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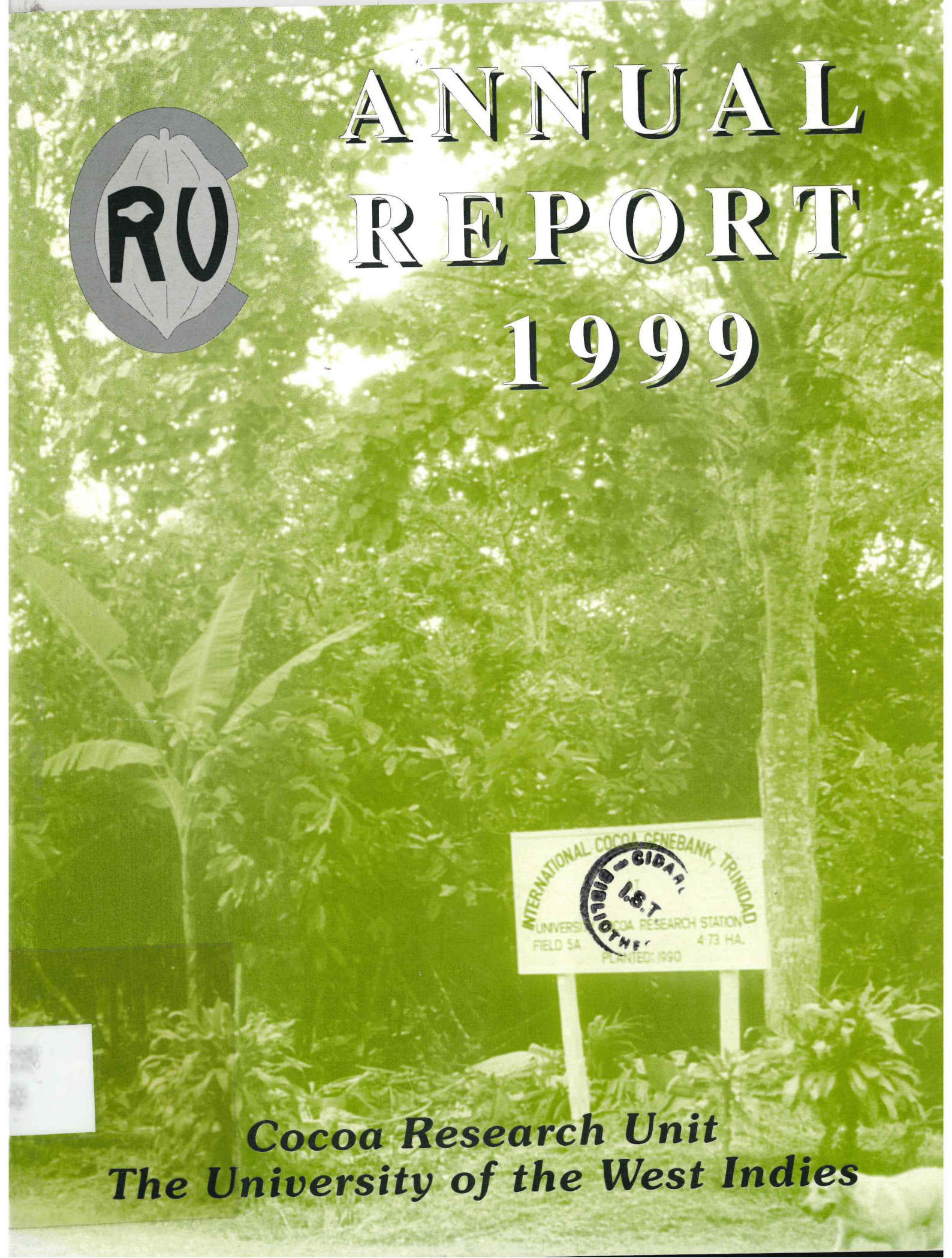

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Cover photograph. Field 5A in the International Cocoa Genebank, Trinidad.

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Evaluation of Cacao Germplasm for Resistance to Witches' Broom Disease: Preliminary Screening Experiments

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Introduction

Witches Broom disease, caused by *C. perniciosa*, is one of the major diseases faced by cocoa producers in South America and the Caribbean. Currently, germplasm enhancement activities are being undertaken at CRU, as part of the CFC/ICCO/IPGRI Project, with the objective of obtaining populations with increased disease resistance. Consequently, detailed information is required on the level of resistance to WB in accessions at the ICG,T. In order to acquire this information, a project for the mass screening of cacao germplasm is in progress.

Since the inception of this ACRI funded project in July 1998, considerable preparatory work was needed before screening of cacao germplasm for resistance to WB could begin. The spray inoculation method selected by ACRI for use in this project is based on an automated belt spray system developed by Purdy *et al.*, (1997), which was designed for inoculation of large numbers of seedlings. CRU is adapting the system for screening grafted clones rather than seedlings. The plant material to be screened is collected from the ICG,T as budwood and top-grafted onto TSH rootstocks, and then kept for three months to allow the grafts to become established. Grafting began in October 1998. Eight grafting series have been completed with a total of 221 clones.

Screening of grafted clones was initiated in April, 1999, and four series of plants; A, B, C, and D, representing a total of 650 plants, were inoculated with *C. perniciosa* using the methodology described below. However, after four months of fortnightly observations, no symptoms developed on the inoculated plants belonging to grafting series A-D. The inability to obtain symptoms in these experiments was attributed to one or more of the following factors:

- Elevated greenhouse temperatures.
- Unstable relative humidity in the incubation room during the infection process.
- The sensitivity of the stage of shoot development at the time of inoculation to infection.

This report contains an account of all the modifications being made to the inoculation system to adapt it for use at CRU. In order to address the problems cited above as reasons for the absence of symptoms after screening of the first four series of grafts, further improvements were made. These are also outlined below. In addition, the results of one preliminary experiment, conducted after implementation of the improvements, are presented.

Adaptations to the Spray Inoculation System

Humidity chambers

One of the main criteria necessary for attaining artificial infection by *C. pernisiosa* is maintenance of high relative humidity so that the plants remain sufficiently moist after inoculation to allow germination of the germ tubes and the penetration of the host by the pathogen. To achieve this, Purdy *et al.*, (1997) used a system comprised of a pump to circulate heated water, humidifiers and lengths of fabric (curtains) to control temperature (25-27°C) and humidity. Since environmental conditions in Trinidad are quite different from those in Florida, an air-conditioning system (AC) was installed to keep the temperature in our incubation chambers between 25-27°C. However, Purdy's temperature and humidity control system did not function well with the AC. As an alternative, chambers were constructed out of PVC frames, which were covered in heavy plastic to enclose the plants on the benches. Near saturation conditions were maintained within the chambers by using water-baths to generate water vapour. The water baths were made cost effectively from large stainless steel vessels, each fitted with a heating element and thermostat from household water heaters.

Spray System

The use of grafted plants instead of seedlings has proven to be problematic for a number of reasons, requiring major adaptations to the spray system. Grafted plants are much taller and heavier than young seedlings and there is much more variability in their size. There is also considerable variation in the physiological stages of grafted plants compared to seedlings thus it is difficult to achieve synchrony when plants are flushing.

In the original automated system, a conveyor belt was used to move the seedlings under atomising spray nozzles, which dispense the inoculum. To accommodate the relatively large grafted plants, we have replaced the conveyor system by benches on wheels. Instead of moving the benches under the spray nozzles, the spray system is being modified to move over the plants. The nozzles are moved on a system of tracks suspended from the ceiling of the inoculation room and controlled manually by strings through a pulley.

Improvements to Experimental conditions

Greenhouse

The greenhouse where inoculated plants are kept for symptom development previously had a fibreglass roof, which restricted air circulation and caused elevated daytime temperatures. This roof was replaced by netting that provides 73% shade and allows better air circulation, thereby maintaining the temperature within a suitable range for plant growth. After changing the roof, the plants were observed to undergo increased flushing prior to inoculation and the improved environment also allows for better post-inoculation conditions, and symptom expression.

Humidity

The water-baths, though able to produce the required level of humidity, initially proved not to be very reliable. Often, the thermostat malfunctioned, causing the heating elements to remain either continuously on or off. In addition, it was observed that the plants, placed directly above the water baths, became dry when most of the adjacent plants had moist leaves. Nevertheless, when the water baths functioned properly, they worked well in conjunction with the AC.

In an effort to improve their reliability, the heating elements and the thermostats were completely upgraded with help from the Electronics Workshop, UWI. To solve the problem of dessication of plants above the water-bath, frames with slatted wood tops were built around each water bath to force the steam to disperse sideways, and reduce convective air currents above the water baths.

Preliminary Experiment

A preliminary experiment was conducted to determine whether it is possible to induce symptoms after applying all of the aforementioned modifications to maintain relative humidity and modify the greenhouse temperature. A standard inoculum concentration of 350,000 basidiospores mL^{-1} (bs mL^{-1}) of *C. perniciosa* (Laker, 1987) was used. The inoculation technique and preparations prior to inoculation were the same as those used for inoculation of Series A-D.

Preparation of grafted plants for inoculation

Prior to inoculation, five plants per clone were selected from three resistant clones (IMC 3, IMC 6, and SCA 6) and three susceptible clones (AMELONADO, ICS 95 and M 8). Selected plants were heavily pruned and fertilised to stimulate the growth of new shoots. Daily observations were made for flushing and shoot development and inoculations were carried out when most of the plants showed signs of bud initiation. On the day before inoculation, shoots on each plant were counted and tagged to record their stage of development.

After tagging, the plants were watered adequately, placed on benches in a completely randomised design and left overnight in conditions of high relative humidity in the incubation room, until the time of inoculation.

Inoculation

Each plant was inoculated with 2 ml of spore suspension with a concentration of 350,000 bs ml^{-1}. The inoculum was prepared using 0.375 % agar to increase the viscosity of the suspension so that when applied, it would remain on the plant and not dry too quickly.

A hand-held atomiser was used to inoculate the plants in *lieu* of the yet to be completed atomiser spray system. Plants were incubated overnight inside the humidity chambers at 25°C after which they were kept in the greenhouse. Fortnightly observations for symptoms were made over a four-month period.

Symptom Evaluation

A rapid assessment of each inoculated plant was made at fortnightly intervals from two weeks after the date of inoculation. In addition to counting the number of swellings, details were noted as follows:

- **Swelling of terminal shoot**: diameters were measured of the stem of the swollen shoot as well as that of the non-swollen shoot below (as a control measurement)
- **Swelling of axillary shoots**: the diameter at the base of the swelling was measured and the diameter of the base of a normal axillary shoot was measured for comparison.
- **Swelling of stem**: the diameters of the swollen stem and the normal stem above and/or below the swollen area were measured.
- **Swelling of petiole**: the diameter of the swollen petioles and those of normal petioles above and below were measured.

Each type of symptom observed (whether on shoots, stems or petioles) was carefully recorded and labelled. In the case of shoot swellings, further observations were made to determine whether a broom developed and the characteristics of the brooms recorded (length, diameter, and number of active shoots, number of non-active shoots, length of largest shoot). Observed brooms were allowed to mature on the plant.

Results and Discussion

All clones developed symptoms after inoculation (Table 1), indicating that the experimental conditions promoted the germination of basidiospores and infection by *C. perniciosa*.

One month after inoculation, swellings and brooms developed on the inoculated clones. Symptoms were seen on both the susceptible clones (ICS 95, AMELONADO and M 8) as well as the resistant clones (SCA 6, IMC 3 and IMC 6), however it was possible to discern differences in the degree of severity of symptoms between resistant and susceptible clones.

While both resistant and susceptible clones produced brooms one month after inoculation, susceptible clones showed a higher frequency of broom production, ranging from 50.0 to 68.2 %, compared with 5.3 to 33.3 % for resistant clones. Conversely, a greater degree of swelling occurred on the resistant clones (10.5 to 33.3 %) than on the susceptible clones (9.1 to 20.0 %). At three months after inoculation, most of the swellings that were initially observed had developed into green brooms. At this time, the results indicated that the susceptible clones produced a greater percentage of brooms (50.0 to 72.7 %) than the resistant clones (26.3 to 44.4 %).

The fact that resistant clones developed symptoms when inoculated with 350,000 bs mL^{-1} of *C. perniciosa* could mean that this is too high a concentration of inoculum to best distinguish differences in the responses of resistant and susceptible clones. However, even with this inoculum concentration, susceptible clones developed more brooms than resistant clones, indicating that the resistant clones were able to slow down or suppress symptom development. This interpretation is also supported by the fact that one month after inoculation, there were more swellings than brooms on the resistant clones, and it took three

months for most of these swellings to develop into brooms. Conversely, broom development was rapid in the susceptible clones, occurring within the first month after inoculation, so that the change in symptoms between one and three months was not as great as it was in the resistant clones.

Further experiments are necessary, however, before firm conclusions can be made.

Table 1. The response of six clones of *T. cacao* inoculated with *C. perniciosa* at 350,000 bs mL^{-1}.

Clone	Resistance status	Swelling Frequency[1] (1 month) (%)	Broom Frequency[2] (1month) (%)	Broom Frequency[2] (3 months) (%)
IMC 3	R	2/8 (25.0)	1/8 (12.5)	3/8 (37.5)
IMC 6	R	3/9 (33.3)	3/9 (33.3)	4/9 (44.4)
SCA 6	R	2/19 (10.5)	1/19 (5.3)	5/19 (26.3)
AMELONADO	S	1/10 (10.0)	5/10 (50.0)	6/10 (60.0)
ICS 95	S	2/10 (20.0)	2/10 (25.0)	5/10 (50.0)
M 8	S	2/22 (9.1)	15/22 (68.2)	16/22 (72.7)

R = resistant S = susceptible

[1]Number of shoots showing swelling out of the total number of shoots tagged

[2] Number of brooms developed out of total number of shoots tagged.

Future direction

There is still a need to determine the optimal concentration needed to carry out future mass screenings, so further preliminary experiments are planned to fulfil the objectives outlined below:

- To compare different concentrations of inoculum and different host plant stages, to pin-point conditions that would best discriminate between resistant and susceptible material.
- To identify symptoms that best describe the degree of susceptibility.
- To document the symptom response of cacao clones which are known to be resistant and susceptible to Witches' Broom disease based on field observations.

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