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SOME DIFFERENCES IN ISOLATES OF THE BLACK POD DISEASE ORGANISMS FROM SIX COCOA GROWING COUNTRIES

by

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SUMMARY

Studies using 34 isolates from Sao Tome, Cameroon, Togo, Ghana, Ivory Coast and Brazil showed that vegetative growth of the A2 isolates irrespective of their country of origin were faster on carrot or V8 Juice agar at 10°C–35°C than the A1. At the higher end of the growth curve moreover, the rate of growth of A1 isolates was more severely reduced than A2 resulting in a bimodal curve when the entire collection was considered.

Deviations from de Barry's classic description of the sporangiophore of the genus *Phytophthora* occur within isolates recognized as *P. palmivora* by workers from the different countries. Thus intercallary and terminal sporangia occur in Lop, an isolate from the Ivory Coast, while branched sporangiophores with terminal sporangia occur in B1 and B2 both isolated from Itabuna, Brazil.

The time taken to produce oospores varied. Oospores occurred 4–5 days after pairing with *P. palmivora* (CMI Nos. 106326/106327 or TG2 from Togo) and 10 days in the case of *P. drechsleri* (CMI No. 136535). With Lop, an isolate from the Ivory Coast, oospores appeared not earlier than 16 days after pairing and the mycelia of Lop bore very few oospores which were echinulate.

Pathogenicity tests on pregerminated West African Amelonado seeds showed that virulence of the isolates depended mainly on their ability to produce sporangia in darkness. The only *P. drechsleri* isolate tested produced sporangia in darkness and was also highly pathogenic. The ability to produce sporangia in darkness was not related to mating type nor to morphological types.

QUELQUES DIFFERENCES DES ISOLATS D'ORGANISMES DE LA POURRITURE BRUNE DANS SIX PAYS CACAO CUTEURS

RESUME

Des études portant sur 34 isolats en provenance de six pays: Sao Tome, Cameroun, Togo, Ghana, Côte d'Ivoire et Brésil, ont montré que la croissance végétative des isolats A2, indépendemment de leur pays d'origine, était plus rapide que celle de l'A1 sur agar au jus de carotte de V8 à une température entre 10°C–35°C. A l'extrémité la plus élevée de la courbe de croissance, l'on remarque en outre que le taux des isolats A1 était plus gravement réduit que celui des A2, provoquant la formation d'une courbe bimodale lorsque l'on envisage l'ensemble de la collection.

Des déviations de la description classique de Bary, sur la sporangiophore du genre *Phytophthora* se produisent parmi les isolats reconnus comme *P. palmivora* par des chercheurs d'autres pays. C'est ainsi que des sporanges intercalaires et terminaux se produisent dans le Lop, isolat de Côte d'Ivoire, tandis que des sporangiophores à embranchement et sporanges terminaux se produisaient chez B1 et B2, tous les deux isolés à Itabuna, Brésil.

Le temps nécessaire pour produire des oospores variait. Les oospores se produisaient quatre à cinq jours après l'appariement avec *P. palmivora* (CMI Nos. 106326/106327 ou TG.2 du Togo) et dix jours dans le cas de *P. drechsleri* CMI No 136535). Chez Lop, isolat de la Côte d'Ivoire, les oospores ne paraissaient pas avant seize jours après l'appariement et le mycelium du Lop ne présentait que très peu d'oospores échinulées.

Les tests de pathogénicité sur des semences d'Amenolado ouest africaines pré-germées montraient que la virulence des isolats dépendait surtout de leur aptitude à produire des sporanges dans l'obscurité. Le seul isolat de *P. drechsleri* mis à l'épreuve produisait des sporanges dans l'obscurité et était aussi très pathogène. L'aptitude à produire des sporanges dans l'obscurité n'avait de rapport ni avec la fertilisation, ni avec les types morphologiques.

ALGUNAS DIFERENÇAS EM ISOLADOS DOS ORGANISMOS DA PODRIDÃO PARDA DE SEIS PAÍSES PRODUTORES DE CACAU

RESUMO

Estudos de 34 isolados de São Tomé, Camarões, Togo, Ghana, Costa do Marfim e Brasil mostraram que o crescimento vegetativo de isolados A2 foi mais rápido em agar de cenoura ou suco V8 entre 10°C e 35°C do que de isolados A1, independentemente do país de origem. Na extremidade mais alta da curva de crescimento, a taxa de crescimento de isolados A1 foi reduzida mais significativamente que a dos A2, resultando numa curva bi-modal quando se considera a coleção toda.

Ocorrem desvios da descrição clássica de Barry de sporangióforos de gênero *Phytophthora* nos isolados reconhecidos como de *P. Palmivora* por pesquisadores em vários países. Os esporângios intercalares e terminais ocorrem em Lop, um isolado da Costa do Marfim, enquanto esporangióforos ramificados com esporângios terminais ocorrem em B1 e B2, ambos isolados de Itabuna, Brasil.

O tempo necessário para a produção de oósporos variou. Oósporos ocorreram quatro a cinco dias após o pareamento com *P. palmivora* (CMI No. 106326/106327 ou TG2 de Togo) e dentro de 10 dias no caso de *P. drechsleri* (CMI No. 136535). No Lop, um isolado da Costa do Marfim, os oósporos apareceram só no 16° dia depois do pareamento e os micélios de Lop continham muito poucos oósporos, que eram.

Ensaios de patogenicidade com sementes prégerminadas de Amelonado da África Ocidental mostraram que a virulência dos isolados dependia principalmente de sua capacidade de produzir esporângios no escuro. O único isolado de *P. drechsleri* testado produziu esporângios no escuro e também foi altamente patogênico. A capacidade de produzir esporângios no escuro não era função do acasalamento nem do tipo morfológico.

ALGUNAS DIFERENCIAS EN AISLADOS DE LOS ORGANISMOS DE LA PODREDUMBRE NEGRA PROVENIENTES DE SEIS PAISES PRODUCTORES DE CACAO

RESUMEN

Estudios efectuados utilizando 34 aislados de Santo Tomé, Camerún, Togo, Ghana, Costa de Marfil y Brasil, demostraron que el crecimiento vegetativo de los aislados A2, independientemente de su país de origen, fué más rápido sobre agar con zumo de zanahoria o V8 a 10°C hasta 35°C que el de A1. Además, en el punto más alto de la curva de crecimiento, la tasa de crecimiento de los aislados A1 fué más drásticamente reducida que la de los A2, dando como resultado una curva bimodal al ser considerada la colección completa.

En aislados reconocidos como *P. palmivora* por trabajadores de los distintos países, se presentan desviaciones de la descripción clásica de de Barry de las esporangióforas del género *Phytophthora.* Así, se encuentran esporangias intercalar y terminal en Lop, un aislado de la Costa de Marfil, mientras que se encuentran esporangióforas ramificadas con esporangia terminal en B1 y B2, aislados ambos de Itabuna, Brasil.

El tiempo empleado para producir oosporas fué variable. Las oosporas aparecieron de cuatro a cinco días después de la cópula con *P. palmivora* (CMI Nos. 106326/106327 o TG2 de Togo) y diez días en el caso de *P. drechsleri* (CMI No. 136535). Con Lop, un aislado de la Costa de Marfil, las oosporas aparecieron no antes de dieciseis días después de la cópula y las micelas de Lop presentaron muy pocas oosporas equinuladas.

Las pruebas de patogenicidad sobre Amelonado de Africa Occidental pregerminado mostraron que la virulencia de los aislados dependia principalmente de su capacidad de producir esporangias en la oscuridad. El único aislado de *P. drechsleri* sometido a prueba produjo esporangias en la oscuridad y fué también altamente patógeno. La capacidad de producir esporangias en la oscuridad no mostró relación con la cópula ni con los tipos morfológicos.

INTRODUCTION

In 1972, Amponsah and Asare-Nyako demonstrated that root infection of cocoa seedlings by *Phytophthora palmivora* (Asomaning, 1964) could be used not only to identify parents and progenies of cocoa resistant to the black pod disease but also to screen populations or progenies for various levels of resistance to the disease. Preliminary field records on establishment of screened seedlings are encouraging but a final assessment will have to await four years of cropping.

Work along similar lines by Partiot (1975) confirmed the findings of Amponsah and Asare-Nyako while Tarjot (1975) working on the Cameroon could not duplicate these findings.

Tarjot's work together with other known epidemiological differences in the black pod disease syndrome in Ghana and in the Ivory Coast on the one hand and in Nigeria and especially Cameroon on the other, led to the suspicion that there might be fundamental differences in the climate, the cocoa types or the pathogen in these countries.

The confirmation of the existence of morphological types of *P. palmivora* (Tucker, 1931; Turner, 1961) by Griffin and Anderson in Nigeria (1975) and the limited distribution of these morphological types in cocoa farms and in the cocoa growing areas of West Africa have been supplemented by differences in chromosome numbers and sizes as well as pathogenicity and thus further strengthened the belief that the fungal types might account for recorded differences in the epidemiology of the black pod disease of cocoa in the different countries.

Phytophthora palmivora (Butl) Butl; the causal agent of the black pod disease of cocoa, occurs on several tropical and subtropical plants (McFarlane, 1968); Ribeiro, 1978). In some instances, it has been isolated simultaneously with *P. cactorum* (Madeiros, 1977), *P. drechsleri* (Waterhouse, 1974; Babacauh, personal communication) and *P. heveae* (Chee, 1974) from the same plants. It is therefore necessary to re-identify isolates of any collection of *P. palmivora* especially when these come from different workers in different countries.

While the genus *Phytophthora* as delimited by de Bary in 1875 may not be a homogeneous or natural group, keys to the species classification by Waterhouse (1963) based on the morphology and production of sporangia, oospores and antheridia are fraught with overlapping characters which complicate their use in certain cases.

As a supplement to the systems of Waterhouse, therefore, Boccas and Laville (1976) differentiated between *P. parasitica, P. palmivora* and *P. citrophthora* on the basis of their optimum and maximum growth temperatures. *P. citrophthora* has an optimum growth temperature around 26°C and a maximum around 32°C; *P. palimvora* at 30°C and 35°C while *P. parasitica* has 32°C and 37°C as optimum and maximum temperatures respectively. The fact that these cardinal temperatures tend to vary for isolates of the same species is admitted by all workers. Thus a range of temperatures is often quoted.

Recent investigations with *P. cinnamomi* in Australia (Shepherd *et al.,* 1974) and in America (Zentmyer *et al.,* 1976) have shown that the variations in the growth rates at various cardinal temperatures may be explained in part, at least, by differences in mating types.

This work investigates some differences in vegetative response to temperature, differences in sporangiophore and sporangium types as well as pathogenicity of various isolates of the cocoa black pod disease organisms from six (6) different countries.

MATERIALS AND METHODS

The sources of the isolates of *P. palmivora* used in this work were as follows: Sao Tome (2), Cameroon (9),

Togo (2), Ghana (6), Ivory Coast (10) and Brazil (2). These form part of the *P. palmivora* culture collections in Dr. Muller's laboratory at Montpellier, France. With the exception of G3 and G5 which came from infected peduncles all isolates came from cocoa pods.

Temperature

For the vegetative growth, a 0.5 cm disc from a 2-week old culture of *P. palmivora* was placed centrally on a 20 ml agar medium in a 9 cm petri dish and allowed to grow in darkness. Three such cultures of each isolate were incubated simultaneously and the mean colony diameter taken at the end of incubation. The first test involved seven isolates, viz. Lop 19, C12 from the Ivory Coast; B1 from Brazil; 6 and 11.2 from Sao Tome; YN from the Cameroon and G7 from Ghana. The incubation temperatures were 5°, 15°, 20°, 25°, 30° and 35°C. V-8 Juice agar was the medium used and colony diameters were taken three days after incubation and then 18 hours later. In the second test 25 isolates including all but G7 used in Test 1 were grown on V-8 agar for five days at 25°C. The excess $CaCO_3$ was filtered from the hot V-8 juice before the agar was added. The colony diameters were recorded two days after incubation and three days later. In the third test, 31 isolates including 20 in Test 2 were studied. The medium, this time, was Carrot Agar (200 g carrot/litre) and the temperatures of incubation were 15°, 20°, 25°, 30° and 37.5°C.

The mating types were obtained by crossing each isolate separately with CMI *P. palmivora* isolates No. 106327 and or No. 106326, CMI *P. drechsleri* isolate No. 136535 and with *P. palmivora* isolate No. TG1 obtained from Togo. The CMI isolates were of the A2 while TG1 was of the A1 mating type. Crosses were made on Carrot Agar (CA) and the cultures incubated in darkness at 20°C.

Morphology

In this work, the various isolates were grown on V-8 Juice agar or on Carrot Agar and the sporangia and sporangiophores observed in 2-week to 4-week old cultures. Drawings were made with camera lucida whenever possible or else free-hand drawings were made. Length/breadth ratios as well as pedicel lengths were also recorded.

Pathogenicity on Amelonado

Amelonado cocoa used for these tests came from the Ivory Coast as demucilaginated beans packed in sterile saw dust in plastic bags. 300 beans were sent at a time by air and reached the Montpellier laboratory within five days. The *P. palmivora* isolates used for this work formed part of the collections in Dr. Muller's laboratory in Montpellier.

The beans were pregerminated (Amponsah and Asare-Nyako, 1975) and inoculated with two 2-week old petri dish cultures grown on Carrot Agar (CA) in the dark. The cultures were comminuted in a Waring Blender in 200 ml of sterile distilled water for two to three minutes and then poured into a 450 ml beaker. Seeds to be inoculated were submerged for a period of about two minutes with intermittent shaking. The inoculum was then decanted and the seeds planted in moist, sterile vermiculite.

Records were kept of emergence and seedling deaths and at the end of each test, plant heights and root infections were noted. Using the inoculum as prepared above, the type and relative proportions of germinations of the sporangia were studied. In this

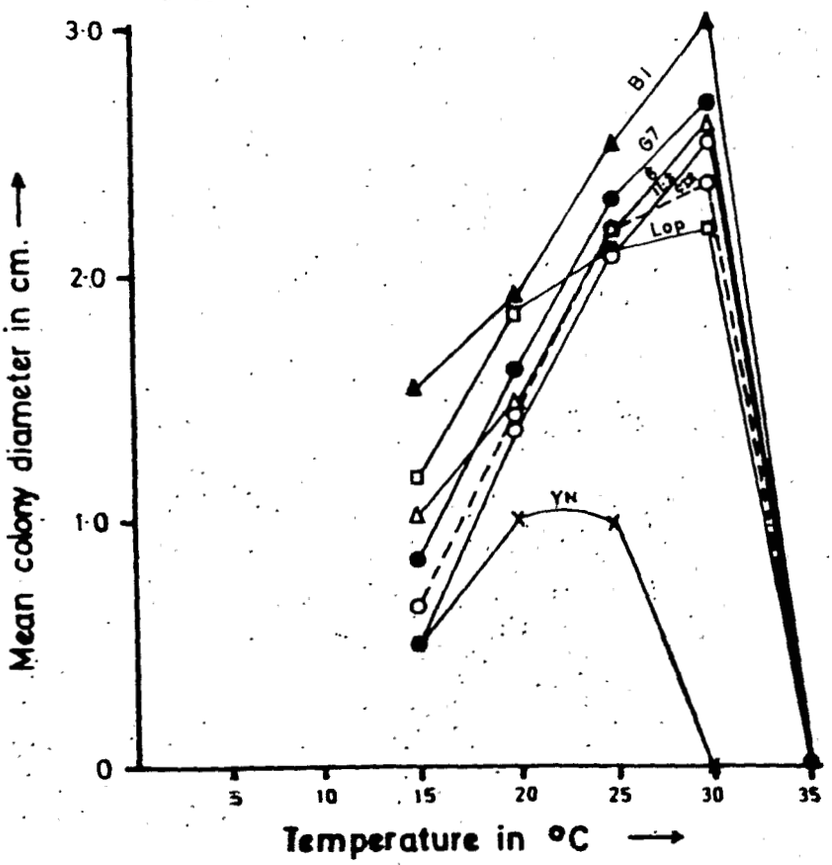

FIG. 1. Increase in colony diameter over 18 hours of seven isolates of *Phytophthora palmivora* (Butl) Butl grown on V-8 agar in darkness at 15°, 20°, 25°, 30° and 35°C.

regard, direct and indirect germinations were recorded in three samples of 0.3 ml of the inoculum left to dry slowly on a glass slide kept in an open metal box of dimension 23 cm × 23 cm × 12 cm. The dried inoculum was stained with lactophenol in cotton blue and all spores observed.

RESULTS

Temperature

In the first test, there were no growths at 5°C but the isolates survived the four days exposure. Growth between 15° and 25°C (Figure 1) appeared to be linear for 11.2, C12 and G7. It is interesting, however, to note the decline in growth rates for Lop 19 and YN between 20° and 25°C. At 30°C YN failed to grow but it was not killed.

Thus when later kept at room temperature (about 24°C) the YN cultures started growing after four days. The optimum growth temperature for the other six isolates was 30°C and it is pertinent to note that at that temperature Lop 19 was the slowest of the six isolates which were growing. Only YN did not survive at 35°C at which temperature only C12 showed any signs of growth.

In the second test (Table 1) as in the first, the Brazil isolates, B1 and B2, were the fastest-growing while the isolates from the Cameroons with the exception of BAF 39 and SK 53 were slow-growing. Figure 2 shows that the distribution of the colony diameters at 25°C had two peaks: one peak within the range 4.1 cm and the other within 6.1–7 cm.

No growths occurred at 37.5° in the third test and none of the isolates survived this temperature after the four days exposure. The differences in rates of growth of the Brazil and the Cameroon isolates were confirmed (Table 2) but the bimodal distribution of colony diameters obtained in Test 2 was only evident at 25° and 30°C and not at 15° and 20°C (Figure 3).

All the isolates from the Cameroon were of the A1 mating type while all those from Ghana, Brazil and Sao Tome were of the A2. One of the two isolates from

TABLE 1

Origin, mating types and mean colony diameters in cm. of isolates of Phytophthora palmivora *grown at 25°C for four days on V8 juice agar*

Isolate/ Strain	*Source — Country of Origin*	*Mating Type*	*Colony diameter in 4 days*
	Cameroon		
BN 52	2° Forest	A1	5.1
BOT 38	Semi Savanna	A1	3.8
OK 30	2° Forest	A1	3.8
SK 53	Forest	A1	6.6
OMB 35	Semi Savanna	A1	3.7
EV 28	2° Forest	A1	4.5
BAF 39	Semi Savanna	A1	6.2
DZ 51	Forest	A1	4.3
SA 18	Semi Savanna	A1	4.0
BAF 30	Semi Savanna	A1	4.7
OK 4	2° Forest	A1	3.8
NT 33	Semi Savanna	A1	4.0
YN 8	2° Forest	A1	3.7
	Ivory Coast		
CI 1	Forest	A2	2.0
CI 2	Forest	A2	6.1
CI 3	Forest	A2	6.0
CI 4	Forest	A2	6.0
Bong 1	Forest	A2	5.9
Soub 20	Forest	A2	6.1
Lop 19	Forest	A1	6.4
Mag 7P	Forest	A2	6.4
	Sao Tome		
11.2	Noy Principe	A2	6.4
6	Portoreal (St. Joaquin)	A2	6.7
	Brazil		
B1	Itabuna	A2	7.2
B2	Itabuna	A2	7.7

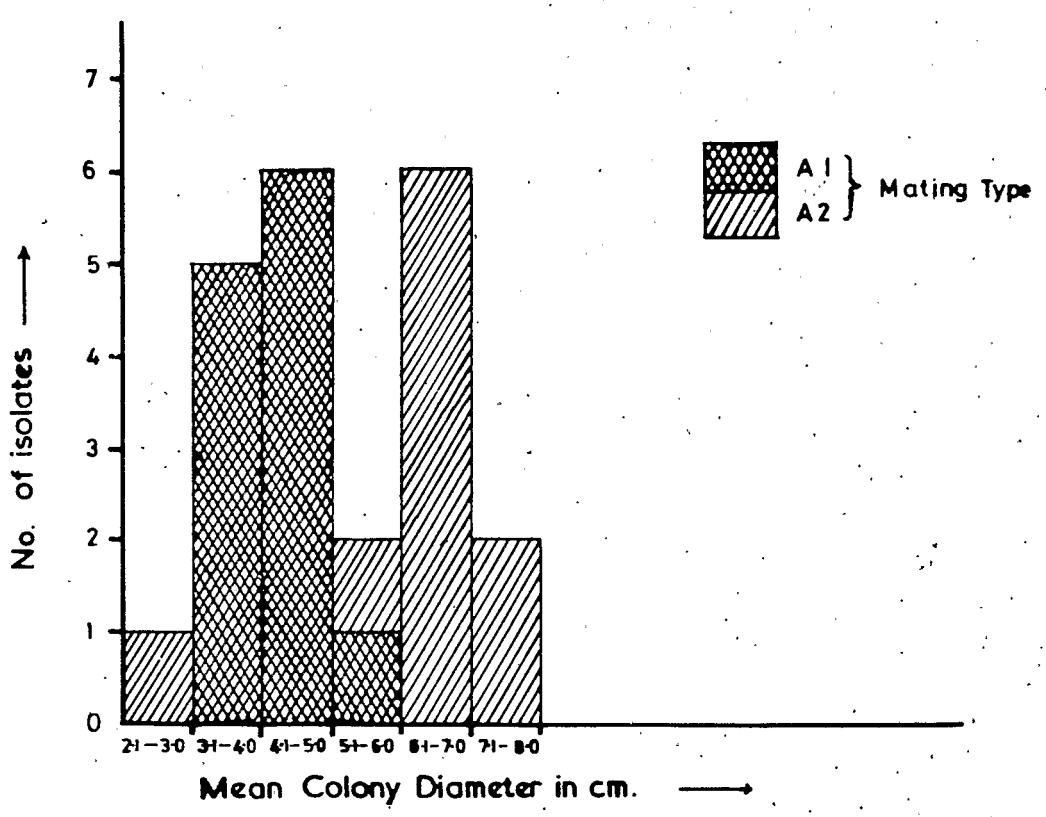

FIG. 2. Colony diameters of 25 isolates of *Phytophthora palmivora* grown for 4 days at 25°C on V8 agar — Distribution of A1 and A2 mating types.

TABLE 2

Mating types and mean colony diameters of 31 isolates of Phytophthora palmivora *grown on carrot agar for four days at 15°, 20° and 30°C*

Country of Origin	*Isolate*	*Colony Diameter in cm. at*				*Mating Type*	*Difference in colony diameter between*		
		15°C	20°C	25°C	30°C		15–20°C	20–25°C	25–30°C
Ghana	G 1	0.9	3.7	5.6	6.0	A2	2.8	1.9	0.4
	G 2	0.3	3.1	5.7	6.6	A2	2.8	2.6	0.9
	G 4	0.8	3.7	5.6	6.0	A2	2.9	1.9	0.4
	G 5	0.5	3.5	5.8	6.3	A2	3.0	2.3	0.5
	G 6	0.0	3.2	6.1	6.5	A2	3.2	2.9	0.4
	G 7	0.2	3.7	5.7	6.5	A2	3.5	2.0	0.8
Ivory Coast	CI 4	0.5	3.5	5.1	6.0	A2	3.0	1.6	0.9
	DIN 10C	0.9	3.3	6.1	6.4	A2	2.4	2.8	0.3
	CI 3	1.9	3.7	5.5	6.2	A2	1.8	1.8	0.7
	Lop 19	1.3	3.9	5.1	5.3	A1	2.6	1.2	0.2
	Za II	1.3	3.5	5.1	5.8	A2	2.2	1.6	0.7
	Bong 1	1.8	4.1	5.1	5.3	A2	2.3	1.0	0.2
	Soub 20	1.0	3.4	5.3	5.7	A2	2.4	1.9	0.4
	4 C	1.3	3.3	5.8	6.3	A2	2.0	2.5	0.5
	Mag 7P	1.4	3.3	6.3	7.4	A2	1.9	3.0	1.0
Cameroon	YN	0	1.7	2.4	2.7	A1	1.7	0.7	0.3
	EV 28	0	2.3	3.1	3.4	A1	2.3	0.8	0.3
	NT 33	0	2.1	2.9	2.5	A1	2.1	0.8	0.4
	BOT 38	1.1	2.4	3.2	3.3	A1	1.3	0.8	0.1
	OMB 35	1.5	2.5	3.7	3.6	A1	1.0	1.2	0.4
	SK 53	2.5	4.4	6.2	6.3	A1	1.9	1.8	0.1
	BN 52	0.6	2.4	3.5	2.9	A1	1.8	1.1	0.6
Brazil	B 1	3.1	4.7	5.9	6.4	A2	1.6	1.2	0.5
	B 2	2.2	3.6	4.3	4.7	A2	1.2	0.7	0.4
Togo	TG 1	1.6	3.2	4.0	4.0	A1	1.6	0.8	0.0
	TG 2	1.0	3.1	3.4	4.0	A2	2.1	0.3	0.6
Sao Tome	6	0.4	3.0	4.6	5.3	A2	2.6	1.6	0.7
	11.2	2.0	3.8	5.7	6.5	A2	1.8	1.9	0.8
Cameroon	SA 18	1.1	3.3	5.2	0	A1	2.2	1.9	5.2
	OK 30	1.4	3.3	4.8	—	A1	1.9	1.5	—
	BAF 30	1.1	2.9	3.7	3.9	A1	1.8	0.8	0.2
Mean difference in colony diameter of A1 & A2							0.56	0.80	0.92

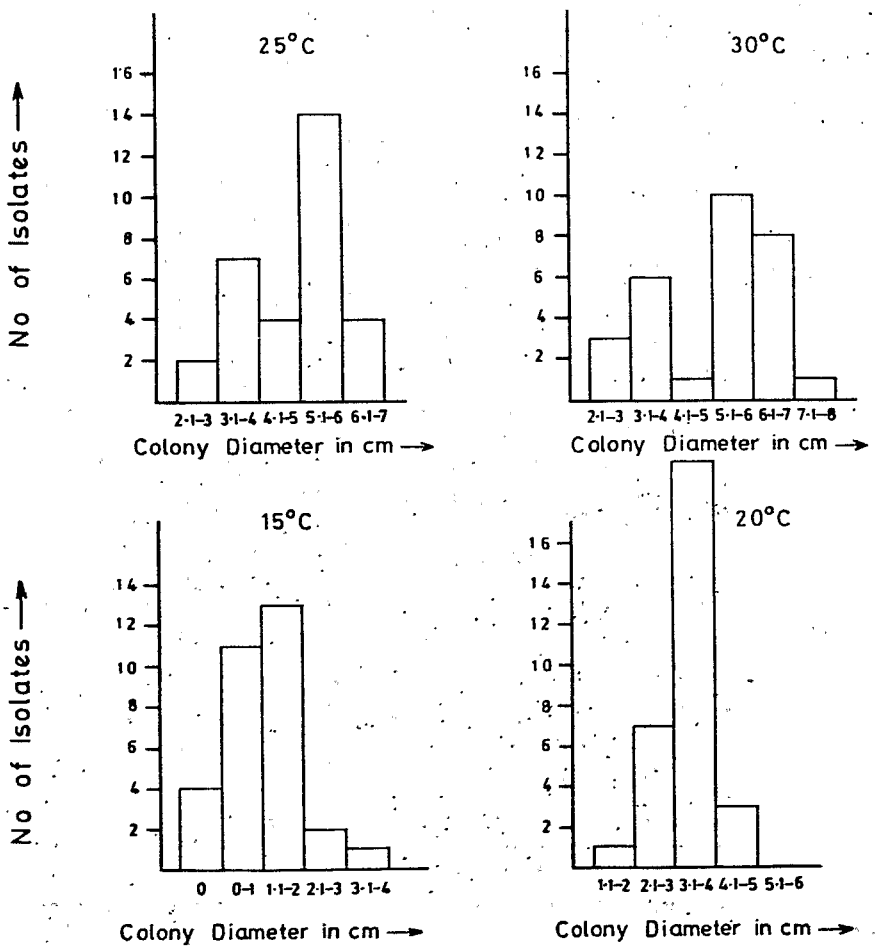

FIG. 3. Distribution of colony diameters of isolates of *Phytophthora palmivora* (Butl) Butl grown for four days on Carrot Agar at 15°, 20°, 25° and 30°C.

Togo (TG1) was A1 as also was one of the nine isolates from the Ivory Coast (Lop 19), Tables 1 and 2.

In Figures 4 and 5, the distribution of the colony contrast to 410 per ml in 44C.
plotted separately and these show that A1 at all the temperatures studied, on the average, grew more slowly than A2. In fact, the mean difference between the colony diameters of A1 and A2 cultures when the temperature was increased from 15°–20°C, 20°–25°C and 25°–30°C was 0.56 cm, 0.80 cm and 0.92 cm respectively (Table 2). Thus on changing from 15–20°C the mean difference in colony diameters of A1 and A2 cultures was 0.56 cm while on changing from 20°–25°C or 20°–30°C the mean difference increased by 43% and 64% respectively.

Morphology

There were four main types of sporangiophores, viz. the typical indeterminately growing sporangiophores producing sympodially and alternately a succession of single sporangia as represented by Figure 6 obtained from G7, a Ghanaian isolate. The pedicel was short and the sporangia rather globose.

Lop 19, an isolate from the Ivory Coast had both terminal and intercallary sporangia. The intercallary sporangium, in some cases, appeared to be formed initially as a terminal sporangium. As the sporangium matured, a hypha, not unlike a germ-tube, developed from the sporangium at a spot opposite to the original subtending hypha. An operculum then developed in a region normal to the two subtending hyphae. Where the operculum developed early, i.e. before the second subtending hypha, there appears to be an inflection such that the operculum is placed normal to the subtending hypha before the second subtending hypha was developed. In other cases, the sporangium developed from a cell in the middle of an otherwise coenocytic hypha. Lop 19 also had terminal sporangia with very long sporosphores (up to 2 cm) with no discernible pedicels. The sporangiophore in this case was not different from a normal coenocytic hypha. It appeared that sporangia were not shed in this isolate; a fact supported by the formation of zoospores in zoosporangia still subtended by sporangiophores. Distribution of spores on the agar surface was uniform.

From Brazil, Itabuna, came B1 and B2. Two types of sporophores have been identified on B2. One type has a main hypha which throws off opposite branches; the successive opposite branches apparently being at right angles as in the branching of the Rubiaceae stem. The sub-branches may also bear single branches. All the branches as well as the main hypha may bear terminal

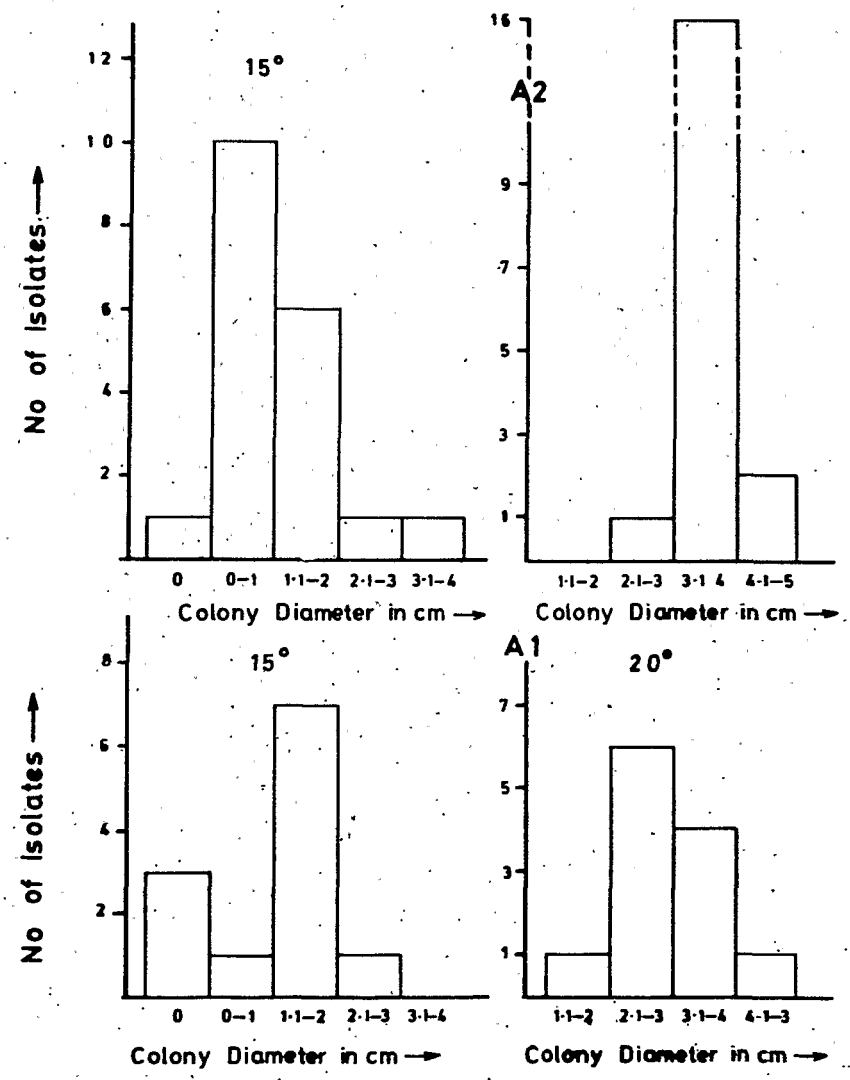

FIG. 4. Frequency distribution of mean colony diameters A1 and A2 mating types of *Phytophthora palmivora* (Butl) Butl grown on carrot agar for 4 days at 15° and 20°C.

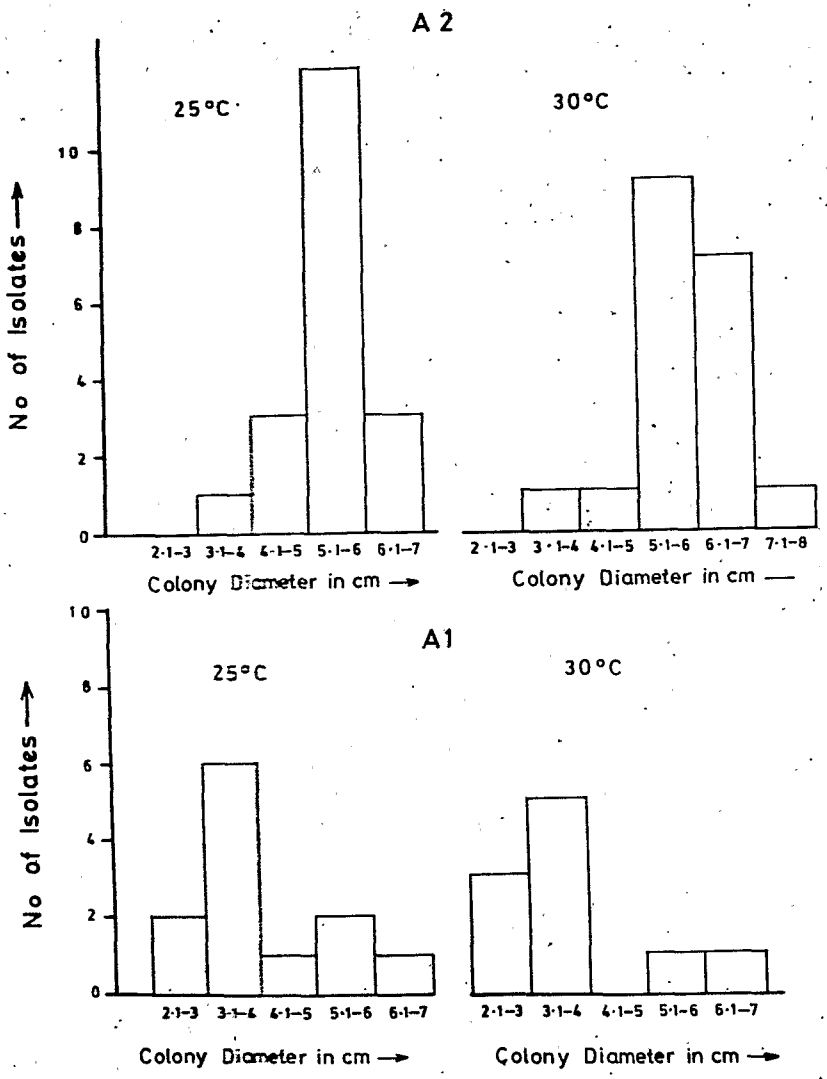

FIG. 5. Distribution of mean colony diameters of A1 and A2 mating types of *Phytophthora palmivora* (Butl) Butl grown on carrot agar for 4 days at 25° and 30°C.

sporangia thus presenting a clumped or grouped type of distribution on the agar surface. Figure 6 illustrates another sporophore type in which rather short stubs which may form a network occur on vegetative hyphae on the agar surface. The pedicels which arise from these short stubs are rather thin and may be long or very short. The attachment appears to be very delicate and the pedicel easily breaks off at the stubby branch end. It is worth noting that although Figure 6 shows direct attachment of one sporangium with one stub sporophores with branches may occur and that the sporangia are at different stages of maturity. Apparently, as one sporangium matures, another is formed on a branch below the older; a situation analogous to de Bary's description. Again, this type of sporophore produces clumped sporangia.

YN, a Cameroon isolate which has more than one sporophore type. The stubby branch and thin long pedicels occur alongside branched ones bearing apical sporangia. The sporangia are grouped in this isolate.

BOT 33, another Cameroon isolate where several sporophores may come off one vegetative at intervals. In a 30-day old culture, on V-8 agar each sporophore (Fig. 6), bore a maximum of two sporangia of different ages. Where only one sporangium remained attached, there were swellings on the sporophore reminiscent of de Bary's description.

Pathogenicity on Amelonado

Emergence was completed from 10-15 days after planting depending on the temperature in the laboratory where inoculated beans were kept. Considering the inoculated seed lots as a group, there was significantly ($P = 0.01$) less emergence and survival of seedlings in inoculated than in non-inoculated lots. Within the inoculated seed lots, the various *P. palmivora* isolates also differed in their effects on emergence and survival of seeds ($P = 0.01$).

The mean height of surviving plants in Test 1 were not different from the non-inoculated plants (controls). Interesting enough, seedlings inoculated with CI 1 had 100% survivals, no visible infections but were markedly shorter than seedlings treated with the other isolates and the controls. It should be noted also that seedlings immersed in Carrot Agar before planting were the tallest although not significantly so at the end of the test. Inoculations with CI 2, SK 53, G1 and TG 1 resulted in no survivals. In Test 2, Bong 1 and EV 28 significantly reduced seedling height while all the isolates reduced survival ($P = 0.01$). Inoculations with

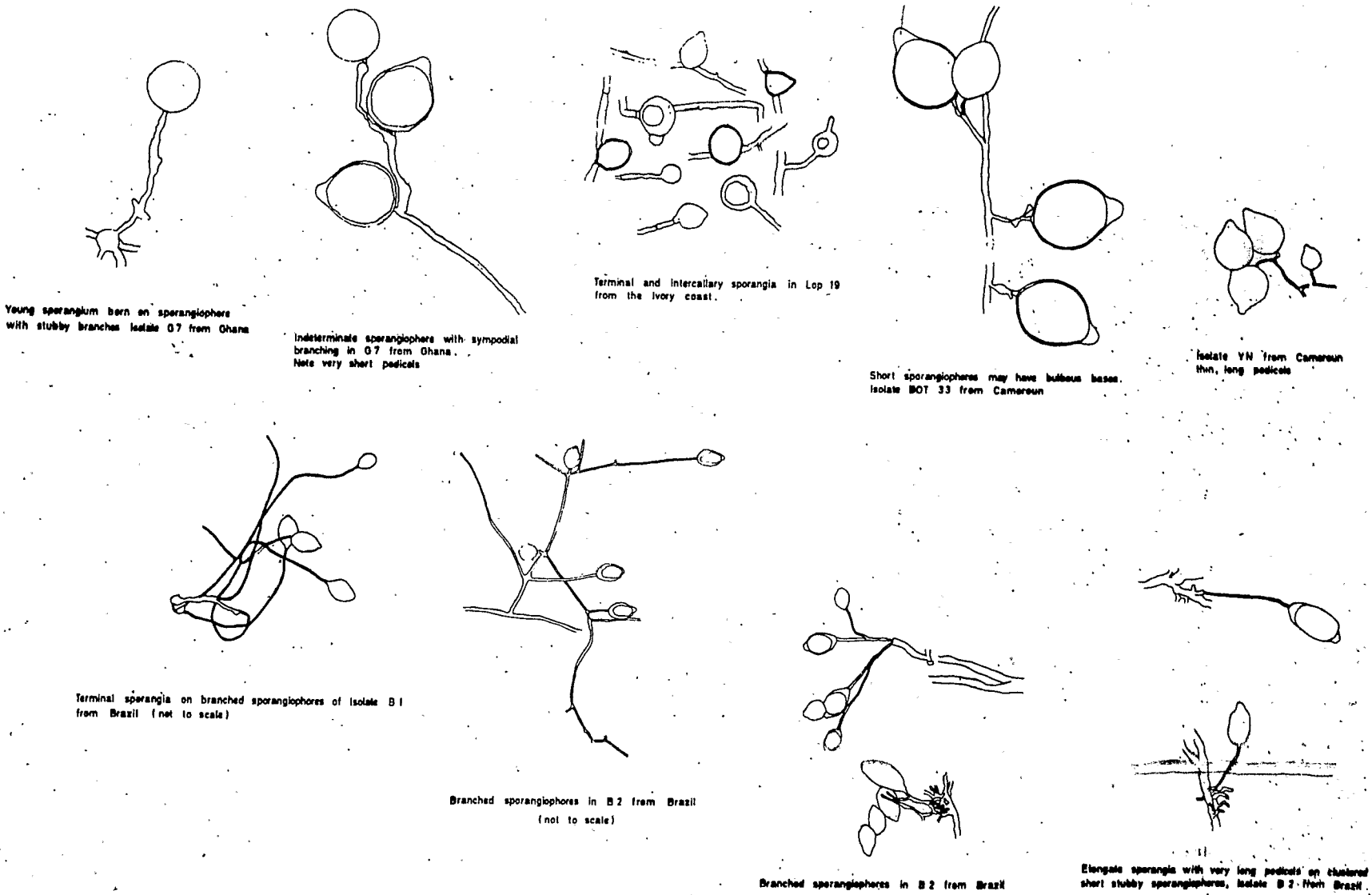

FIG. 6. Sporangium and sporangiophore types.

G5, G1, CI 3, CI 2, 11.2 and 6 produced no survivals. In test 3 also, eight isolates—TG1, G7, G4, G2, ZAII, Son 20, 44C and the only *P. drechsleri* isolate tested (CMI No. 136535)—killed off all seedlings of the two which left survivals the reduction in plant height was not significant.

From the sporangial counts, three categories of isolates could be identified, according as these produced *(a)* not any, *(b)* a few or *(c)* relatively high numbers of sporangia in darkness. Isolates that did not produce sporangia in darkness were YN, Bot 38, CI 1, EV 28 and TG2. EV 28 and Lop produced a low number of chlamydospores (3 and 10 per ml respectively) which germinated to produce sporangia or ramifying hyphae when left on the slide. Furthermore, isolates such as OMB 35 and EV 28 could within 1–3 days of exposure on the slide produce sporangia from the comminuted hyphae. About 80% of these rather small sporangia (20×25–30 m) were observed to have matured and liberated zoospores within an 18 hour period. Another phenomenon first observed in TG1 but later found to occur in other isolates such as G7, G1, G4, G2, Soub 20 and 44C is the germination of the residual cytoplasm after liberation of zoospores. The hyphae produced from such cytoplasm developed into either nycelia or secondary and even tertiary sporangia.

Among the isolates producing sporangia in darkness, there were differences in the concentrations of sporangia produced and in the relative proportions of direct and indirect germinations (Table 3). Thus B1 and B2 produced 20 and 30 respectively as compared with 590 and 243 per ml by SK 53 and G1 respectively. In test 2, BN 52 had 37 sporangia while CI 2 had 1,540 per ml of the inoculum and in Test 3, NT33 contained 53 in contrast to 410 per ml in 44C.

Isolates producing no sporangia or those producing very low numbers in darkness such as CI 1 and BOT 38 and B1, B2 and OMB 35 produced very little infection. In fact, isolates with high zoospore production gave high infection rates as measured by seedling deaths. In this regard, it is interesting to note that B1, B2 and OMB 35 had comparatively few sporangia but a high proportion of zoospore production and gave appreciably high infections. Moreover, B1 with lower sporangial numbers than B2 had a large proportion of zoosporangia and a higher level of plant infection than B2. Of these three isolates, OMB 35 had the highest proportion of zoosporangia and also the highest seedling infection percentage.

While this pattern generally occurred in the two other tests, there were isolates like YN which produced no sporangia in the dark but did infect seedlings (Test 2).

DISCUSSION

Temperature

Working in Australia with local isilates of *Phytophthora cinnamomi,* Shepherd and Pratt (1974) obtained a mean growth rate of 12.25 mm/day at 25°C.

TABLE 3

The presence of sporangia, their type of germination and pathogenicity of 27 isolates of P. palmivora *on Amelonado cocoa*

		Surviving plants			*Sporangia*	*Sporangial Germination Type*	
Isolate	*Number planted*	*% of number planted*	*Mean height mm*	*Visibly infected %*	*per ml. of inoculum*	*Zoopores %*	*Germ tube %*
Test 1							
CI 1	23	100.0	169	0	0	—	—
CI 2	23	0.0	—	—	207	83.9	16.1
SK 53	23	0.0	—	—	590	95.5	4.5
B 2	23	69.9	188	1	30	66.7	33.3
OMB 35	23	17.4	183	1	20	83.3	16.7
G 1	23	0.0	—	—	243	83.6	16.4
B 1	23	34.8	183	3	20	80.0	20.0
TG1	23	0.0	—	—	67	86.6	13.4
Bot 38	23	73.9	172	5	0	—	—
Control m	35	100.0	192	0	0	—	—
Control	35	100.0	185	0	0	—	—
Test 2							
G5	20	0.0	—	—	1200	31.1	66.4
G1	20	0.0	—	—	952	62.2	32.9
Lop 19	20	45.0	116	100.0	10	0.0	100.0
C13	20	0.0	—	—	1754	75.7	22.1
Bong 1	20	20.0	83	100.0	90	63.0	33.3
EV 28	20	15.0	59	100.0	3	0.0	100.0
CI2	20	0.0	—	—	1540	45.0	38.1
11.2	20	0.0	—	—	1060	37.1	28.6
6	20	0.0	—	—	1186	11.2	82.6
BN 52	20	25.0	104	100.0	37	63.6	0.0
YN	20	25.0	128	100.0	0	—	—
Control	21	100.0	124	0	0	—	—
Test 3							
TG2	27	78.8	100.1	14.3	0	—	—
TG1	27	0.0	—	—	43	84.6	7.7
G7	27	0.0	—	—	400	59.2	40.8
G4	27	0.0	—	—	340	82.4	5.9
G2	27	0.0	—	—	397	12.6	47.9
ZA II	27	0.0	—	—	350	64.8	33.3
Soub 20	27	0.0	—	—	397	76.5	22.7
44C	27	0.0	—	—	410	66.7	30.9
NT 33	27	44.4	80.4	41.7	53	12.5	56.3
35	27	0.0	—	—	357	36.4	34.6
Control	24	100.0	98.0	0	0	—	—

Zentmyer *et al.* (1976) working in California and using *P. cinnamomi* isolates from eight countries and 15 hosts, however, obtained a mean of 6.25 mm/day at 25°C on CV8-agar. The indications, therefore, were that the Australian isolates of *P. cinnamomi* were faster growing than the isolates used by Zentmyer *et al.* In fact, the growth rates of the Australian isolates in the population studied by Zentmyer *et al.* were higher than average regardless of their mating types on "Minimal medium" but on PDA A2 isolates were significantly faster growing than A1 isolates.

The differences in vegetative growth rates of *P. cinnamomi* isolates depending apparently on country of origin although suggested in the present work with *P. palmivora* was not substantiated when mating types were considered. Thus, although the work being reported showed that the isolates from the Cameroon were, on the average, significantly slower growing at all the cardinal temperatures than isolates from the other five countries, it must be noted that these isolates from the Cameroon were all of the A1 mating type. Also noteworthy is the presence of two A1 isolates SK 53 and BAF 39 from the coastal forest and semi-savanna belts respectively of the Cameroon which were as fast growing as the isolates from the Ivory Coast, Sao Tome or Ghana. From the same belt and country as BAF 39 and BAF 30 which was of the slow growing A1 type.

The Ivory Coast and Togo collections contained both A1 and A2 mating types. At 25°C Lop 19—the only A1 isolate from the Ivory Coast—grew at the same rate as the A2 isolates while the growth rate of TG1—the A1 isolate from Togo—was about the same as TG2—the A2 isolate also from Togo.

On the strength of the data available, therefore, the differences in growth rates could not be imputed to the country or vegetation belt where the isolate was collected.

What appeared to be consistent was the fact that growth rates declined after 20°C and that this decline became more drastic the higher the temperature was above 20°C. In all cases, the decline at the higher temperatures was more drastic for A1 and A2 isolates. Thus while for A1 the mean increase in growth rate on increasing the temperature from 25°C to 30°C ranged from −13 mm to +0.5 mm, in A2 the range was from +0.5 mm to 2.5 mm and the mean difference in increase of growth rates between the two mating types on increasing the temperature from 25°C to 30°C was 2.3 mm/day.

It must be pointed out that A2 mating type isolates occur in the Cameroon (Zentmyer *et al.*, 1973) and it would be interesting to compare A2 and A2 from the Cameroon. The presence of A1 mating types in the Ivory Coast and in Togo is very interesting because these countries are separated by Ghana where no A1 has, so far, been obtained.

The A1 isolates did have the same optimum growth temperatures as A2, i.e. at 30°C. The three exceptions obtained in this regard were not related to sex. Isolates SA 18 and YN were A1 but CI 1 was A2. The bimodal effect (Figs 2 and 3) may thus be explained by the fact that at 25° and 30°C the growth rate of A1 isolates declines more than that of A2 and, as a result, the differences in the growth rates of the two mating types become greater at the two higher than at the two lower temperatures. This observation agrees with findings in Test 1 (Figure 1) which showed marked decline in growth rates of Lop 19 and YN, both of which are A1, after 20°C as compared with the continued nearly linear growth of C12, 6, G7 and B1 which are all of the A2 mating type.

Morphology

When de Bary in 1875 created the genus *Phytophthora*, distinguished it from the genera *Peronospora* and *Cystopus* by its "having not one but several conidia successively formed at the end of a tree-like conidiophore. When the first conidium is ripe, it is pushed to the side by an unequal swelling of the point to which it is attached. The top of this swollen portion then begins to grow in the original direction of the branch into a new conidial point; and when this has reached a length equal to that of a conidium or a conidium and a half, a new conidium is formed at its apex".

de Bary was of the opinion that in a vigorous specimen, the same process might be repeated 10–15 times. Waterhouse in a more recent work (1974) 5 and 6 considered that up to 20 sporangia could be produced by a sporangiophore. According to de Bary "the conidia were easily shed and as many swellings remained on each branch of the conidiophore as there had been conidia. These conidiophores are absent from Peronospora".

This definition would make the isolate G7 a typical *Phytophthora* species. The absence of swellings in Figure 1 may be because there had not yet been any loss of sporangia on the young sporangiophore but Figure 2 did show the presence of three swellings and according to de Bary three sporangia might have been shed. Such typical *Phytophthora* sporangiophores were present in the eight Ghana isolates of *P. palmivora* and in isolates from the Ivory Coast (CI 2, CI 3, CI 4). There appeared, however, to be various degrees of deviations from this typical type described by de Bary when cultures obtained from cocoa and recognized as *P. palmivora* by local experts were examined. There is a case for revising the genera of such isolates as Lop 19, B1 and B2.

Pathogenicity on Amelonado

Notice must be taken of the fact that isolates that produced no sporangia in darkness occurred in the Cameroon, Togo and in the Ivory Coast. These three countries as well as Brazil also had isolates with few sporangia produced in the dark. The point must be stressed, however, that the number of isolates included in these studies from the various countries especially those from Brazil, Sao Tome and Togo are too few to be representative. What appears to be obvious is that isolates not sporulating in darkness are not limited to any one country.

The ability to infect in the initial absence of spores as occurred in YN would indicate the possibility of infection by hyphae. The observation of sporangial production by comminuted hyphae in OMB 35, however, seems to suggest that the mycelia may not be the ultimate infecting organs. While the indication in the example of B1, B2 and OMB 35 is that zoospore production *in situ* influenced level of seedling infection,

it was evident that not all chlamydosphores and sporangia germinated to produce zoospores. What is very interesting is the marked adaptability of *Phytophthora palmivora*.

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