A TECHNIQUE FOR THE PRODUCTION OF BACTERIA-FREE PLANTING STOCK OF CASSAVA (Manihot esculenta Crantz)

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ABSTRACT

A method of rooting shoot tips is described whereby plants free from Cassava Bacterial Blight were produced. The use of this method in addition to cultural practices is proposed for producing pathogen-free foundation stock for a planting material certification programme.

Cassava Bacterial Blight (CBB) (7) is becoming an increasingly significant disease in tropical America and West Africa, and causes extensive losses to cassava, an important carbohydrate source for human, livestock and industrial consumption.

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.

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.

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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 (Fig. 1) 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	38.8
Gravel	383	93.5

The cuttings used in this propagation technique were 10-cm-long shoot tips (Figs. 2 and 3). 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. After 12-15 days the rooted cuttings were transferred into plastic plant pots containing sterile sandy soil and maintained in a screen house 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 throughout 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 9. 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 block 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 produce 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 approximates 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 wind-borne rain-splash, 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.

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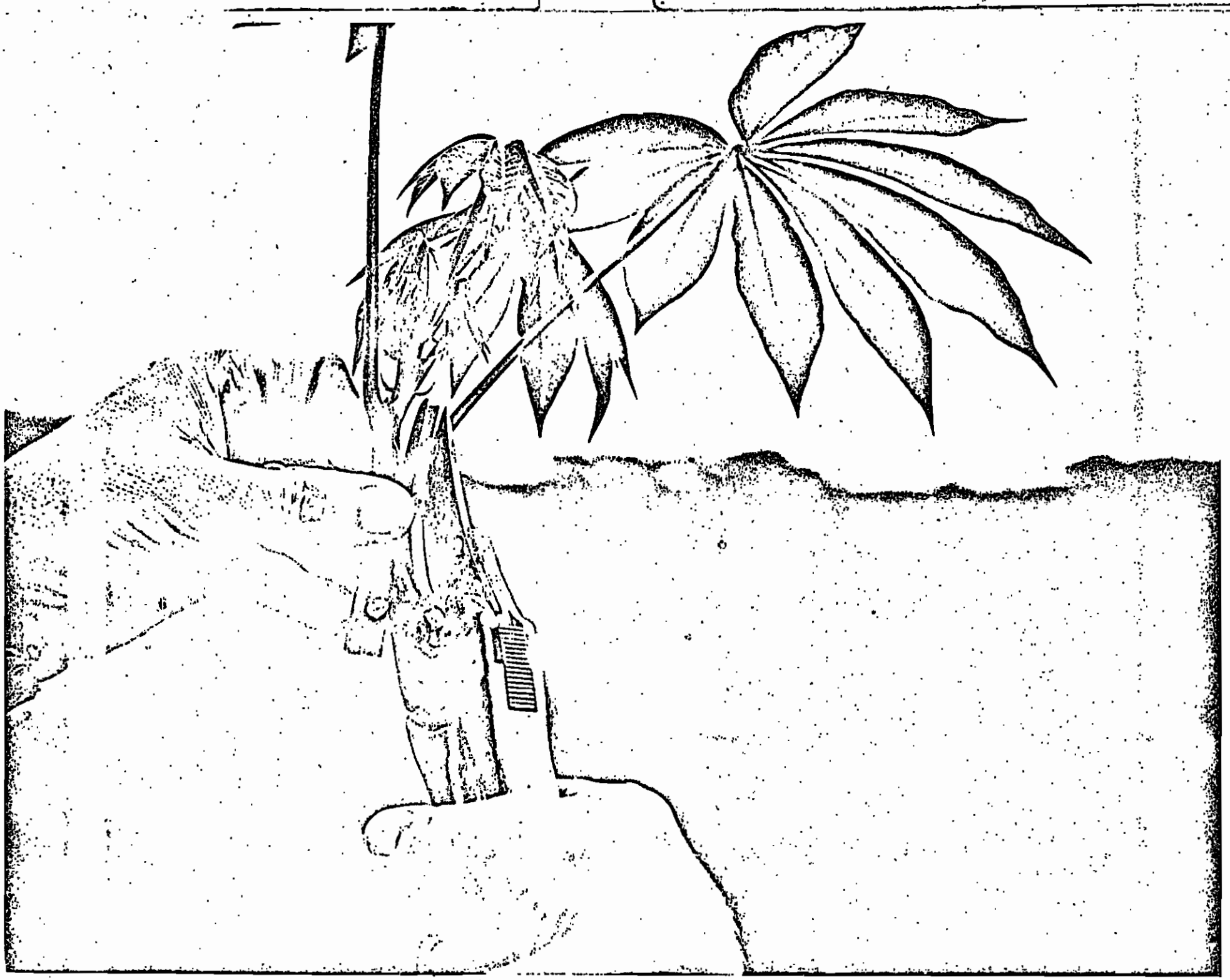

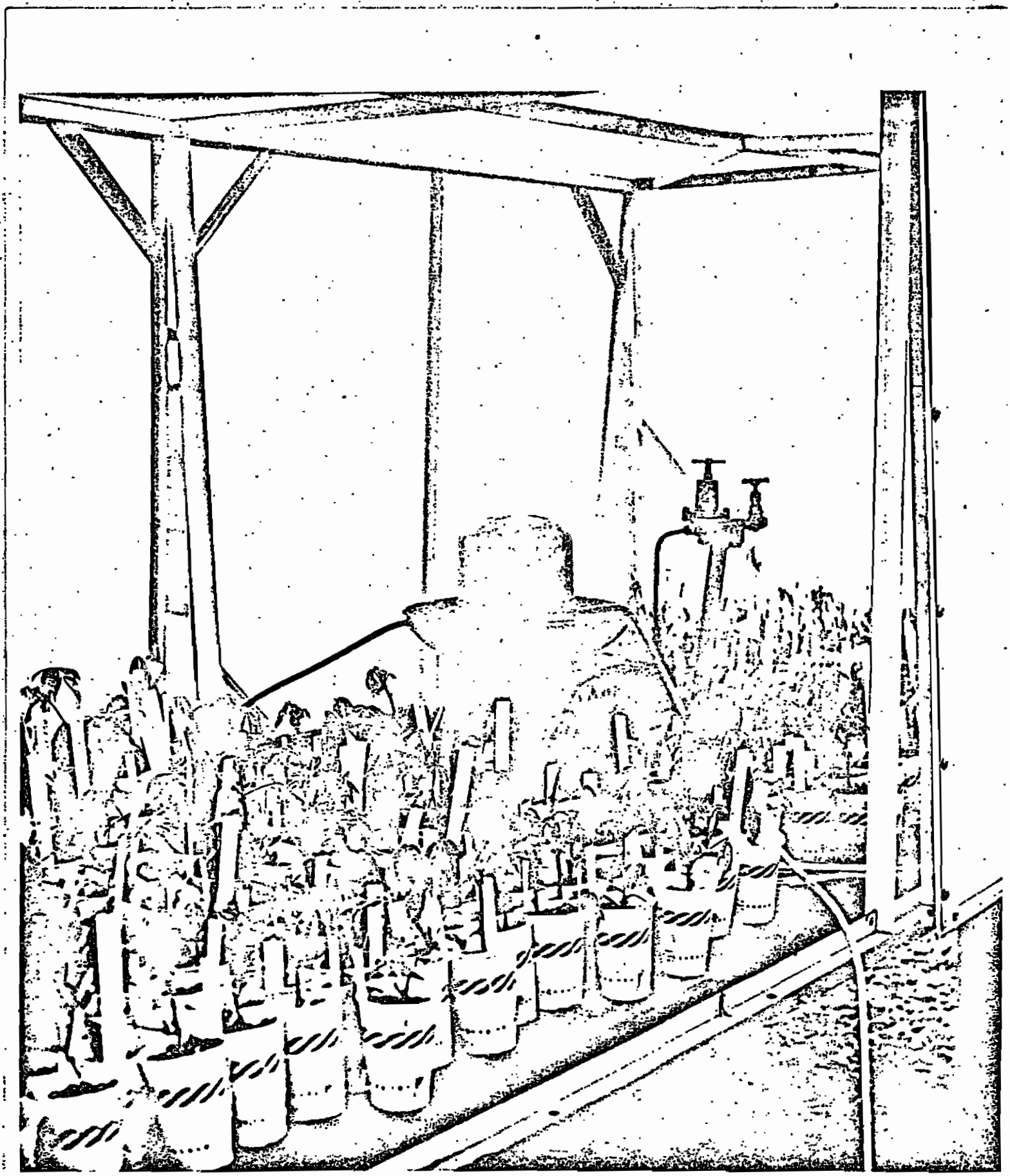

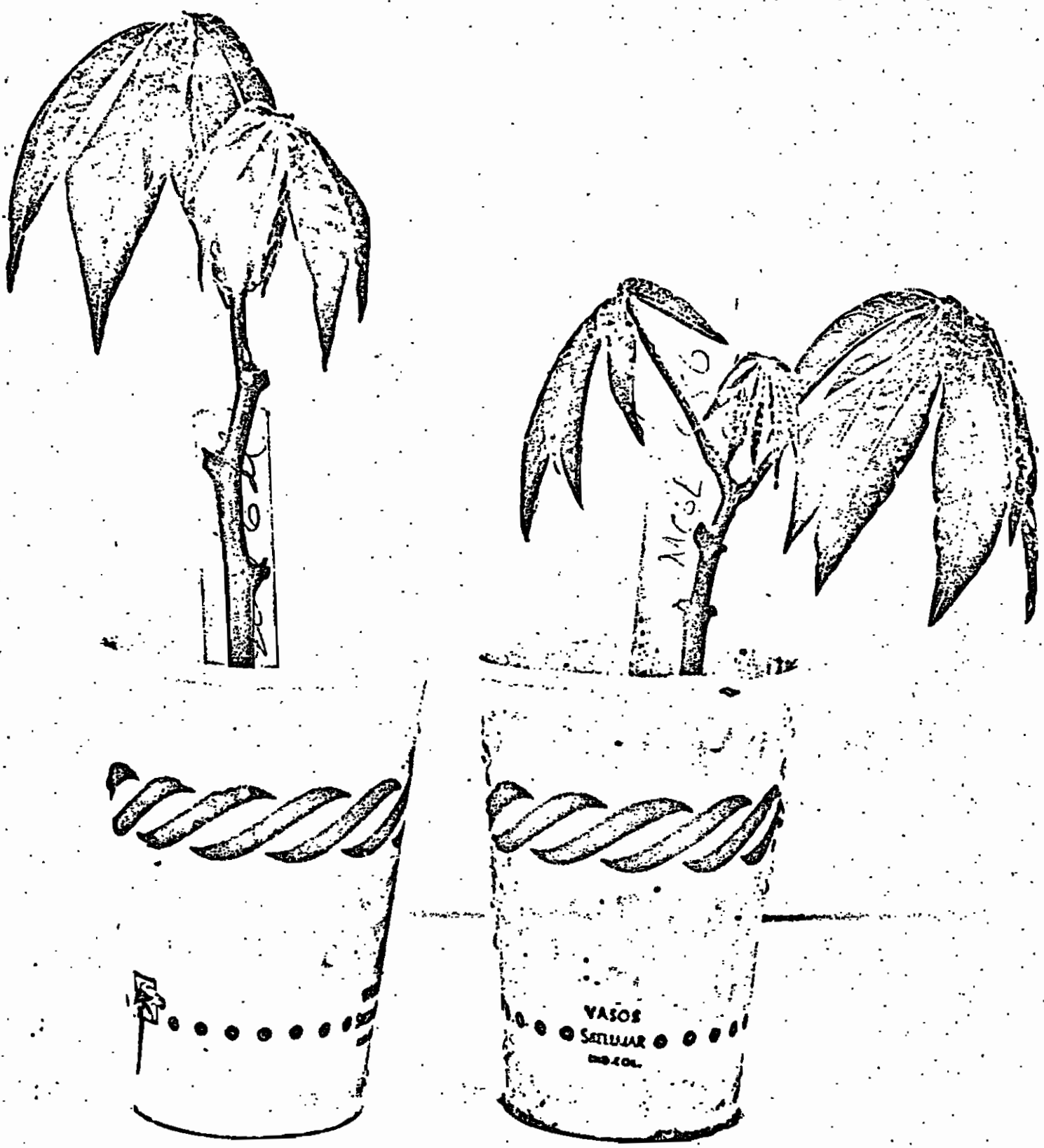
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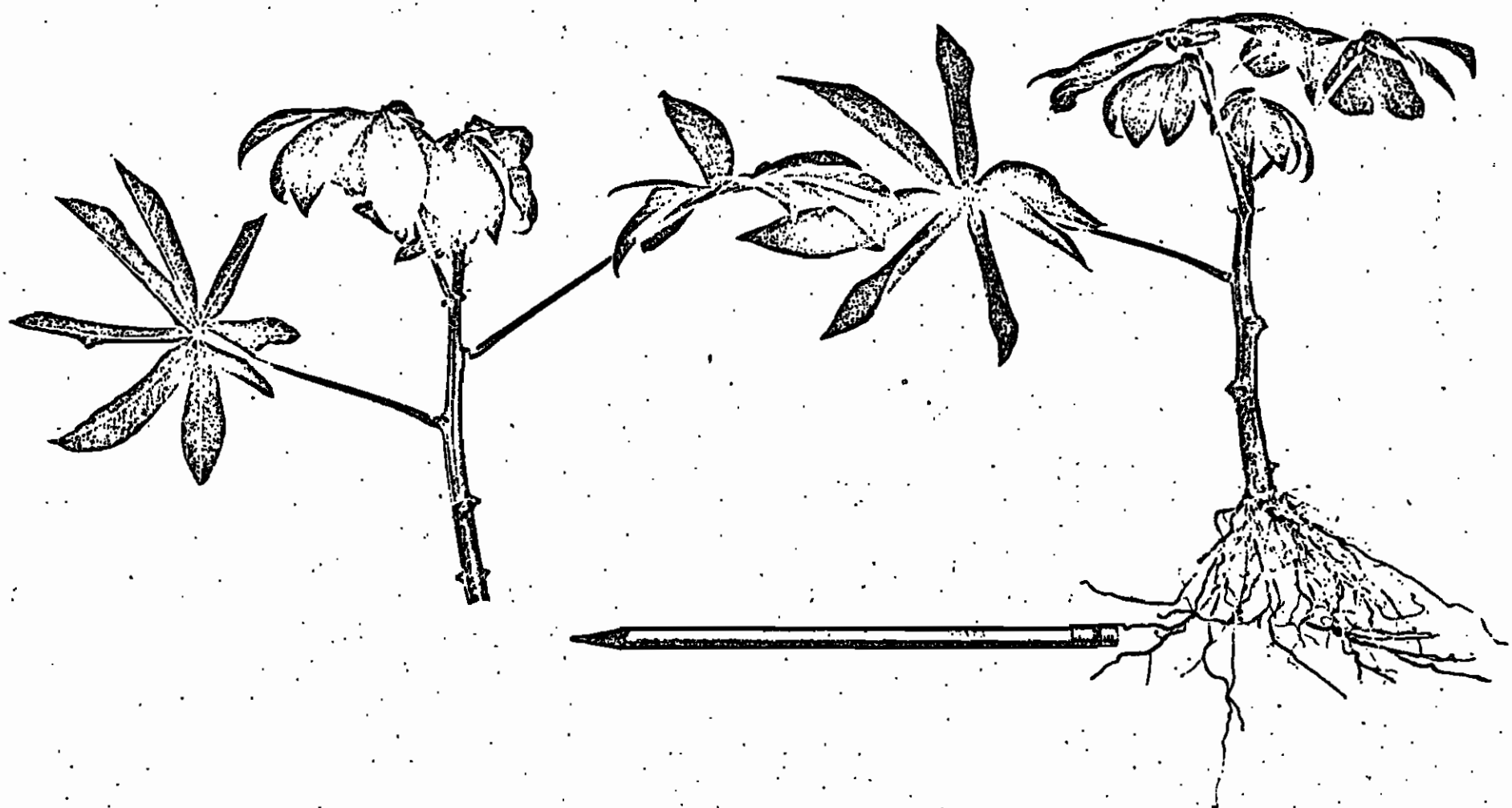

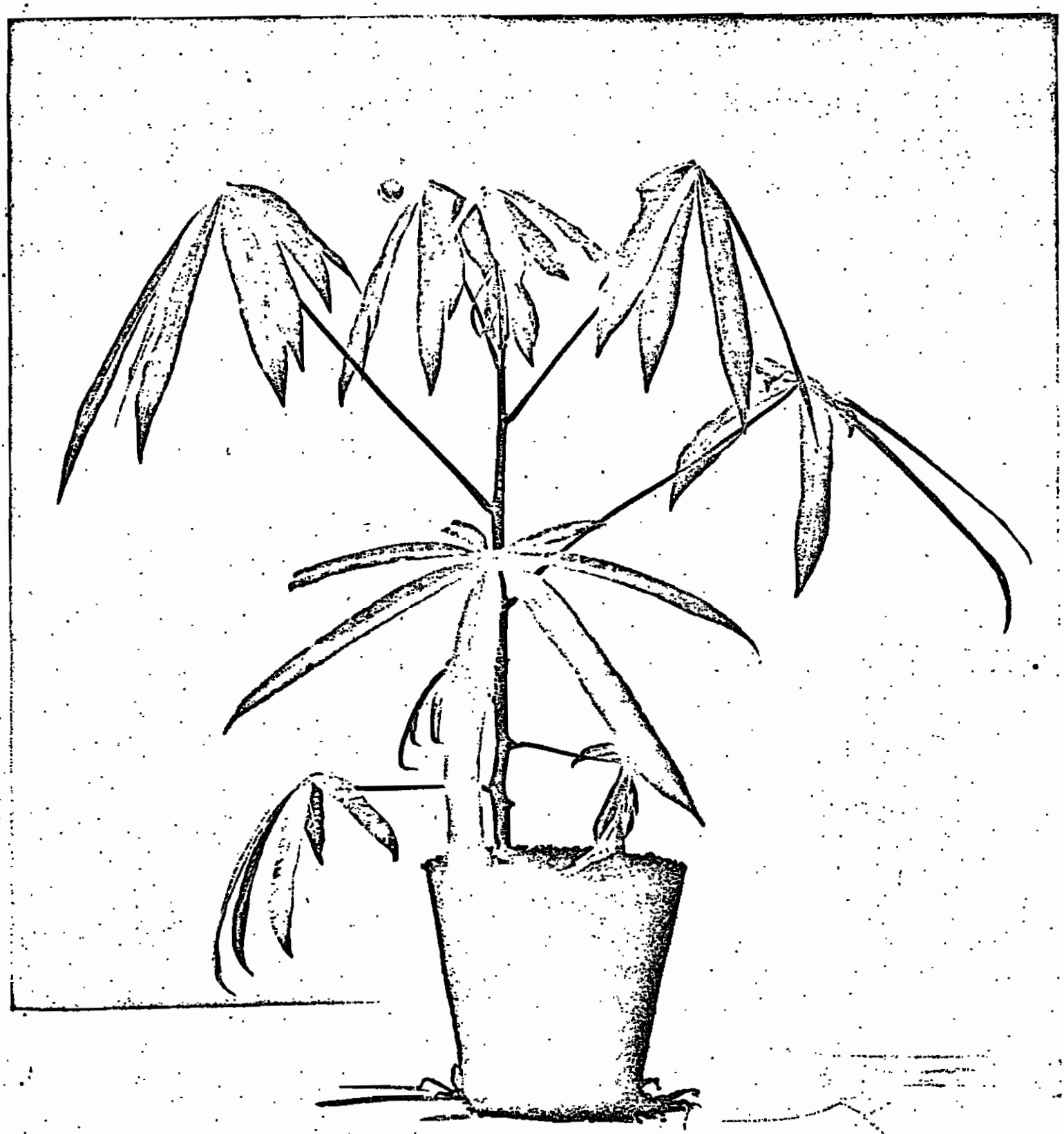

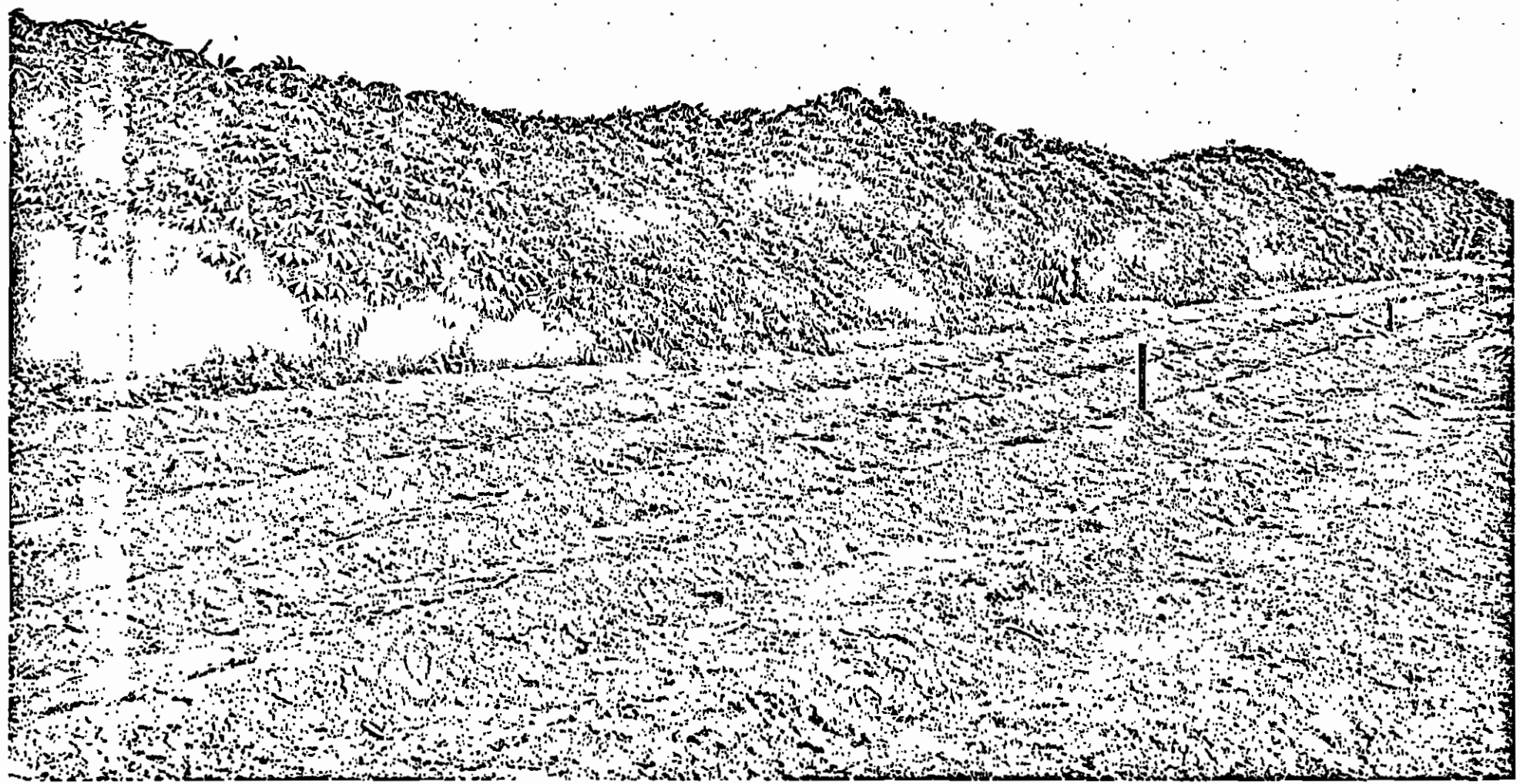

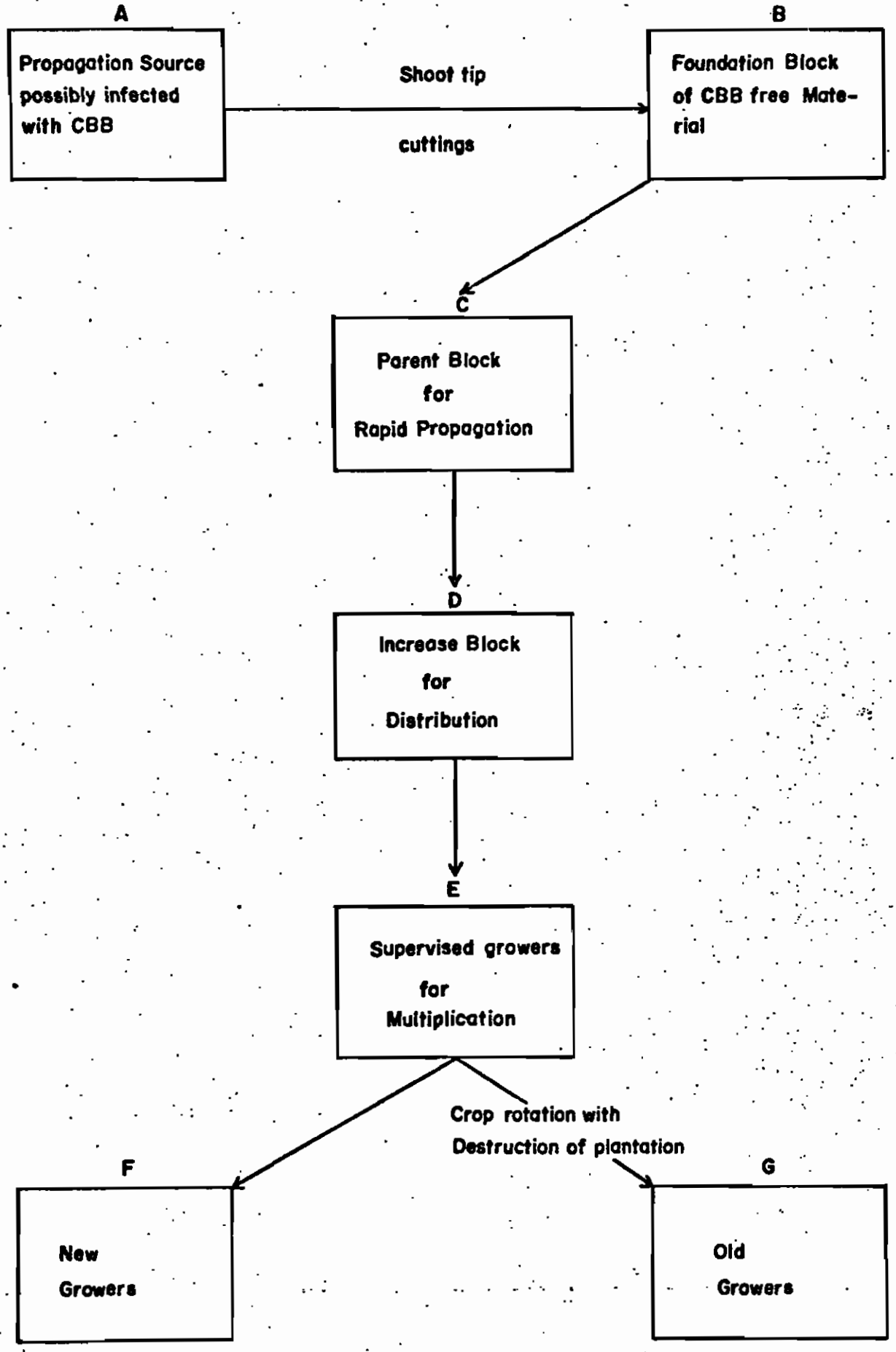
A
Propagation Source possibly infected with CBB
Shoot tip
cuttings
B
Foundation Block of CBB free Material
C
Parent Block for Rapid Propagation
D
Increase Block for Distribution
E
Supervised growers for Multiplication
Crop rotation with Destruction of plantation
F
New Growers
G
Old Growers