot-water treatments. Temperatures above 53 °C seriously reduced germination of cuttings of both varieties. Hot-water treatment at 53 °C reduced the germination of Llanera cuttings more than that of CMC 39 cuttings (Table 2, 3). It is considered that the best hot-water treatment is 52 C for 30 minutes, since germination was higher than 80% in both cultivars (CMC 84 and Llanera) (Table 4 and 5).

2. Microwave treatments.

Germination of both Llanera and CMC 39 was seriously reduced by more than 105 seconds of microwave exposure. Seventy five to ninety seconds of exposure seem to be the best treatment-periods (Table 6).

3. Ultraviolet-light treatments.

Germination was not affected by u.v. treatments even with 6 hrs. exposure. No differences in germination between Llanera and CMC 84 were observed (Table 7).

4. Inhibitory or inactivatory effects on the host-parasite relationships.

In each case CBB was isolated from hot-water treated cutting. A bacterial population closely similar to the untreated cuttings was found in mature and old stem portions after being treated at 65°C for 60 minutes. Similarly, 90 and 105 seconds of microwave exposure or 6-7 hours of u.v. exposure did not induce any inactivatory or inhibitory effects of CBB.

In contrast, Botryodiplodia sp., Glomerella sp. and the causal agent of the superelongation disease were controlled when early infections both by 52C/30 min. hot water treatment and 60 seconds plus microwave exposure. When cuttings were old infected (with necrotic woody tissue) these pathogens were not controlled by these treatments. However, u.v. exposure did not control any of the above pathogens infecting old cassava cuttings. Table 8 shows the inhibitory effects on Botryodiplodia sp. by treatment with hot-water (52°C/min), microwaves (60 seconds) and u.v. (6 hours).

5. Sensitivity in vitro of some cassava pathogens.

CBB's highest sensitivity to hot-water was at 54°C/30 min. After forty five seconds exposure to microwaves CBB was disrupted. Ultraviolet light did not induce any inactivatory effect on CBB suspension in sterile distilled water, even after 7 hrs. of treatment.

Botryodiplodia sp., Glomerella sp., and the causal agent of the superelongation disease were inactivated by 30 seconds of microwave exposure. Seven hrs. of ultraviolet light exposure did not induce any effect.

DISCUSSION

Comparing the three methods one can immediately say that u.v. did not show any promise as a method for disinfectation of planting material, as it is well known that u.v is reflected from dense surfaces. However, both methods of heat treatment, either through hot-water immersion or exposure to microwaves, resulted in positive methods for the possible control of certain pathogens in planting material under field conditions.

Results of hot-water and microwave treatments to healthy and diseased cuttings suggest that these treatments can be lethal to both host and pathogen during relatively short periods of exposure. Since diseased cuttings still contained viable CBB cells after treatment with hot-water at 65°C for 60 min. or microwave exposure for 105 seconds, it is concluded that in spite of previous reports (2) CBB can not be eliminated from inside the cuttings by these methods because both treatments result in the death of the cuttings. It is considered that both the woody structure of the cuttings and possibly the polysaccharides produced by CBB (7) play an important role in preventing the transmission of the heat throughout all the tissues of the cutting.

Considering other vascular pathogens, the "superbrotamento diseases" (witches broom), caused by a mycoplasma, has been successfully inactivated by hot-water treatments to cuttings at 50°C for 1 to 1 1/2 hrs. or by storing infected cuttings at 38°C for a weed (3, 4). A component of the African mosaic syndrome seems to be heat-sensitive, since it was inactived by hot-water treatment at 45°C for 1 hr. or hot-air at 39°C for 6 weeks. However, the causal entity of the mild stage in the syndrome of

this disease seems to be heat-stable (Omuemu and Berbee, University of Wisconsin, personal communication) supporting earlier investigations on the African mosaic disease (1). As earlier investigations suggest that in the disease syndrome at least two components are involved, hot-water or microwave treatments could be only partially effective. As the causal agents of the African mosaic disease are still unknown it is not yet possible to prove the efficiency of heat treatments on this disease until the purification of the pathogenic agents can be performed.

The common mosaic virus of America, one of the potato virus X group, can not be eradicated by heat therapy since it has been shown to be heat-stable (thermal death point = 65-70 °C/ 20 min) (3, 4). Investigations carried out by Costa and Kitajuna (3) report no inactivation of the virus inside the cuttings after hot-water treatments at 55 °C for 30 min. This temperature has been shown to cause death to a large proportion of cuttings (Table 2 and 3).

Other virus-like diseases of cassava, vein-mosaic, brown streak, and latent virus (3), do not exist within Colombia, therefore it was not possible to investigate the effects of these treatments at CIAT. Also there are no reports in the literature which provide information about the physical properties of these causal agents. Therefore, it is not possible to extrapolate from the results of the experiments performed at CIAT.

No fungal vascular pathogens have been reported in cassava, however the superelongation disease is known to be transmitted by planting material. In contrast with the results of the experiments with vascular pathogen

(CBB), where it was proved impossible to heat the interior of the cuttings to the critical temperature without damaging the buds, it was found that good control of the superelongation disease was possible because the causal agent is found within the cortex or epidermal tissues of the planting material. Our results suggest that early infections of other common epidermal or cortical pathogens of cassava can be controlled when the cuttings are treated at the optimal point of sensitivity (52°C/60 min). Results on the eradication of early infections of Botryodiplodia sp. and Glomerella sp. support this assumption. However, when the infection of these two pathogens was advanced with the woody tissues invaded and necrosed, their control by using these treatments was not effective. Since advanced stem infections can be easily detected, selection of the cuttings suitable for planting is advised. A chemical treatment combined with a hot-water or a microwave treatment will prevent the possiblility of reinfection during storage before planting.

Although definite conclusions can be drawn from these investigations it is recommended that more investigations are required before complete understanding is possible for the control of all known stem pathogens of cassava. However, considering the difficulties of critical temperature control and the penetration of heat to all tissues, it seems more worthwhile to investigate on the possibilities of shoot-tip propagation or tissue culture in order to produce disease-free planting material for international interchange.

CONCLUSIONS

1. Treatments using hot-water or microwaves can seriously reduce

cassava cutting germination. Ultraviolet light for 7 hrs. did not have any effect on germination.

2. The optimum temperature for hot-water treatment to cassava cuttings was 52°C/30 min., and the optimum microwave treatment, 75 to 90 seconds of exposure.

3. CBB, a vascular pathogen, was not controlled by hot-water or microwave treatments. Its inactivatory or inhibitory point, when associated with its host, is higher than the optimum point of sensitivity of cassava cuttings to these treatments.

4. Botryodiplodia sp., Glomerella sp., and the causal agent of the superelongation disease (a lower Ascomycete) were controlled by hot-water or microwave treatments only in the case of early infected cutting. Ultraviolet light exposure did not control these pathogens.

5. Since green cuttings were shown to be very sensitive to hot-water and microwaves, these treatments are unsuitable for this type of cutting.

C. Training

A Colombia technician was trained in Campinas, Brazil. Her main training was concerned with electromicroscopy, both histology and management. A Nigerian student, from the Imperial College of Science and Technology, London (under the supervision of Dr. R.K.S. Wood), has been working since April, 1973, on his Ph.D. Thesis.

A new Brazilian trainee from Brazilia University will arrive at CIAT

during March, 1974, to work on the general pathology of cassava. His training will be enphasized on CBB epidemiology and control, since serious problems are present in those areas were the disease is endemic. It is hope that after his training he will apply CBB control methods devised at CIAT to eradicate this pathogen from these zones in Brazil.

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Table 2. Cutting germination (CNC 39) after hot-water treatment at different temperatures and periods of exposures.

Type of cutting	Temperature (°C)	Percentage of germination after time of exposure (min.)						
		10	20	30	40	50	60	CK[2]
old	65	0[1]	0	0	0	0	0	100
mature	"	0	0	0	0	0	0	100
green	"	0	0	0	0	0	0	70
old	60	0	0	0	0	0	0	100
mature	"	0	0	0	0	0	0	100
green	"	0	0	0	0	0	0	65
old	58	0	0	0	0	0	0	100
mature	"	0	0	0	0	0	0	100
green	"	0	0	0	0	0	0	70
old	56	10	0	0	0	0	0	100
mature	"	0	0	0	0	0	0	100
green	"	0	0	0	0	0	0	70
old	53	80	80	60	40	20	10	100
mature	"	70	70	60	10	0	0	100
green	"	0	0	0	0	0	0	65
old	50	100	100	100	80	50	50	100
mature	"	100	80	80	60	40	30	100
green	"	20	10	0	0	0	0	60
old	47	100	100	100	100	100	100	100
mature	"	100	100	100	100	100	100	100
green	"	50	50	40	40	30	20	60

1 = average of 2 replications with 10 cuttings each.

2 = Control (CK) untreated cuttings.

Table 3. Cutting germination (Llanera) after hot-water treatment at different temperatures and periods of exposures.

Type of cutting	Temperature (°C)	Percentage of germination after time of exposure (min).						
		10	20	30	40	50	60	CK[2]
old	65	0[1]	0	0	0	0	0	90
mature	"	0	0	0	0	0	0	70
green	"	0	0	0	0	0	0	40
old	60	0	0	0	0	0	0	60
mature	"	0	0	0	0	0	0	80
green	"	0	0	0	0	0	0	30
old	58	0	0	0	0	0	0	80
mature	"	0	0	0	0	0	0	80
green	"	0	0	0	0	0	0	35
old	56	0	0	0	0	0	0	80
mature	"	0	0	0	0	0	0	60
green	"	0	0	0	0	0	0	40
old	53	60	40	20	10	0	0	80
mature	"	60	40	20	10	0	0	80
green	"	40	0	0	0	0	0	45
old	50	80	70	40	20	20	10	80
mature	"	90	90	50	50	50	30	75
green	"	30	20	0	0	0	0	45
old	47	80	80	80	80	60	40	75
mature	"	80	80	70	80	50	70	80
green	"	45	50	50	40	30	30	50

1 = average of 2 replications with 10 cuttings each.

2 = Control (CK) untreated cuttings.

Table 4. Cutting germination (CMC 84) after hot-water treatment at different temperatures and periods of exposures.

Type of cutting	Temperature (°C)	Percentage of germination after time of exposure (min).						
		10	20	30	40	50	60	CK[2/]
old	53	77[1/]	73	53	50	40	23	100
mature	53	67	60	43	33	33	13	100
old	52	100	97	87	63	50	23	100
mature	52	97	93	83	70	60	37	100
old	51	100	90	83	67	53	40	100
mature	51	97	87	87	67	47	33	100

1 = Average of 3 replications with 10 cuttings each.

2 = Control (CK) untreated cuttings.

Table 5. Cutting germination (Llanera) after hot-water treatment at different temperatures and periods of exposures.

Type of cutting	Temperature* (°C)	Percentage of germination after time of exposure (min).						
		10	20	30	40	50	60	CK[2/]
old	53	83[1/]	77	70	43	23	10	93
mature	53	60	60	47	27	13	10	80
old	52	90	83	80	73	50	33	97
mature	52	87	83	77	57	47	17	83
old	51	93	87	83	57	40	33	93
mature	51	87	83	77	60	47	20	83

1 = Average of 3 replications with 10 cuttings each.

2 = Control (CK) untreated cuttings.

* A more accurate water bath was used in this study which gave slightly better rates of germination than shown in Table 3. This shows the importance of accurate temperature control for cutting treatment.

Table 6. Cutting germination after microwave exposures for different periods of time.

Time of exposure (seconds)	Cultivar Llanera		Cultivar CMC-39	
	Type of cutting and % of germination		Type of cutting and % of germination	
	old	mature	old	mature
0	83[1]	87	100	100
15	87	83	100	100
30	93	93	100	100
45	83	83	100	100
60	87	87	100	100
75	90	87	100	97
90	70	67	100	93
105	3	0	33	10

1 = Average of 3 replications with 10 cuttings each.

Table 7. Cutting germination after ultraviolet exposure for different periods of time.

Time of exposure (hours)	Cultivar Llanera		Cultivar CMC-84	
	Type of cutting and % of germination		Type of cutting and % of germination	
	old	mature	old	mature
0	90 1/	90	100	100
1	90	100	100	100
2	90	90	100	100
3	100	90	100	100
4	90	80	100	100
5	90	90	100	100
6	90	90	100	100

1/ Average of 2 replications with 10 cuttings each.

Table 8. Presence of Botryodiplodia sp. in CMC 84 cuttings after treatments with hot-water, microwaves and ultraviolet light. Cuttings were early infected (10 days) by putting a disc of micelial growth on the surface of the cutting and incubate at 28 °C.

Cutting number	Treatments and exposure periods hot-water (52 °C/30 min).	microwaves. (60 seconds)	u.v. (6 hours)
1	+[1]	-	+
2	-	-	+
3	-	-	+
4	±	-	-
5	-	-	+
6	-	-	+
7	-	-	+
8	-	-	+
9	-	-	+
10	-	-	+
CK[2]	+	+	+

1: + = positive isolation; ± isolation from only one of there samples.

- = negative isolation. These results are means of 3 samples taken to each infected cutting.

2: Control (CK) untreated cuttings. Isolation from 3 samples/cutting.