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FIRST REPORT IN CUBA OF GREEN POINT GALL OF COCOA CUSHION CAUSED BY *ALBONECTRIA RIGIDIUSCULA* (*FUSARIUM DECEMCELLULARE*)

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ABSTRACT

Galls and witches broom like symptoms were found in trees in a small house backyard of Cienfuegos city, Cienfuegos province and in two cacao farms in Guantanamo province, during surveys on cacao diseases carried out in plantations in the central and west cacao producing areas of Cuba,. Field symptoms consisted in a disorganized meristem growth in plant branches and stem cushions which develop as a super production of vegetative primordia developing to vegetative gnarl galls as small green points cushion or to an uncontrolled production of flowers in the gall. Affected cushions can stay as green point galls or eventually developed to shoots with some 15-20 cm length that finally die and dry, with an occasional white-grayish fungal mycelia growth on the surface. Samples of tissues incubated in humid chambers and isolations from cushion galls tissues in PDA and CLA agar uniformly yielded a Fusarium *sp. with conspicuous macroconidia developed from sporodochia, ovoid microconidia developed in chains from long phialides and colonies which yielded develop an intense and conspicuous rose pigment. Symptoms and isolates fungal morphology agree with description for* Albonectria rigidiuscula*, causal agent of cacao cushion gall disease. This is the first report of the disease in Cuba. A discussion on the life cycle and preliminary ways of management is carried out.*

Key words: *cacao cushion gall, buba, Albonectria rigidiuscula, Fusarium decemcellulare, cacao*

RESUMEN

Durante el desarrollo de una encuesta de enfermedades en cacao en la región central y oriental de Cuba se encontraron síntomas de agallas similares a escobas de brujas en árboles de un patio de una casa en la ciudad de Cienfuegos, provincia del mismo nombre, y en dos fincas cacaoteras de la provincia de Guantánamo. Los síntomas consistían en un crecimiento desorganizado del meristemo de las yemas de ramas y tallos en forma de una superproducción de primordios vegetativos que se desarrollan como una agalla de pequeños puntos verdes, o como una producción descontrolada de flores en la agalla. Los cojinetes afectados pueden permanecer como pequeñas agallas de punto verde o eventualmente desarrollar brotes de unos 15-20 cm de largo que finalmente mueren y se secan, y que desarrollan ocasionalmente un micelio blanco grisáceo en su superficie. De las muestras de tejido incubadas en cámaras húmedas y de los aislamientos realizados de las agallas de cojinete, en PDA y CLA agar, se desarrolló una especie de Fusarium *con macroconidios característicos que se desarrollan sobre esporodoquios, cadenas de microconidios ovoides sobre fiálides largas y colonias con un intenso pigmento rosa característico. Los síntomas y la morfología de los aislamientos concuerdan con la descripción de* Albonectria rigidiuscula *agente causal del la enfermedad de la agalla del cojinete del cacao. Este es el primer informe de la enfermedad en Cuba. Se realiza una discusión sobre el ciclo de vida y las vías preliminares del manejo de la enfermedad.*

Palabras claves: *agalla del cojinete del cacao, Albonectria rigidiuscula, Fusarium decemcellulare, cacao*

INTRODUCTION

According to FAO statistics [FAOStat, 2010], there were in Cuba in 2010, 8900 ha of cacao (*Theobroma cacao)* with a total production of 1709 ton, distributed in 16 municipalities of Guantanamo, Santiago de Cuba, Holguín, Granma and Sancti Spíritus provinces [Ministry of Agriculture, Cuba 2010, unpublished data].

During national 2009-2011 cacao diseases surveys, cushion galls and witches broom like symptoms were found in cacao trees in a house backyard in Cienfuegos city, Cienfuegos province, and in two farms La Guayaba farm belonging to the UBPC 'Héctor Grimón Gainza' in Baracoa and in CPA 'Abel Santamaría' in

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Lagunita, Caujerí valley respectively, both in Guantánamo province.

Present paper describes the procedures carried out to establish the etiology, and reports the presence in Cuba of cushion gall disease and symptoms it causes.

MATERIALS AND METHODS

Galls and witches broom like symptoms found in affected cacao trees of the localities already mentioned, they were photographed in the field and samples were collected taking part of branches with symptoms. In the case of large branches or stems, samplings were carried out cutting out the gall and part of the bark under the cortex which were placed in bags. All samples were sent to the Central Plant Quarantine Laboratory in Havana for description and analysis.

Symptoms were divided in two groups a) green points or buba alike galls symptoms and b) short outgrowth shoots resembling small witches broom symptoms.

Fungal outgrowths were directly transferred to agar. Pieces of branches with galls tissues were disinfected with 3 % $NaClO_3$, placed in humid chambers plated on water agar plus 100 µg/mL chloramphenicol, and incubated at 28 °C for seven days in darkness. Fungal growths obtained from humid chambers and water agar plates, were transferred to PDA to determine morphology of colonies and diffusible pigments, and to carnation leaf agar (CLA) to observe conidiogenesis and conidia morphology. Microscopic slides with lactophenol cotton blue were prepared from colonies in PDA and CLA and observed in phase contrast and direct transmission with an Axioscop 40 (Carl Zeiss) microscope, to observe conidiogenic structures and to describe, measure and photograph phialides, macro and microconidia. All characteristics observed were compared with descriptions reported by Booth and Waterson (1964), Booth (1971) and Leslie and Summerell (2006).

RESULTS AND DISCUSSION

Symptoms. Field symptoms consisted in a disorganized meristem growth in cacao plant branches and stem cushions with a vegetative and flower super production of primordia which developed to vegetative gnarl galls as small green points cushion balls (*Fig. 1*), to some caulinar witches broom like offshoots (*Fig*. 2 A to C) or to an uncontrolled production of flowers in the gall (*Fig. 3*). Symptoms in trees started as individual apparition of multiple shoots in isolated cushions of a branch or a trunk and as disease progress, the presence of affected cushions in branches and trunks is more frequent. Affected cushions can stay as green point galls or eventually developed to short shoots with about15-20 cm length that finally die and dry (*Fig*. 2 C), with an occasional white-grayish fungal mycelia growth on the surface.

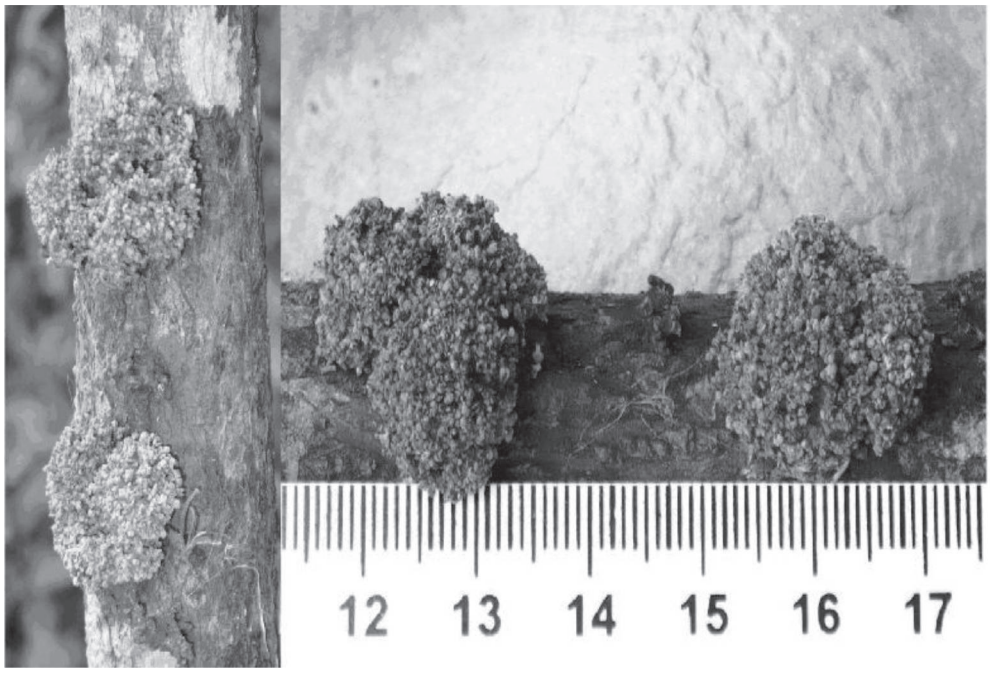

Figure 1. Green point cushion galls or buba in cacao main stem and branch.

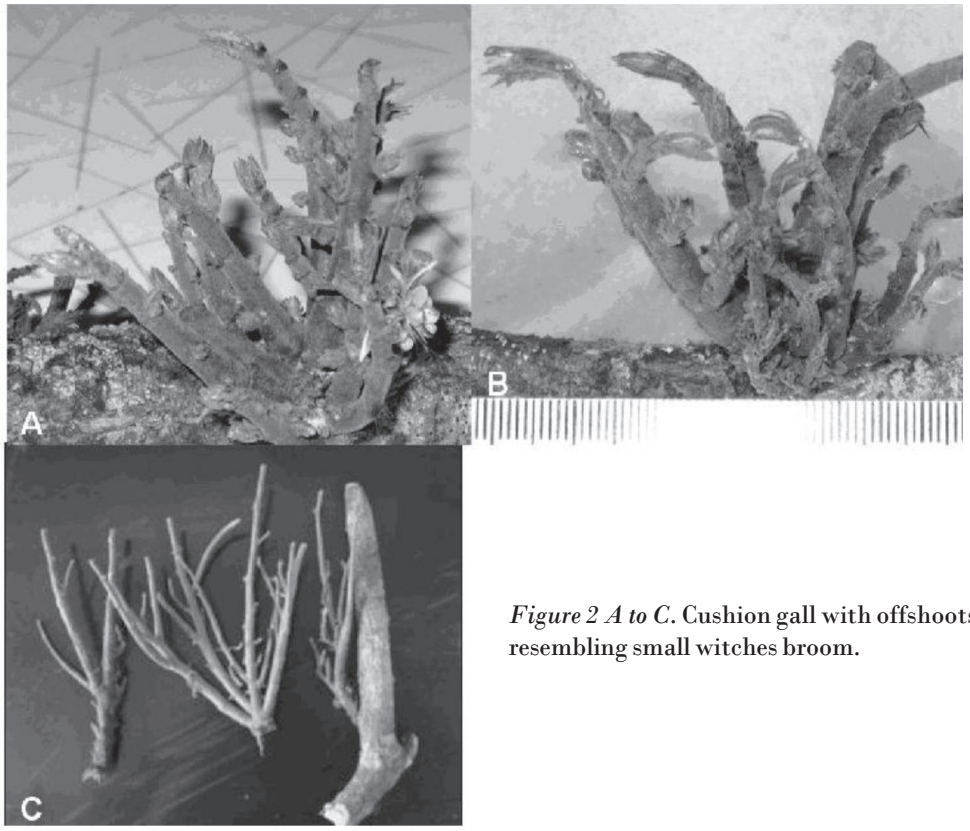

Figure 2 A to C. Cushion gall with offshoots growth resembling small witches broom.

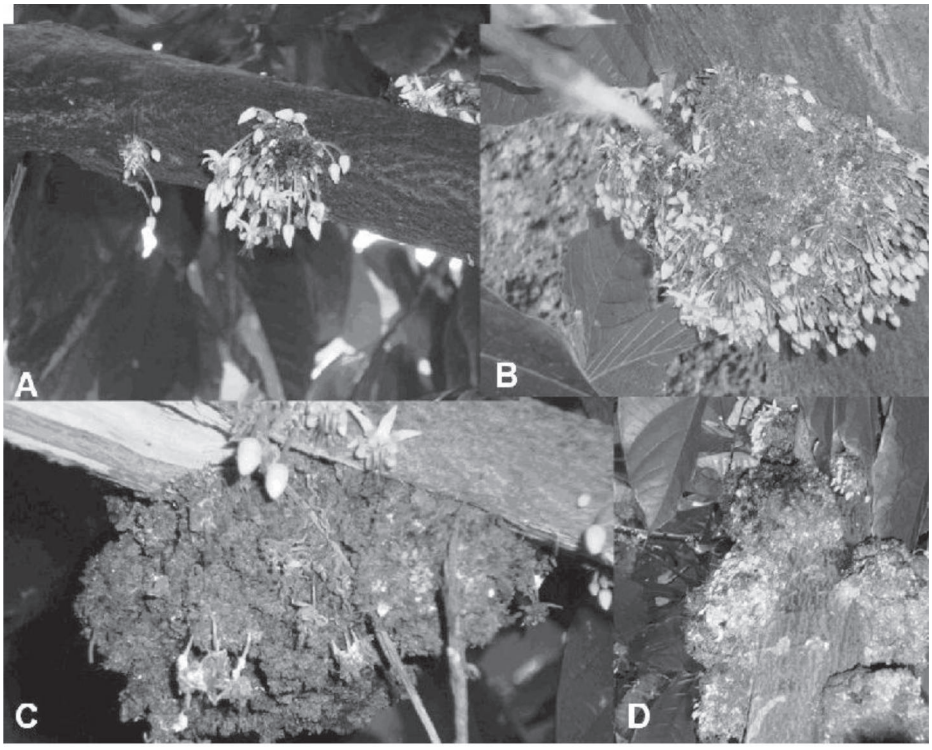

Figure 3. Flowered cushion galls. *A. rigidiuscula* outgrowth in the surface.

Fungal structures. From cushion galls, superficial witches broom like symptoms and agar plates, were obtained an uniformly and consistently growths a *Fusarium* sp. with conspicuous macroconidia developed from sporodochia and monophialides, tubular in shape, with straight and curved thick walls in both sides of the conidia, 3-9 septa (more frequently 7-8 septa), with a hooked and rounded apical cell and a footed basal cell, 27-77 x 3-5 µm (*Fig. 4 A*). Microconidia develop in chains from monophialides in simple and ramified conidiophores (23-40 µm), oval, 0-1 septa, 6-16 x 3-5 µm, with a plain papilae in the basal cell, (*Fig. 4 B-C-D*). Chlamydospores absent. Colonies on PDA are slow growing, with white cream mycelia firstly and after powdery with an intense rose pigment and abundant production of phialides and microconidia (*Fig.* 5).

Symptoms and fungus morphology are coincident with descriptions of cushion gall disease of cacao [Brunt and Wharton, 1962; Booth and Waterston, 1964; Ploetz, 2006; Leslie and Summerell, 2006] and its causal agent *Fusarium decemcellulare* Brick 1908, anamorph of *Albonectria rigidiuscula* (Berk & Broom) Rossman & Samuels 1999, basyonim: *Nectria rigidiuscula* Berk & Broome (1875). This disease was firstly reported in British Guyana in 1905 [Ploetz, 2006]. Since this initial report to 1959 it was considered be caused by different agents until Brunt and Wharton (1962) when they inoculated buds in Ghana, shown that *F. decemcellulare* was the causal agent of cacao cushion gall and that disease was not systemic. Although these authors used the teleomoph name *Calonectria rigidiuscula* they only report the work with the anamorph stage *F. decemcellulare*.

Cushion gall is considered a minor disease in most countries. However, epidemics outbreaks have been reported in Nicaragua and Costa Rica with a 50-70 % incidence of infected trees [Kevorkian, 1951].

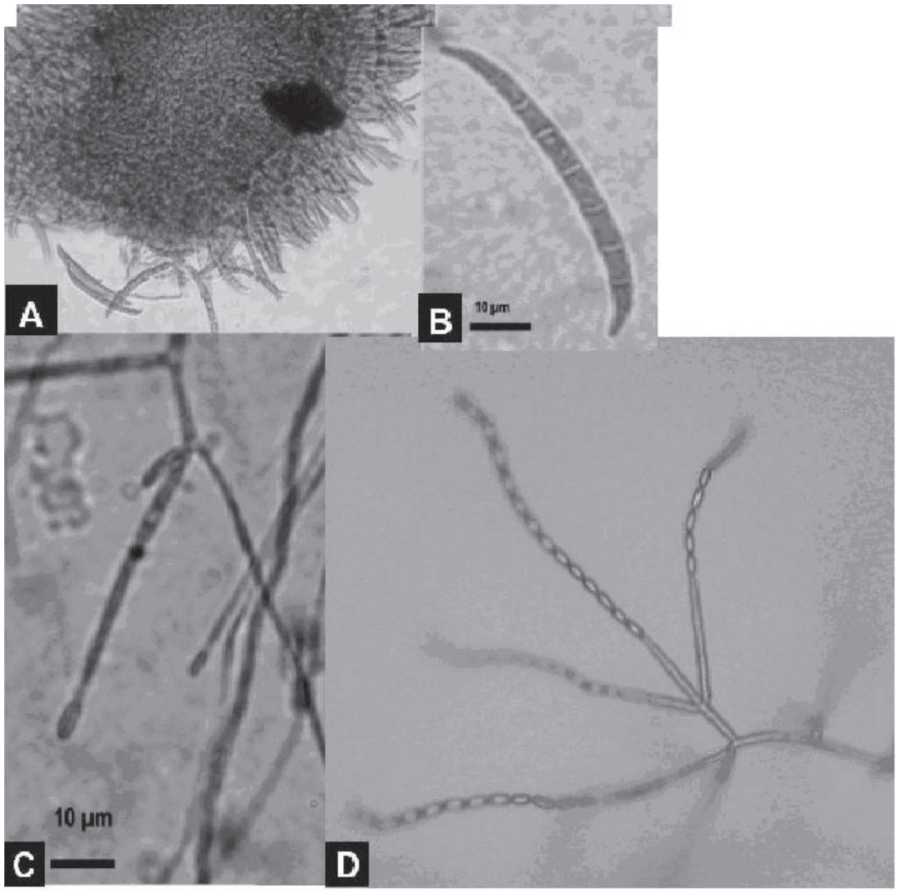

Figure 4. Morphology of anamorph stage *F. decemcellulare*: A) Sporodochia and macroconidia; B) Macroconidia; C) Phialide and microconidia and D) Phialides and chains of microconidia

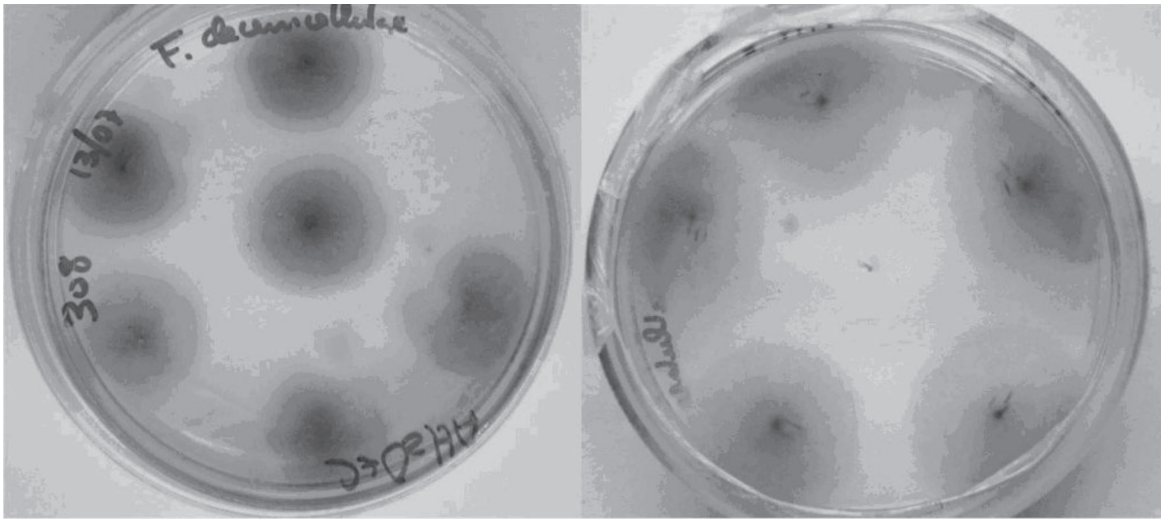

Figure 5. Colonies of *A. rigidiuscula*

Lasiodiplodia theobromae, (Pat.) Griffiths & Maubl was isolated from a high number of cushion galls especially from dry branches and stems similarly as reported by Brunt and Wharton (1962) and Ploetz (2006). As this fungus has a very fast growth in culture soon overgrowth the isolates of *F. decemcellulare*.

Cushion gall disease symptoms has been from time to time confused by cacao growers with those reported for witches broom disease caused by *Moniliophthora perniciosa* (Stahel) Aime (syn. *Crinipellis perniciosa* Stahel Singer) which is in the A1 list of Cuban quarantined pests and which its presence has not so far been confirmed in the country. In Cuba [Matos *et al.*, 2002; 2003], listed the presence of a witches broom disease in cacao in Mosquitero locality in Baracoa, but do not shown or reported its etiological agent. Cushion gall has been confused in many places with witches broom disease, because some galls develop short shoots 15-20 cm long in a witches broom like fashion that soon dry and die [Purdy, 1999; Ploetz, 2007]. A study commission for the improvement of cacao production in Haiti (SECID/Auburn University PLUS) reported that witches broom disease by *M. perniciosa* was not present, and that similar symptoms found in plantations belonged to cacao cushion gall caused by *Albonectria rigidiuscula* [Purdy, 1999]. *Moniliophthora perniciosa* causal agent of cacao witches broom disease is readily isolated of brooms produced in cacao branches, and commonly produce basidiocarps and basidiospores under adequate humidity conditions [Purdy and Dickstein, 1990; Purdy and Schdmidt, 1996], which can develop in pure cultures in appropriate culture media [Merchan, 1979]. Brooms caused by *M. perniciosa* can reach a great length; meanwhile those present in cacao plantations in Cuba dry and die after reach a maximum of 10-20 cm length. Samples collected in the field and kept in humid chambers neither developed basidiocarps of *M. perniciosa* or typical basidiomycete mycelia, basidiospores and clamidospores were obtained from isolates and cultures.

Fusarium decemcellulare had been previously reported in Cuba (as *Nectria rigidiuscula)* as an endophyte growing fungus on different forest trees in provinces of Havana, Matanzas and Granma [Castañeda and Rodríguez, 1998; Minter *et al.*, 2001]. The present article is the first report of cacao cushion gall disease caused by *Albonectria rigidiuscula* in Cuba.

A. rigidiuscula has been primarily reported as a saprophyte that lives in death cortex tissues and parasite wounds of weak trees that frequently develops following infections by *Phytophthora* sp., *L. theobromae*, other plant pathogens and insect damages [Crowdy, 1947; Owen, 1956]. The fungus can not only prevent healing of necrotic lesions caused by other plant pathogens, but also retain its viability in healed lesions caused by other agents from which it can produce new infections when environmental conditions are favorable [Booth and Waterston, 1964].

A. rigidiuscula incidence is higher during humid months of the year when the disease is also more evident. The fungus is disseminated through macroconidia produced on small sporodochia in died stalks and branches, microconidia developed from mycelia growing in

recently infected tissues and ascospores produced in fallen pods and died branches in decomposition in soil and has besides a saprophytic phase in soil [Booth and Waterston, 1964]. Ford *et al.* (1967), shown that galls inducing isolates, are heterotallic producing asci with four to eight ascospores, meanwhile those homothallic which produce perithecia from single ascospore cultures, do not induce the disease. Perithecia are produced in small groups of stromata in long time died branches, and emerge through cortex producing ascospores during the rainy season, which are disseminated by wind or rain drop splashes to other wounds or exposed tissues. The fungus can survive as vegetative mycelia in the cortex and edges of canker lesions of the host and as spores, fructification bodies and as saprophyte of cortex.

As *A. rigidiuscula* is a common endophyte in many plant species including fruit and forest trees of different families [Booth and Waterston, 1964] it probably has different physiological races, aspect that have to be confirmed in the future.

In sampled plantations cushion gall was only found in isolated trees under poor cultural management and growing conditions coincidently with previous reports [Kevorkian, 1951; Brunt and Wharton, 1962]; according to this, it is so far considered a minor disease of cacao in Cuba.

Preliminary considerations regarding management can be derived from the observations of affected fields and the reports on biological cycle of *A. rigidiuscula*: a) as disease is more common in stressed trees and insect damaged tissues [Kevorkian, 1951; Brunt and Wharton, 1962], improving growing conditions of cacao and reduction of insect damages by different ways is customary; b) frequent sanitation of galls by pruning e:to eliminating cortex down to cambium and at least 2.5 cm outside borders of galls; c) avoid pruning during the rainy season and favor exposed tissue healing by adequate nutrition and protection with a protectant fungicide.

CONCLUSIONS

- The present is the first report in Cuba of cacao cushion gall disease caused by *Albonectria rigidiuscula* (anamorph, *Fusarium decemcellulare*). The fungus had been previously reported as endophyte in forest trees in Cuba.
- The fungus induce in stressed trees in humid environments, vegetative and flower super production of primordia developing to vegetative gnarl galls as small green points cushion balls as well as witches broom like offshoots in cacao branches.
- The disease is associated to weak trees and due to its frequency is considered a minor cacao disease in Cuba
- The witches broom like offshoots symptoms previously associated by the growers to *Moniliophthora perniciosa* uniformly yielded *A. rigidiuscula*. Due to this and that never has been observed or isolated in culture *M. perniciosa*, witches broom disease is considered not to be present in Cuba.

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