Budwood Deterioration and Germplasm Transfer in *Theobroma cacao*[1]

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ABSTRACT

Propagation failures at the cacao quarantine facility in Miami prompted study to determine the causes. Fungi were isolates from budsticks from CATIE, Turrialba, Costa Rica. Various treatments of budsticks were applied at CATIE and Miami, and buds were placed in rootstocks in Miami, CATIE, and Gainesville. A low percentage of buds survived in Miami and Gainesville for two months, and only 22% survived at CATIE. The five most common fungi associated with cacao budsticks were: *Botryodiplodia, Theobromae, Fusarium decemcellulare, Fusarium oxysporum, Pestalotiopsis* ssp., and *Phomopsis* spp. Certain fungi were found to be residents of the woody vascular cylinder of apparently healthy cacao. Fungi within and on cacao budsticks become active as a result of moisture loss by budsticks following their removal from donor trees. In as few as two days, certain fungi invaded the woody vascular cylinder. Moisture loss from budsticks can be reduced or delayed by applying molten paraffin to the ends of budsticks and to the petiole stubs if they remain attached. Petioles should be cut to remove leaves 10-15 days before removing budsticks from donor trees. If patch or chip buds cannot be used, the side veneer graft resulted in high survival of germplasm.

COMPENDIO

Las fallas en las labores de propagación, ocurridas en la estación de cuarentena de cacao localizada en Miami, Florida, indujeron a estudiar las causas de tales fallas. Para ello, se aislaron algunos hongos obtenidos de estacas procedentes del CATIE, Turrialba, Costa Rica. Se establecieron varios tratamientos experimentales tanto en CATIE como en Miami; se colocaron yemas en portainjertos en CATIE, Miami y Gainesville, Florida. Un bajo porcentaje de yemas sobrevivieron en Miami y en Gainesville y solamente el 22% en CATIE. Los cinco hongos más frecuentemente asociados con yemas injertadas fueron: *Botryodiplodia, Theobromae, Fusarium decemcellulare. Fusarium oxysporum, Pestalotiopsis* sp., y *Phomopsis* sp. Algunos hongos estaban localizados en el cilindro vascular leñoso y en el portainjerto se reactivan como resultado de la pérdida de humedad de las estacas portainjertos, al ser separadas de los árboles donantes. En períodos tan cortos como dos días, algunos hongos invadieron el cilindro vascular leñoso. La pérdida de humedad en el portainjerto puede ser reducida o demorada con la aplicación de parafina derretida a los cortes del portainjerto y a las porciones de los peciolos que pudieran haber quedado adheridos; estos debieran ser podados para remover las hojas, 10 ó 15 días antes de cortar las estacas portainjertos de los árboles donantes. Si no se pueden utilizar yemas en injertos de escudete o bien en injertos de aproximación, se comprobó que el injerto de cuña lateral fue eficaz en preservar el germoplasma deseable de cacao.

INTRODUCTION

Germplasm of *Theobroma cacao* L. collected in its center of origin, the Amazon Basin, is moved to quarantine or to germplasm collections where it is propagated by budding or grafting for conservation and eventual use for cacao improvement. Clonal accessions are transferred by air shipment to quarantine facilities as budsticks, 20-30 cm, that are hardened stem pieces with stem diameters of 0.5-1.0 cm with dormant buds in leaf axils [Plate 1, (4)].

Guidelines for the safe movement of cacao germplasm have been published (2). Following arrival at the destination, propagation of the germplasm is by grafting or budding to a rootstock plant. Thus, propagation of cacao germplasm from its collection in the wild, entry into quarantine, establishment in germplasm collections, and its increase for various purposes is dependent on vegetative propagation by budding or grafting. Occasionally clones of cacao have been moved to quarantine as rooted plants (1), but this practice is hazardous from the standpoint of the movement of pathogens, and is discouraged for transfer of germplasm (2).

Low percentages (5%) of survival of cacao clones from Ecuador, after attempted propagation in England, caused serious concern (1). Likewise, germplasm received at the cacao quarantine facility of the United States Department of Agriculture, Subtropical Horticulture Research Station, Miami, Florida, also had low rates of survival (personal communication from P.K. Soderholm). Budsticks received appeared healthy, and were grafted onto rootstock plants of various age and in various growth stages. Connection of grafts with rootstocks appeared to take place, but often the entire graft died and fungal growth developed on its surface, indicating that fungi were associated in some way with the failures of propagation. According to Wellman (9), there are more than 100 pathogens associated with diseases or disorders of cacao, but deterioration of cacao budwood was not included. Purdy and Soderholm (3) reported that five fungal species were associated with budwood that failed to become established on rootstock plants. The fungi they mentioned were also among the more than

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80 fungi associated with cacao that exhibited die-back symptoms (8). Turner described the many stresses to which cacao is subjected that are in some way related to die-back, and concluded that it is not so much the resistance of the trees to fungal infection but resistance to those conditions under which they (the trees) become susceptible and predisposed to attack by fungi. Cacao die-back is a stress-related syndrome resulting from various stresses, and with which many different fungi are associated.

Cacao germplasm is transferred a) from natural habitats to a collection site, b) from germplasm collections to quarantine, and c) from quarantine to various sites for reproduction. Local transfer of clonal plant materials for vegetative reproduction also falls under the broad definition of germplasm transfer, but most often the removal from donor trees to grafting or budding to rootstocks is minimal, usually the same day (4).

Soria (7) cited the short viability of budwood as the "greatest difficulty" associated with germplasm collection, because budwood may die if too much time elapses between its collection and application to a rootstock plant. When budsticks are removed from donor trees they immediately beging to lose moisture from the exposed cut surfaces. This is compounded by the removal of leaves resulting in moisture loss through the petiole stubs that are attached to the budstick. If the cut ends of budsticks are dipped into molten paraffin, moisture loss may be slowed. Nevertheless, when budsticks are removed from the donor trees they are subjected to the most serious moisture stress possible, yet the budwood is expected to survive.

Budwood deterioration, like cacao die-back, appears to be a stress-related syndrome, and stresses to which budwood are subjected seem to predispose it to fungi that destroy its viability. Purdy and Soderholm (3) demonstrated that species in five genera of fungi were associated with dying or dead budwood. In research reported here, additional fungi have been associated with cacao budwood, and procedures to prolong budwood viability and increase propagation success are presented.

MATERIALS AND METHODS

Experiments to identify the cause of budwood deterioration included treatments applied at CATIE, Turrialba, Costa Rica, and at the U.S. Department of Agriculture, Subtropical Horticulture Research Station, Miami, Florida. For experiment 1, treatments were: a) Benlate + Dithane M–45 (1:10) applied to budsticks as an undiluted dust; b) Kocide 101 applied undiluted as a dust; c) an antisenescent chemical (aminooxy acetic acid, 0.01 M and 0.001 M) in which budsticks were soaked for 30 minutes. Non-treated UF 29 was the control.

For experiment 2, Benlate + Dithane M–45 was applied as in experiment 1. Bayleton was applied to budsticks as a dust. Budsticks were soaked for 30 minutes in an antisenescent growth-promoting mixture consisting of 50 mg indole butyric acid; 25 mg 1–phenyl–3–methyl–5–pyrazolone; 10 mg streptomycin sulfate; 50 g sucrose; 50 mg spermine, dissolved in one liter water. An additional treatment included in experiment 2 was girdling of budwood five and 15 days before its removal from the donor tree. Budwood of SCA 6, SCA 12, and UF 29 were included in experiment 2 as controls.

Hardened budsticks of UF 29 were cut from trees in the field at CATIE, leaf petioles were cut about 0.5 cm from the stem, and treatments were applied in the field immediately, and after one hour in the laboratory. One set of budwood was carried to Miami as cabin baggage and the treatments were applied at the cacao quarantine facility in Miami. In addition, a duplicate set of untreated budwood was mailed to Miami, and treatments were applied after its arrival. Ends of budsticks were dipped in a mixture of molten paraffin and bee's wax, wrapped in damp newspaper, and placed in a plastic bag for transport to Miami. Budwood that remained at CATIE was treated, wrapped in newspaper, and placed on the laboratory bench for seven days. Propagation of treated budwood at CATIE was by patch budding and grafting, whereas at Miami only a wide-angle approach graft was used.

Isolation of fungi associated with the budsticks was done a) in the field immediately after treatments were applied, b) in the laboratory about one hour after treatment, c) in Miami, and d) in Gainvesville. Small pieces of budsticks were surface-sterilized by immersion in 70% ethyl alcohol for two minutes, followed immediately by immersion in 5% sodium hypochlorite for 10 minutes, and then the treated stem pieces were allowed to dry under sterile paper towels. One end of treated budsticks about 1-2 mm thick was removed, and then cross-sections (0.5 mm thick) of stems were cut and placed on 1.5% water agar slants in screw-top vials that were transported to Gainesville for identification of the fungi. Similar isolations were made in Miami.

The alcohol treatment was eliminated for subsequent experiments and budsticks were surface-sterilized by immersion in 10% chlorox for 10 minutes, and then rinsed twice in sterile deionized water. To determine if fungi reside in the woody vascular cylinder of cacao, budsticks were immersed in 10% chlorox for 10 minutes after which the soft cortical tissues were removed; the remaining woody vascular cylinder was immersed in 10% chlorox for

10 minutes, and then rinsed twice in sterile deionized water. One end of surface-sterilized budsticks, or woody vascular cylinders, was removed and thin cross-sections were then cut and placed on the isolation medium. Three or more budsticks of each treatment, or clone, were sampled by placing three to four cross-sections on the isolation medium. There were five replicates for each budstick.

Isolation media were 1.5% water agar, and a selective medium of potato dextrose agar, streptomycin sulfate, tergitol NP–10, and chlorotetracycline (PDASTC). Fungi that grew from the tissue pieces were transferred to PDA for later identification.

The bark of budsticks several days old adhered tightly to the wood and patch buds could not be used in Miami, so the angled approach graft was used in experiments 1 and 2. The advantage of a side veneer graft (10) over the angled approach graft was demonstrated and the technique was adopted. Initially, side veneer grafts were wrapped in clear plastic grafting tape, but emerging buds had difficulty in growing if the tape was not removed from directly over the bud. This problem was solved by using budding rubbers to wrap the grafted stem piece, making certain that the bud was not covered by the budding rubber. The rubber-wrapped graft was then covered with tightly-wound parafilm that deteriorated in about two to three weeks so that the bud could emerge and begin growth. The budding rubber was removed later.

Propagation attempts in Miami with budsticks with origins in the vicinity of Belem, Brazil, Apartado, Colombia, and Port of Spain, Trinidad resulted in only a 10% success rate. Isolations as described were made from these budsticks and from budsticks of nine clones in Gainesville from the cacao germplasm collection at Mayaguez, Puerto Rico.

To determine if certain fungi isolated from budwood could invade the vascular cylinder of cacao, sections of cacao stems from trees growing in a greenhouse in Gainesville, with the leaf petiole cut about 0.25 to 0.5 cm from the stem, were inoculated with five fungi isolated from cacao budwood. The cacao stem pieces were placed in paper troughs and covered with suspensions of spores and mycelium of *Botryodiplodia theobromae, Fusarium decemcellulare, Fusarium oxysporum, Pestalotiopsis* spp., and *Phomopsis* spp. that had grown on PDA. After 20 minutes, the stem pieces were removed from the suspensions and wrapped immediately in moist sterile paper towels, placed in a plastic bag, and kept on the laboratory bench at 22-25°C. Isolation was after 0, 2, 4, 8, and 16 days on PDASTIC from non-surface-sterilized stems, surface-sterilized stems with the cortex in place, and twice-sterilized stems with the cortex removed.

RESULTS

All budwood in experiment 1 grafted in Miami failed to survive, whereas 22% survived in Turrialba. Also, 73% of patch buds from budwood in experiment 1 survived in Turrialba. Patch buds were not used in Miami. There seemed to be greater survival with the application of Benlate + Dithane M–45 and girdling five or 15 days before removal of budwood from the tree. But only 12% of grafts in Miami and 10% of those in Gainesville survived for only two months. No treatment was any better than any other with respect to survival of budwood grafted in Miami. Undiluted Kocide 101 applied to budsticks as a dust was phytotoxic.

From the dead and dying grafts in experiment 1 and 2, fungi in 15 genera were isolated, and in descending order of frequency of isolation they were:

Pestalotiopsis
Phomopsis
Fusarium
Botryodiplodia
Paecilomyces
Verticillium
Anthromyces
Trichoderma
Colletotrichum
Torula
Gleocladium
Gleosporium
Stilbum
Stachybotrys
Sphaeropsis

Botryodiplodia isolates fit the description of *B. theobromae*, and several isolates of *Fusarium* were identified to species: *F. decemcellulare, F. graminearum, F. moniliformae, F. oxysporum, F. subglutinans*, and *F. semitectorum*.

A total of 60 budwood pieces of four clones received in Miami from the vicinity of Belem, Brazil were sampled for fungi. The most frequently isolated fungus was *Botryodiplodia theobromae* from 31 budsticks pieces, followed by *Fusarium* spp. from 21, *Diplodia* spp. from nine, *Phomopsis* spp. from three, and *Aspergillus* spp., *Pestalotiopsis* spp., and *Trichoderma* spp. each from one budstick. All budsticks were very dry, and were rotted badly on the ends and nodes. Nodal rot presumably developed through the basal portions of the petioles that were attached to the budwood when it was packed for shipment.

Eight TSH clones from Trinidad to Miami failed to survive following grafting to rootstock plants. Almost pure cultures of *Botryodiplodia theobromae* developed from "wood only," chlorox, and nonsterilized treatments of four clones (TSB 654, TSH 728, TSH 1082, and TSH 1188). The other four clones developed no growth from the "wood only" treatment, but several unidentified fungi developed from the other treatments.

Botryodiplodia theobromae was isolated 32 times, and *Fusarium* spp. were isolated seven times from the

woody vascular cylinder from budsticks of five clones collected at the Tulenapa Experiment Station of the Instituto Colombiano Agropecuario near Apartado, Colombia. Other fungi isolated from cross sections that retained cortical tissues were *Gleosporium* spp., *Virgrospora* spp., and *Trichoderma* spp.

Budsticks of nine cacao clones in the Cacao Germplasm Collection in Mayaguez, Puerto Rico were transported to Gainesville where species in 12 genera of fungi were isolated from nonsterilized, surface-sterilized with cortex, and surface-sterilized after the cortex was removed. *Pestalotiopsis* spp. was isolated 11 times from woody vascular cylinders, 33 times from the surface-sterilized treatment with the cortex in place, and only nine times from nonsterilized budwood. In contrast to the fungal residents of budwood from other locations, *Botryodiplodia theobromae* was isolated two times each from woody vascular cylinders and the surface-sterilized treatments, and once from nonsterilized budwood. Fungal genera in descending frequencies of isolation were: *Pestalotiopsis*, *Trichoderma*, *Fusarium*, *Rhizopus*, *Penicillium*, *Aspergillus*, *Cephlosporium*, *Botryodiplodia*, *Phoma*, *Phomopsis*, and *Nigrospora*.

Four of the five fungi applied to stem pieces of greenhouse-grown cacao trees invaded the woody vascular cylinder in only four days after inoculation. These fungi, *B. theobromae*, *F. Decemcellulare*, *F. oxysporum*, and *Phomopsis* spp., were also isolated after eight and 16 days. However, it is most interesting that *Pestalotiopsis* spp. did not invade the vascular cylinder even after 14 days, but as with the other four fungi, it was isolated from surface sterilized budwood with the cortex in place after only two days. There were no fungi isolated from the woody vascular cylinder of the controls, but nine common airborne fungi were isolated from the surface-sterilized controls with the cortex in place, and from the nonsterilized treatment.

In a comparison of propagation methods, almost 100% successful propagation resulted from patch buds, chip buds, approach grafts, and side veneer grafts, when the budwood was removed from the donor tree and immediately applied to a flushing rootstock plant. Budwood used in the comparison was green, green/brown, and brown, with brown being hardened portions of stems. In addition, buds of grafts wrapped with plastic grafting tape, or budding rubbers and covered with parafilm, had equally high rates of survival.

DISCUSSION

The low survival rate of cacao germplasm following attempts to propagate it at shipment destinations has resulted in considerable concern about germplasm conservation and utilization. There may be several reasons why propagation failed, but it was logical to consider fungi as causal agents because of their association with dead or dying buds and grafts. There were many fungi associated with propagation failures. Several fungi have been isolated from the woody vascular cylinders of cacao budsticks, and seemingly they are residents in or on apparently healthy budsticks selected for germplasm transfer. Two hypotheses are proposed: a) that there are fungal residents of healthy cacao stems, and b) that if these fungi are not residents of healthy cacao stems, then they have the capability to invade the wood of cacao stems in as few as two days after the stem (budstick) is removed from the tree.

Fungicides applied to budsticks after their removal from trees failed to increase survival. Similar observations were made by Allen (1) regarding applications of fungicides to budsticks and, in addition, he stated that fungicides may even reduce budwood viability. There may be benefit from girdling of budsticks before removing them from trees, but results presented here are inconclusive.

Fungi isolated from cacao stems or cacao budsticks are saprophytes, or weak parasites, that flourish on almost any substrate. In association with a growing plant, they may be surface contaminants that fail to induce a response from the plant on which they reside. Also, they may invade a plant through various openings, but again they may fail to induce a response from the invaded plant. However, the plant with these resident fungi becomes symptomatic rapidly when the fungi are stimulated into rapid growth by some change in the host plant, such as the removal of the budstick from the donor tree.

Time and location may influence the predominating fungal species associated with cacao. For example, almost pure cultures of *Botryodiplodia theobromae* were isolated from the woody vascular cylinder of cacao from Trinidad, and this species was almost common in budwood from Belem and Apartado, whereas *Pestalotiopsis* spp. was most common in budwood from Costa Rica.

The most serious water stress begins with the cutting of budsticks from the tree, and is compounded further by the cutting of the leaf petioles somewhere near the basal pulvinus. These cut surfaces lose moisture rapidly and probably never heal to form a barrier to moisture loss. It is a recommended practice that the cut ends of budsticks be dipped into molten paraffin to retard moisture loss (2). However, there are no procedures used to retard or reduce loss of moisture through cut petioles. Moisture loss, by whatever means it occurs, obviously affects the physiological status of the budstick, either through a direct influence on fungi present in or on budsticks,

or by an affect on the budwood *per se*. The resulting interaction between the saprophytic fungi that are facultative parasites and the cacao budwood results in loss of budwood viability. This pathological condition occurs if a budstick is stressed by loss of moisture, but the condition is avoided if the moisture stress in insufficient to trigger an increased activity by the resident fungi. This latter situation apparently occurs if buds or grafts are placed in rootstocks during the same day that budsticks are removed from donor trees (4).

All of the fungi observed to be associated with cacao budwood and its loss of viability were also reported to be associated with cacao dieback (8). Turner discussed the various stresses that induce the dieback syndrome, and water stress may be most important. Likewise, the most important stress associated with budwood failures is loss of water from budsticks.

To transfer cacao germplasm, apparently healthy budsticks are selected from an apparently healthy tree. Certain procedures may prolong viability of cacao budwood so that germplasm transfer can be completed as a result of its successful propagation. It may be beneficial if budsticks are girdled 10-15 days before the budwood is removed from the donor tree, although Soria (5, 6) stated otherwise. A. J. Kennedy, formerly Head, Cocoa Research Unit, University of the West Indies, St. Augustine, Trinidad (personal communication), pointed out that leaf petioles cut 10-15 days before removal of budsticks induced natural abscision of the petiole stub and development of a natural scar that prevents moisture loss at the point of leaf attachment. Also, the stub of the petiole when attached to the stem becomes a substrate for fungi on the surface of the stem, and it could provide nutrients for fungi within the stem. When wrapped with plastic grafting tape, parafilm, or other water-holding material, a moist chamber is created, within which fungi that normally do not affect cacao flourish, enter the axillary bud, and destroy it.

Several methods of budding or grafting offer reasonable potential for success providing that the time from removal of the budstick from the donor tree to its application to a rootstock plant is as short as possible. The most effective grafting technique establishes maximal contact between the cambium of the rootstock and the cambium of the scion. The side veneer graft provides this relationship. Regardless of the budding or grafting method, only vigorous healthy rootstock plants should be used (4).

For cacao germplasm collected in its center of origin, budsticks must be removed from a tree when it is encountered, so any pre-removal practice cannot be used. Evertything possible should be done to reduce moisture loss from budsticks, such as dipping cut stem ends and cut ends of petiole stubs into molten paraffin. It is important to reduce to a minimum the number of days from removing budsticks from donor trees to grafting or budding to a rootstock plant by arranging in advance the most rapid transport channels possible (6).

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El Género *Theobroma* en el Territorio Federal Amazonas (Venezuela). I. Notas Etnobotánicas y Consideraciones Agronómicas[1]

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ABSTRACT

In order to assess the agronomic potential of *Theobroma* species in the Brazilian Amazonas territory, we collected live plants and botanical samples of cacao (*Theobroma cacao*), copo-azú (*T. grandiflorum*), montero cacao (*T. subincanum*), himare (*T. bicolor*), and kayani (*T. af gileri*). In each case, indigenous names and uses were recorded. Improved agronomic and phytosanitary practices for the commercially exploitable cacao species in the region are suggested, in view of forthcoming projects. Recommendations are also given for more efficient use of the resources involved.

COMPENDIO

A los fines de conocer el potencial agronómico de especies del género *Theobroma* en el Territorio Federal Amazonas, se colectaron muestras vivas y/o botánicas de cacao (*Theobroma cacao*), copo-azú (*Theobroma grandiflorum*), cacao montero (*Theobroma subincanum*), himare (*Theobroma bicolor*) y kyani (*Theobroma af gileri*). Para cada caso se especificó los usos y nombres autóctonos asignados por cada etnia indígena en particular. Para cacao, que es la especie del género explotada comercialmente, se presentan algunas consideraciones agronómicas y fitosanitarias, orientadas a mejorar las actividades de los programas cacaoteros de la región. Finalmente, se aportan sugerencias respecto al buen uso de los recursos en beneficio del hombre y su entorno.

INTRODUCCION

En su totalidad el gran bosque Amazónico es el más extenso del mundo (10), en nuestro caso, el área referida se ubica al sur del río Orinoco y tiene una superficie de 180 000 km^2 (15), presentando extensos sistemas montañosos. En razón a su ubicación, configuración espacial, distribución geográfica y las características culturales, sociales y económicas de sus pobladores, se han creado espectativas estadales que han generado proposiciones de actividades con miras a su futuro desarrollo. Esta actitud ha generado comentarios polarizados, destacando la fragilidad del ecosistema y la necesidad de formular estrategias claras e integrales con carácter participativo de las instituciones competentes.

Respecto al género *Theobroma*, se sabe que es de origen tropical (2, 3, 4, 5, 7, 13, 14, 16, 17) distribuido entre los 18° de latitud norte y 15° de latitud sur, restringido a zonas de alta precipitación y ubicado en el estrato medio de los bosques húmedos tropicales que mantienen una vegetación siempre verde. Algunos trabajos (2, 3, 4, 5, 6, 7, 12, 17) señalan como posible centro de origen de este género las cuencas de los ríos Amazonas y Orinoco.

En el caso específico del Territorio Federal Amazonas, Venezuela, se evidencia que hasta la fecha se

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