International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Influence of Altitudes on Sporangia Size and Aggressiveness of *Phytophthoracolocasiae* Isolates in Cameroon

G.R. Tsopmbeng[ab]*, J.A. Lienou[b] , and D.A. Fontem[c]

[a,b,c]*Phytopathology Laboratory, Faculty of Agronomy and Agricultural Sciences, University of Dschang, Box 208 Dschang Cameroun*

[a]*Laboratory of Applied Botany, Faculty of Science, University of Dschang, Box 67 Dschang, Cameroon*

grnoumbo@yahoo.fr

dfontem@yahoo.fr

lienjules@yahoo.fr

Abstract

A study was carried out in four altitudes in Cameroon to assess the size of the sporangia and the aggressiveness of *Phytophthoracolocasiae* isolates on taro cultivars. The study was conducted in the West and Littoral regions, which include high, medium and low altitude. Sporangia sizes were measured with the microscope on 50 sporangia collected from each isolate and the cross infectivity of the isolates was assessed on leaf fragments of four cultivars; namely Green Purplish Petiole (GPP), Pink Petiole (PP), "White" Petiole Large Blade (WPLB) and "White" Petiole Small Blade (WPSB). Results showed that sporangia measured 40.2-52.3 x 20.3-24.5 µm with a length into width ratio of 1.9-2.2 and a pedicel of 2- 4.3µm. Sporangia from highland and medium altitude isolate of *P. colocasiae* were larger than those from lowland. Likewise, sporangia from taro cultivar GPP were larger than those from PP cultivar. In term of pathogenicity, the isolates were more aggressive on their cultivar of origin than the other cultivar and those from the lowland formed larger lesion than those from medium and high altitude.

---

* Corresponding author. Tel.: 00237 33 75 73 71
E-mail address: grnoumbo@yahoo.fr

The study indicates that sporangia size and infectivity of *P. colocasiae* varied with the cultivar of origin of the isolate and the altitude.

Keywords: altitude**,** aggressiveness, *Phytophthoracolocasiae*, sporangia size

1. Introduction

Leaf blight, caused by *Phytophthoracolocasiae* is the most destructive disease of taro. The disease affects the leaves and petioles of taro plants, resulting in extensive damage of the foliage[1, 2]. It has become a limiting factor for taro production in all taro growing-countries causing yield loss of up to 30-50 % [3, 4, 5]. It was recently reported in West Africa, especially in Nigeria [6], Ghana [7] and Cameroon [8], where it poses a grave threat to food security. The disease continues to decimate taro cultivation, and is impacting on the livelihoods and welfare of farmers and rural communities where taro is a major staple food and an important source of income.

Taro leaf blight symptoms appear as small, water-soaked spots, which increase in size and number. With the advancement of the disease, lesions enlarge and become irregular in shape and dark brown in colour with yellow margins [2].Under cloudy weather conditions with intermittent rains and temperature around 28 °C, the disease quickly spreads across entire fields giving them a blighted appearance [9].Epidemics are favoured by repeated night time temperatures close to 20 °C and relative humidity of 90-100 % when zoospore release is greatest [4, 9, 10,11]. In Cameroon, this disease is more prominent in the Littoral, Nord-West, South-West and West regions which are potential areas of taro production. In other parts of Cameroon, this disease appears occasionally but in serious proportions [8].The disease spreads within crop areas through zoospores and sporangia. Sporangia are dispersed through leaf exudates, rain splash or windblown rain, within and between plants [9, 12].

Several studies have pointed out the morphological variation of *Phytophthora*spp. in many countries [13].Differences in sporangia size and shape have been shown to contribute in *Phytophthora* identification and to influence disease management strategies. Information on morphological characteristics and aggressiveness of *P. colocasiae* isolates on taro cultivars is needed for efficient disease management. Based on the background, a research was conducted on *P. colocasiase* isolates from four different areas in region that had high, medium and low altitudes, to assess the size of the sporangia and the aggressiveness of the pathogen isolates on taro cultivars.

2. Materials and methods

2.1 Survey and collection of isolates

A survey was conducted between july2011 and November 2011 in the West and Littoral regions of Cameroon. Isolates were collected on four taro cultivars from Djuttitsa, Dschang, Santchou and Djombe localities; differentiated by differences in altitude, namely 2038 m, 1400 m, 700 m and 80 m above sea level respectively. These cultivars were Green purplish petiole (GPP), Pink petiole (PP), “white” petiole large blade (WPLB) and

"white" petiole small blade (WPSB) and were identified based on petiole colour and leaf size adapted from [14, 15]. At each site, leaves containing single lesions of taro blight from each cultivar were picked in the field and placed into ice-box coolers and taken to the Phytopathology laboratory of the University of Dschang.

2.2 Assessment of sporangia size

Young sporulated fresh lesions from each cultivar were brushed out with a small clean brush in 20 ml of sterile distilled water in a 9 cm petri dish and a drop of Tween 80 was added to each plate to dislodge sporangia. Sporangial suspensions obtained were filtered through a double layer of cheesecloth to remove leaf debris. Then a drop of sporangia suspension from each isolate of *P. colocasiae* from different cultivars was mounted in a slide with a drop of lacto phenol to make a specimen more visible, and observed with a calibrated microscope (Olympus brand) at magnification 40X. Fifty sporangia were randomly selected and measured. Length, width and pedicel length of each sporangium were recorded. The experiment was repeated twice.

2.3 Aggressiveness of P. colocasiae isolates on taro cultivars

2.3.1 Preparation of inoculum

Inoculum was prepared from fresh leaves showing taro blight symptoms collected from taro cultivars in different localities. This was to reassure that sporangia keep the environmental condition of the collection site. Sporangia were brushed out from single sporulating leaf lesion of each cultivar with a small clean brush in 20 ml of sterilized distilled water in a 9 cm Petri dish. A drop of Tween 80 was added to each plate to dislodge sporangia. Sporangial suspensions obtained were filtered through a double layer of cheesecloth to remove leaf debris. The suspensions were adjusted to 40,000 sporangia/ml with the aid of a haematocy meter. The sporangia were chilled at 4° C for 30 min to induce zoospore liberation.

2.3.2 Infectivity of P. colocasiae isolates on taro cultivars

Aggressiveness was evaluated on detached leaf fragments obtained from leaves of the middle canopy of 3 months old taro plant. Taro leaves were collected from each of the above four cultivars in the experimental farm of the Faculty of Agronomy and Agricultural Sciences, University of Dschang. Leaf fragments were prepared and washed with running tap water for about 1 min and blotted slightly on paper towels to remove excess moisture. The base of each leaf fragment was covered with a piece of moist cotton to reduce leaf desiccation. They were then placed abaxial side up in 90 cm diameter petri dishes. A single leaf fragment was placed in each Petri dish. Detached leaf fragments of each cultivar were inoculated separately with isolates of the pathogen recovered from different cultivars followed the method used by [16].A drop of 25 µl suspension containing 4 $x10^4$ sporangia/ml was applied to each leaf fragment. Control treatment received the same quantity of sterile distilled water. Inoculated leaf fragments were placed on a laboratory bench and incubated at 24 °C and ambient light for 5 days. Pathogenicity of

the isolates was determined by assessing the lesion area of each leaf fragment by tracing the lesion perimeter on a transparent graph paper and counting the number of square enclosed within the margins recorded [17].

2.4 Data analysis

Data on sporangia size and lesion area were subjected to analysis of variance (ANOVA) using SPSS version 17 software and means were separated using Duncan's test at P = 0.05.

3. Results

3.1.Sporangia size

Sporangia length ranged from 40.2 µm to 52.3 µm (average 46.25 µm) and width from 20.3 to 25.5 µm (average 22.9 µm) in low and high altitude respectively. Pedicel length varied from 2.0 to 4.3 µm (average 3.15 µm) (Table 1). Mean dimension of sporangia of *P. colocasiae* isolates from low altitude wassmaller than thatfrom other altitudes. Also isolates from "white petiole large blade" (WPLB) and "white petiole small blade" (WPSB) cultivar had larger sporangia compared to those from Green purplish petiole (GPP) and Pink petiole (PP) (Table 2). The length**:** width ratio varied from 1.9 to 2.2 and was not significantly different among altitudes and cultivars.

Table 1: Sporangia Size (µm) of *P colocasiae* isolates collected from four locations on different taro cultivars.

Sporangia size	Locations (Altitudes)	Isolates from			
		GPP	PP	WPLB	WPSB
Length	Djutitsa (2080 m)	44.5 aB*	-[z]	47.7 bA	46.7 aA
	Dschang(1400 m)	46.0 aB	46.0 aB	48.5 bA	46.8 aB
	Santchou (700 m)	46.6 aB	46.7 aB	52.3 aA	48.8 aB
	Njombe (80 m)	40.5 bA	40.2 bA	42.5 bA	42.6 bA
Width					
	Djutitsa (2080 m)	22.1 cD	-[z]	24.0 cC	25.5 cC
	Dschang (1400 m)	23.5 cD	23.5 cD	25.0 cC	23.7 cCD
	Santchou (700 m)	23.6 cC	23.7 cC	24.0 cC	24.5 cC
	Njombe (80 m)	20.3 dC	20.3 dC	22.7 dC	21.5 cC
Pedicel length	Djutitsa (2080 m)	4.1 eE	-[z]	3.3 eE	4.0 eE
	Dschang (1400 m)	3.5 eE	3.8 eE	2.8 eE	3.6 eE
	Santchou (700 m)	4.3 eE	3.5 eE	3.5 eE	3.7 eE
	Njombe (80 m)	2.3 fE	2.0 fE	2.1 fE	2.3 fE

GPP: Green purplish petiole, PP: Pink petiole, WPLB: "white" petiole large blade, WPSB: "white" petiole small blade.
*****Means in a column or rowfor each cultivars followed by the same small or capitalletters are not significantly different according to Duncan New Multiple range Test (P = 0.05).
[z] Not determined

Table 2: Mean sporangia size of *P. colocasiae* isolates measured from different taro cultivars in the 4 locations.

	Sporangium (length x width in µm)			
	Isolates from			
Location (Altitude)	GPP	PP	WPLB	WBSB
Djuttitsa (2080 m)	44.5 x 22.1	-[z]	47.7 x 24.0	46.7 x 24.5
Dschang (1400 m)	46.0 x 23.5	46.0 x 23.5	48.5 x 25.0	48.8 x 23.7
Santchou (700 m)	46.6 x 23.6	46.7 x 23.7	52.3 x 24.0	48.8 x 24.5
Njombé (80 m)	40.5 x 20.3	40.2 x 20.3	42.5 x 22.7	42.6 x 21.5

Average of fifty sporangiameasured for each location and cultivars.
[z] Not determined

Table 3: Lesion size (cm^2) of taro blight recorded 5 days after inoculation of *P. colocasiae*isolates from various locations on detached leaf fragments of four taro cultivars.

		Isolates from			
Locations (Altitude)	Primary host	GPP	PP	WPLB	WPSB
Njombe (80 m)	GPP	17.5 a*	14.0 b	13.5 b	16.3 a
	PP	10.0 e	16.5 a	11.3 b	15.5 a
	WPLB	12.0 cd	15.0 a	16.3 a	13.8 b
	WPSB	11.5 cde	13.0 b	12.5 b	15.8 a
Santchou (700 m)	GPP	11.0 de	10.0 cd	10.0 cd	8.5 cd
	PP	9.3 e	12.5 b	6.8 d	6.7 d
	WPLB	10.0 e	8.7 cd	13.0 b	10.0 c
	WPSB	9.5 e	10.0 cd	10.0 cd	12.5 b
Dschang (1400 m)	GPP	13.0 c	9.5 cd	10.0 b	8.3 cd
	PP	7.5 e	13.7 b	6.8 d	8.7 c
	WPLB	10.0 e	10.0 cd	12.5 b	9.0 c
	WPSB	10.5 e	8.7 cd	9.7 cd	12.5 b
Djutitsa (2080 m)	GPP	15.5 b	9.8 cd	9.8 cd	9.5 cd
	PP	-[z]	-[z]	-[z]	-[z]
	WPLB	9.8 e	9.5 cd	12.4 b	7.5 d
	WPSB	9.5 e	7.5 d	6.8 d	11.9 b

GPP: Green purplish petiole, PP: Pink petiole, WPLB: "white" petiole large blade, WPSB: "white" petiole small blade.
*****Means in a row for each location followed by the same letter are not significantly different according to Duncan New Multiple range Test (P = 0.05).
[Z] Not determined.

3.2 Aggressiveness of P. colocasiae isolates

In terms of aggressiveness, blighted lesions formed by isolates from low altitude were significantly larger for all the cultivars tested than those induced by isolates from the other altitudes. Lesion sizes were significantly larger on Green purplish petiole cultivar than on the other cultivars. Generally, isolates of P. colocasiae produced greater

lesion on their original host cultivar than in others (Table 3). A negative correlation was found between sporangia sizeand lesion area formed (table4).

Table 4: Correlation and regression between sporangia size and lesion size formed by *P.colocasiae* isolates on four taro cultivars.

Isolate	Correlation coefficient (R)	Regression equation	Coefficient. of determination (R^2)	Probability
GPP	-0,768	Y= -0,911x + 52,087	0,590	P<0,001
PP	-0,617	Y= -0,701x + 41,509	0,381	P<0,033
WPLB	-0,611	Y = -0,414x + 30,990	0,381	P<0,012
WPSB	-0,536	Y = -0,546x + 52,121	0,287	P<0,033

GPP: Green purplish petiole, PP: Pink petiole, WPLB: “white” petiole large blade, WPSB: “white” petiole small blade.

4. Discussion

4.1 Sporangia size

The dimensions of the sporangia of *P. colocasiae*isolates were located within the intervals defined by several and vary from one country to the other. In Nigeria, [6]found small size sporangia while [7]reported larger sporangia in Ghana. In terms of length, sporangia from New Caledonia were shorter than those reported in Cameroon [18] while pedicel lengths wereshorter than those reported by [7, 19, 20].The sporangia size from high altitude and medium altitude were significantly bigger than those from low altitude. This suggests that altitudes could have an influence on the size of the sporangia of the pathogen which may be due to different climatic conditions prevailing at these altitudes. The influence of climate in the variability of oomycetes pathogen has been reported [21].Although the extent of genetic variability in *P. colocasiae* is unknown in Cameroon, it has been reported to be associated with increased genetic variation including increased variability in virulence and aggressiveness.

Sporangia of isolates collected from cultivars petiole green and large green leaf were also larger than those of isolates from cultivars with pigmented petiole (purplish green and purplish red). This variation could be attributed to the genetic make-up of the plant.

The length/width ratio of the sporangia of different isolates was not significant between isolates and cultivars. It ranged from 1.9 to 2.2 which weregreater than that obtained by [20]in Samoa State. However, it was within the range of 1.2 to 2.4 reported in Java Island [19].In addition, this ratio more than 1 showed that the sporangia of *P. colocasiae* isolates from Cameroon could be more elongated than rounded.

4.2 Aggressiveness of P. colocasiae isolates on taro cultivars

Lesion areas formed on leaf fragments by isolates of *P. colocasiae* from low altitude were larger than those from medium and high altitudes. This suggests that geographic origin of the pathogen may have an influence in its

aggressiveness. In this present studies, isolates of *P. colocasaie* from lowland area were more aggressive than those from other areas. But it is not known whether this variation in aggressiveness is due to the altitudes or to the size of the sporangia. However, variation in host infectivity had been observed in *Phytophthorainfestans* with the geographical origin of the isolates in Cameroon [17].The study also showed that isolates with small sizes sporangia produced larger lesions on all the cultivars. The size of the sporangia could be another factor influencing the aggressiveness of the pathogen. Furthermore, the two variables were negatively correlated; in fact lesion area waslarger with small sporangia size, confirming the aggressiveness of those sporangia over the biggest ones.

Isolates were more aggressive in their original host cultivar than in others. This may be due to physiological characteristics of the original host. Physiological specificity had been reported in isolates of *P. infestans* from tomato, black nightshade and potato in Cameroon [17, 22].Also pathogen isolates have been shown to be adapted to the host cultivars from which they originated than to other cultivar.

5. Conclusions

The study showed that sporangia size and lesions area induced by *P. colocasiae* isolates varied with the altitude. Sporangia from low altitude were smaller than those from medium and high altitudes and were more aggressive. Infectivity of the fungus varied with altitude and its primary hosts. Although the influence of altitudes in sporangia sizeand aggressiveness of the fungus were elucidated, further studies are needed for the characterization of this important plant pathogen so as to develop an efficient disease management strategy.

Acknowledgement

The authors are greatly indebted to God Almighty and to Dr.YaoubaAoudouandLekeufackMartin for their critical review of the manuscript.

References

[1]Paiki F.A. 1996. Symptoms of taro leaf bight disease (*Phytophthoracolocasiae*) and relationship with yield components in Biak, Irain Jaya. Sci. New Guin. 21: 153-157.

[2]Singh D., Jackon G., Hunter D., Fullerton R., Lebot V., Taylor M., Iosefa T., Okpul T. and Tyson J. 2012.Taro Leaf Blight- A Threat to Food Security. *Agriculture* 2: 182-203.

[3]Jackson G.V.H., Gollifer D.E., Newhook F.J. 1985. Studies on the taro leaf blight fungus *Phytophthoracolocasiae*in the Solomon Islands: control by fungicides and spacing. *Ann. Appl. Biol.* 96:1–10.

[4]Thankappan M. 1985. Leaf blight of taro-a review.*J Root Crops*11:1–8.

[5]Misra R.S. and Chowdhury S.R. 1997.*Phytophthora Leaf Blight Disease of Taro*.Technical Bulletin Series 21. Trivandrum, India: Central Tuber Crops Research Institute and St Joseph Press.

[6]Bandyopadhyay R., Sharma K., Onyeka T.J., Aregbesola A. and Kumar L.P. 2011.First Report of Taro (*Colocasiaesculenta*) Leaf Blight Caused by *Phytophthoracolocasiae* in Nigeria.*Disease Notes* 95(5): 618.

[7]Omane E.,Oduro K.A., Cornelius E.W., Opuku I.Y., Akrofi A.Y. Sharma K., Lava kumar S. and Bandyopadhyay R. 2012. First report of leaf blight of taro (*Colocasiaesculenta*) caused by *Phytophthora colocasiae* in Ghana. *Dis. Notes* 96(2): 292.

[8]Guarino L. 2010. Taro leaf blight in Cameroon? 2010. Agricultural Biodiversity Weblog. Available online: http:/agro.biodiver.se/2010/07/taro-leaf-blght-in-Cameroon/ Accessed on 15 May 2012).

[9] Scot N., Brooks F. &Teves G. 2011. Taro Leaf Blight in Hawai'I. *Plant Disease* 71: 1-14.

[10]Gurr, P. 1996. The taro leaf blight situation in American Samoa.Taro Leaf Blight Seminar.*Proceedings.Alafua, Western Samoa, 22–26 November*, 1993. (pp.35–38). Noumea, New Caledonia: South Pacific Commission

[11]Jackson G.V.H. 1999.*Taro leaf blight*.*Pest Advisory Leaflet 3*.Plant Protection Service of the Secretariat of the Pacific Community.2 p.

[12]Erwin D.C. and Ribeiro O.K. 1996.*Phytophthora Diseases Worldwide*. St Paul; MN, USA: APS Press.

[13]Trimanto, Sajidan and Sugiyarto 2010. Characterisation of Taro (*Colocasiaesculenta*) based on morphological and isozymic patterns markers. *Bioscience* 2 (1): 7-14

[14] Robin C.G. 2008. Commercial dasheen (*Colocasiaesculenta*(L.)Schott) var. *esculenta* production and post-harvest protocol for the OECS.Caribbean Agricultural Research and Development Institut- Dominica 31p.

[15] Bambara, H. M. J. 2009. Caractérisation agromorphologique d'une collection de taro (*Colocasiaesculenta* (L.) Schott) originaire des domaines soudanien et soudano-guinéen du Burkina Faso. Mémoire d'Ingénieur du Développement Rural, Université Polytechnique de Bobo- Dioulasso, Burkina Faso. 64p.

[16]Brooks F.E. 2008.Detached-Leaf biossay for evaluating taro resistance to *Phytophthoracolocasiae*.*Plant Disease* 92: 126-131.

[17]Fontem D.A., Olanya O.M., Tsopmbeng G.R. and Owona M.A.P. 2007.Metalaxyl sensitivity and cross infectivity of *Phytophthorainfestans* isolates from garden huckleberry, potato and tomato in Cameroon. *African Potato Association Conferences Proceedings* 7: 102-112.

[18]Brunt J., Hunter D. and Delp C. 2001.A bibliography of taro leaf blight.Secretariat of the Pacific Community, Noumea, New Caledonia.96 p.

[19]Gerrettson-Cornell L. 1994. A Compendium and classification of the species of the genus *Phytophthora* De Bary by the canons of the traditional taxonomy. Research Division, State Forests of New South Wales.Technical Paper (45), 96 p.

[20] Brooks F.E. 2005. Taro leaf blight.*The Plant Health Instructor*.
http://www.apsnet.org/edcenter/intropp/lessons/fungi/, consulted on 17 December 2012.

[21]Vishnu S.N., Muthukrishnan S., Vinayaka M. H. Muthulekshmi L. J., Raj S. M.Syamala S.V. and Mithun R. 2013. Genetic diversity of *Phytophthoracolocasiae*isolates in India based on AFLP analysis. *Biotech 3:297–305.*

[22]Fontem D.A., Tsopmbeng G.R., Owona M.A.P. &Olanya O.M. 2001. Infectivity and sensitivity to métalaxyl of *Phytophthorainfestans* isolates from garden huckleberry, potato and tomato. *Biosciences Proceeding* 8: 341-353.