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A new method by correlation to forecast the optimal time of spore-prints production and collection on sporocarps of *Ganoderma resinaceum* Boud. (Basidiomycota) on natural substrate

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Abstract

Spores features of most fungal species are of prime importance in the process of their identification. These spores could also be used for numerous other studies including fungal genetics, reproduction and molecular biology. Studies involving single spore cultures are numerous in various domains of mycology and these isolated spores could be obtained at best only from spore-prints. These spore prints are most often produced by sporocarps of most Agaricales on field and also in the laboratory after collection. In contrary, *Ganoderma* in general including *G. resinaceum* produce spore-prints only on field and for a limited period of time not covering all their life span. These spore-prints could therefore be collected only on the field during a well defined period. The correlation equation $Y = a+bX$ between size of sporocarps and time of growth now renders possible the forecast of the period of spore-prints production by sporocarps of *G. resinaceum* on natural substrate, thereby enabling collection of these spores at the best time on field for various mycological investigations.

Key words – basidiocarp size – correlation equation – field data – *Ganodermataceae* – life span – spore collection – time of growth

Introduction

The genus *Ganoderma* Karst with over 250 species (Ryvarden 1992, Chang & Buswell 1999) is a cosmopolitan genus recorded worldwide as well in tropical as in temperate climates. It belongs to the family Ganodermataceae with as key characters the shape and size of basidiospores and the texture of the pileus (Ryvarden 2000). Several species are found in tropical Africa (Bresadola 1890; Steyaert 1962, 1967, 1980; Futardo 1965, 1967; Zoberi 1972; Ryvarden & Johansen 1980; Ryvarden 1992; Hjortstam et al. 1993; Moncalvo & Ryvarden 1997; Roberts & Ryvarden 2006; Kinge 2012; Mossebo et al. 2014a; Mossebo & Kengni Ayissi 2014b) and numerous studies have so far been carried out in this genus due to its importance in various

scientific domains such as agriculture, forestry and medicine.

The taxonomy of *Ganoderma* as well as that of other groups of Macromycetes is primarily based on macro- and micromorphological characters of sporocarps, sometimes completed by molecular phylogeny. The micro-morphological features in particular refer among others to the morphology, size and ornamentations of spores and cuticle cells according to Steyaert (1972) and Ryvar den (2000) who warranted that “the two most important characters in the genus are the shape and size of basidiospores and cuticle cells”. Also, Ling-Chie Wong et al. (2012) later on added that “spore length can be used to distinguish *Ganoderma* spp.”. However and contrary to numerous other Macromycetes of other genera including most Agaricales of which the sporocarp pileus is known for most species to be able to keep on producing spores days after collection, sporocarps of *Ganoderma* in general produce spore-prints only on their natural substrate (Figs 1C-D) and for a limited time period most often ranging from maturity to the moment just preceding the beginning of senescence. Also, according to our field and lab monitoring, sporocarps cease producing spore-prints immediately after collection (Fig. 1F) from substrate, what renders absolutely necessary the collection of these spore-prints rather on living sporocarps still on substrate (Figs 1C-D) for taxonomic purposes (spore-print and spore colour(s), size, morphology, ornamentations), or more specific studies needing a great number of isolated individual spores such as genetics of reproduction, polarity in sexual reproduction by crossings of mycelium (Adaskaveg & Giltbertson 1986, Mossebo 1995, Mossebo et al. 1998) from single - or multispores cultures.

However such quantities of single- or multispores are hardly found in basidiocarps tissues (pores or tubes section) during morphological investigations and can only be obtained from spore-prints, which as said earlier and as far as *Ganoderma* in general are concerned, are produced only by living sporocarps still on substrate (Figs 1C-D) and just for a limited period of time in their life span.

The correlation equation $Y = a + bX$ [where Y represents the size (length (L) or width (l) of sporocarp growing on its natural substrate) and X is the time in days of growth] worked out by the authors for the first time as far as *G. resinaceum* is concerned, henceforth enables to determine the approximate time of growth and remaining life span of a young basidiocarp in active growth (Fig. 1E), as well as the approximate best time period for collection of spore-prints (Figs 1C-D) on the field for various mycological investigations.

Materials & Methods

Taxonomy and identification

Specimens of *Ganoderma* monitored on the field were first thoroughly scrutinized in taxonomy in order to determine whether they actually belong to *Ganoderma resinaceum* which is the more widespread and readily available species in the study area. Their macro- and micro-morphological features were first described according to various protocols used for Polyporales description (Ryvar den & Johansen 1980; Mossebo & Ryvar den 1997, 2003; Mossebo 2005; Mossebo et al. 2007) and more specific protocols for *Ganoderma* (Núñez & Ryvar den 2000; Ryvar den 2000, 2004; Kinge & Mih 2011; Kinge 2012; Mossebo et al. 2014a; Mossebo & Kengni Ayissi 2014b). These features were thereafter compared to those of existing taxa of *Ganoderma* described in the most reliable taxonomic studies cited above in order to determine whether the specimen actually belongs to *G. resinaceum*. Other specimens were sent to Ryvar den (personal communication) at the University of Oslo in Norway for cross-checking our preliminary identifications.

Correlation between time and growth parameters of sporocarps of *G. resinaceum* in vivo

Some growth parameters of *G. resinaceum* such as the length and width of 6 different sporocarps as 6 replicates growing on tree stumps and trunks (Figs 1A-B-C-D-E) at 6 different sites (Table 3) in Cameroon were monitored on a regular basis. *G. resinaceum* being the more widespread and readily available species in the study area, the 6 sporocarps were selected among

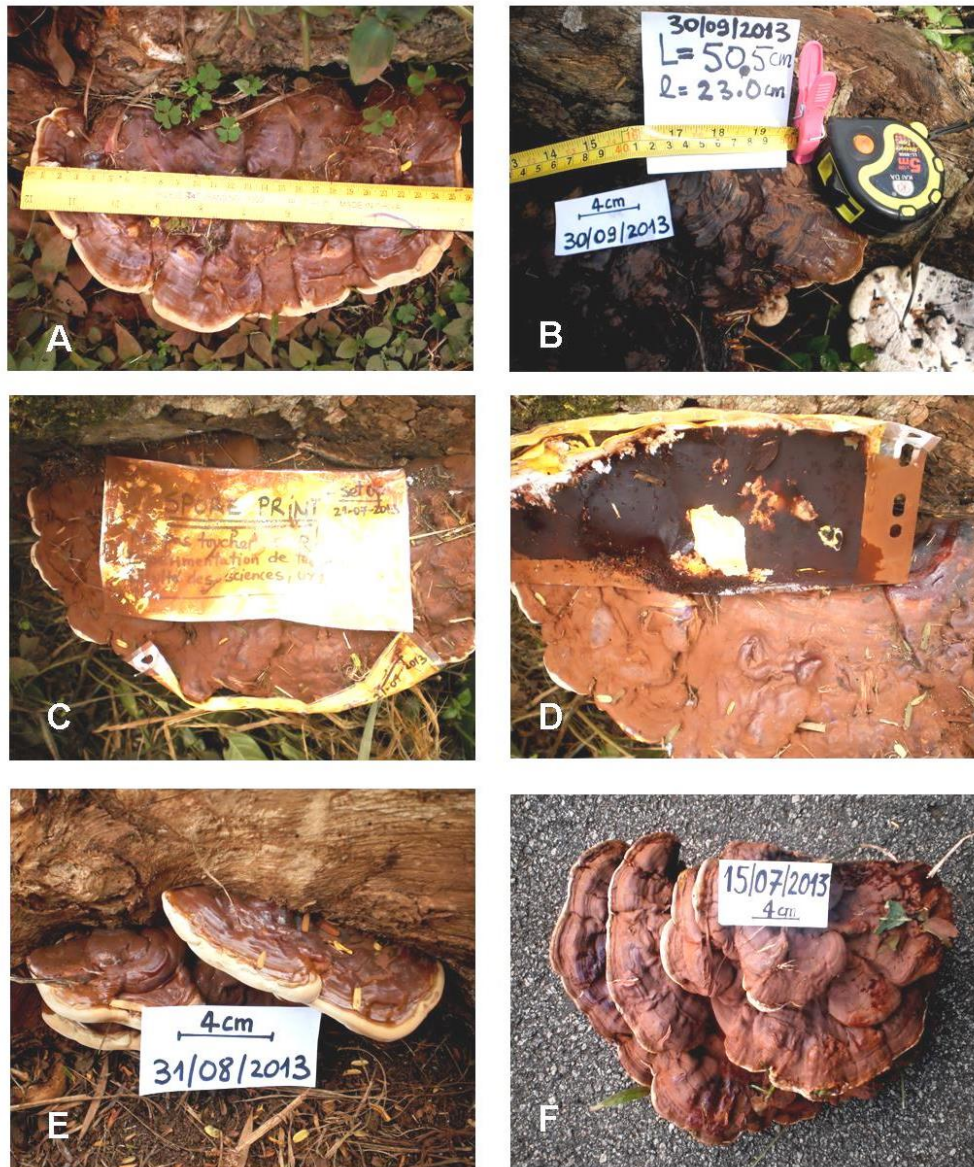


Fig. 1 – Monitoring growth and spore-print production of *Ganoderma resinaceum* on natural substrate (mostly dead wood). A-B Measurements of length and width of sporocarp. C-D Spore-print produced by sporocarp on natural substrate. E Young sporocarps in active growth. F Sporocarp cease producing spore-print once collected from substrate. Scale bars as shown on the figures.

other criteria, that they should be of equal size or nearly so at the launching of the study. Also, a sporocarp was selected only when there was no other fungal species on the same substrate in order to avoid possible negative effects of eventual mycoparasitism or fungal competition. Length and width of sporocarps were measured (Figs 1A-B) every 2 weeks over more than 250 days (Table 1 & 2) and the measurements correlated with time in days of growth, in order to compute for the first time since the first description (Boudier 1889) of *G. resinaceum*, the correlation equation $Y = a + bX$ (Mc. Donald 2014; Parker 1991) between length of sporocarp and time of growth on the one hand, and width and time of growth on the other hand. The natural production of spores as spore-prints was equally closely monitored by attaching with tailor needles pieces of contrast paper (Figs 1C-D) on the upper and lower sides of the 6 basidiocarps (6 replicates) at different sites (Table 3) in Cameroon in order to determine the time of growth corresponding to the beginning and end of production of spore-prints on natural substrate on the field. The spore-print colour was determined according to the colour chart of Kornerup & Wansher (1978).

Results

Correlation between time and some field growth parameters of *G. resinaceum*

Data in Table 1 & 2 and Figures 2 & 3 from the 6 replicates of *G. resinaceum* show that on natural substrate, sporocarps of this species which is annual to perennial (Ryvarden 2000, 2004) grow nearly 2 times faster in length than in width from young till mature stage of growth. Figs 2 & 3 show a continuous and steady increase of sporocarp length from the beginning till mature stage of growth, whereas the growth in width appears more slower to almost stagnant from nearly the 5th month (143 days) of growth. All the 6 replicates monitored started producing spore-prints in form of spore dust (Figs 1C-D) between the 5th and 6th month (150 to 180 days) of growth. The computed correlation equations $Y = 1.11 + 0.225X$ for the growth in length, and $Y = 3.1 + 0.0874X$ for the growth in width with their positive correlation coefficients $r = 0.998$ and $r = 0.98$ (Fig. 3) confirm a positive relationship between time and growth of sporocarp which reaches full maturity at averagely 253 days (about 8 and ½ months) old (Table 1 & 2, Fig. 3). Our data and field monitoring show that the natural production of spore-prints is maximal at this period and just few weeks later, it starts declining when sporocarps enter into senescence. However, still according to our field observations, most sporocarps of *G. resinaceum* live on their natural substrate (mostly dead wood) for about 10 to 12 months, even though some rare specimens stay alive for more than 12 months (365 days) with however no significant increase in length and width, before naturally ceasing all metabolism and start rotting. Walley & Rammeloo (1994) reported a *Ganoderma* sporocarp from Cameroon measuring 84 cm diameter upon collection, a figure which reported to the correlation equation $Y = 1.11 + 0.225X$ (Fig. 3) gives a figure of $X \approx 368.4$ days corresponding to a little bit more than a full year (12 months) of growth, assuming that the collected specimen was *G. resinaceum* which is one of the most widespread species in the area of collection in Cameroon. These figures remarkably corroborate our above mentioned field observations on the approximate life span of sporocarps of this species.

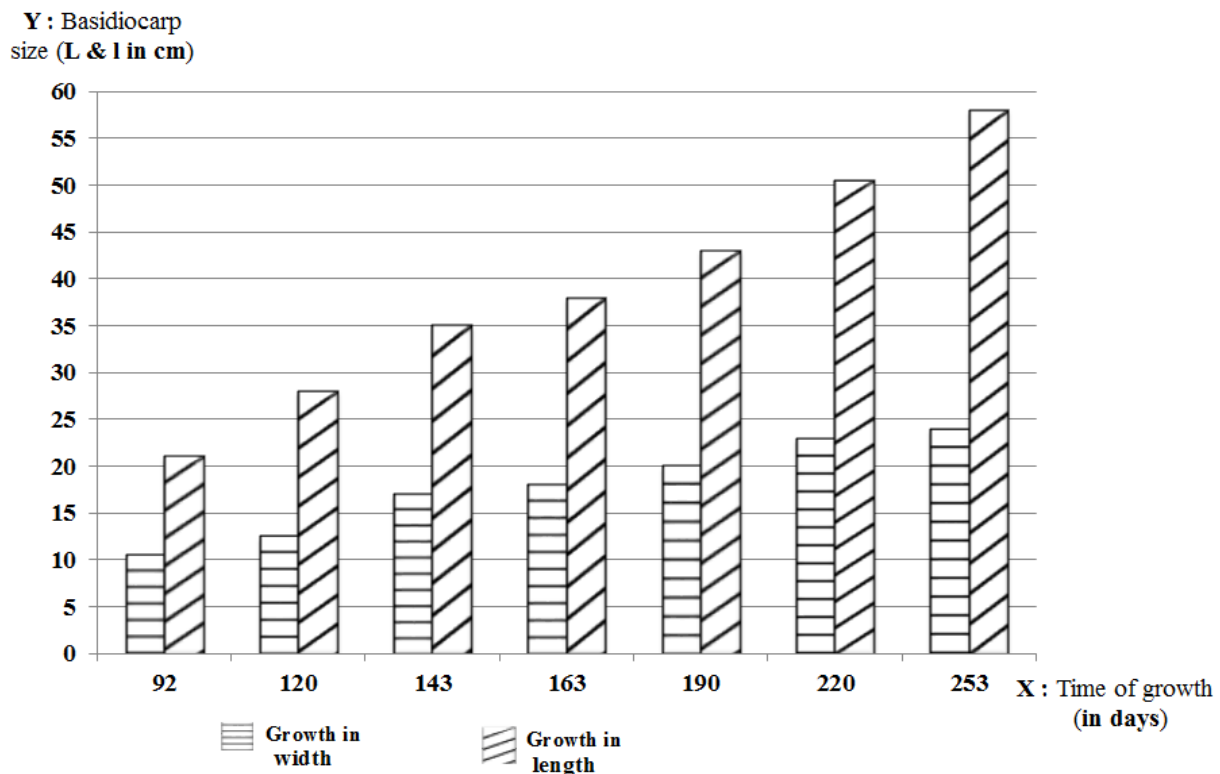


Fig. 2 – Histograms comparing the growth in length and in width of basidiocarps of *G. resinaceum* on natural substrate

All in all, considering that the average life span of a sporocarp of *G. resinaceum* is about 10 to 12 months (rarely more) in tropical climates (Table 3) and that sporocarps start producing spore-prints averagely between 5 to 6 months till about 8 to 9 months old as explained above, it is henceforth possible using the equation $Y = a+bX$ and the sporocarps measurements (L & l as Y) on the field, to work out and forecast - for a young sporocarp in active growth (Fig. 1E) on its natural substrate - several important parameters including:

- the approximate time of growth of sporocarp from its primordium stage till the date its length (L) and width (l) are measured on the field, that is the growth history of the sporocarp in time
- most importantly and using the data obtained in (a), the approximate best period (time on calendar) to start hunting spore-prints on the field, considering that they most often start appearing conspicuously in form of a chocolate-brown (8F5-7) dust (Figs 1C-D) on the upper side of sporocarps when these are about 5 to 6 months old which is about half the average life-span of this species
- the approximate period, as a time range on calendar, when sporocarps senescence will begin considering the above mentioned average life span of this species.

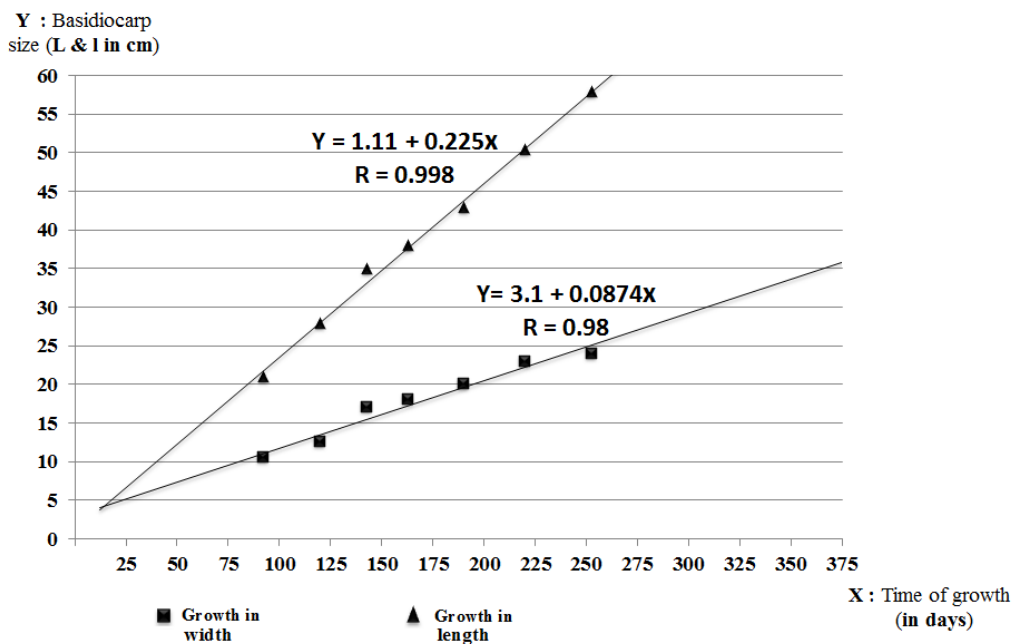


Fig. 3 – Correlation between size (L & l) of basidiocarps of *G. resinaceum* and time of growth in days

Statistical calculations

Table 1 Statistical data and correlation equation between growth in length (L) and time of sporocarp of *Ganoderma resinaceum* growing on natural substrate

X (time in days)	Y (growth in length)	X ²	Y ²	X.Y
92	21	8464	441	1932
120	28	14400	784	3360
143	35	20449	1225	5005
163	38	26569	1444	6194
190	43	36100	1849	8170
220	50,5	48400	2550,25	11110
253	58	64009	3364	14674

Each value of X and Y represents the mean of 6 replicates (6 different sporocarps monitored at different sites)

$$n = 7$$

$$\sum X = 1181; \quad \sum X^2 = 218391; \quad (\sum X)^2 = 1394761$$

$$\sum Y = 273.5; \quad \sum Y^2 = 11657.25; \quad (\sum Y)^2 = 74802.25$$

$$\sum X \cdot Y = 50445$$

Means of X and Y from Table 1:

$$\bar{X} = \frac{\sum X}{n} = \frac{1181}{7} = 168.71 \quad \text{et} \quad \bar{Y} = \frac{\sum Y}{n} = \frac{273.5}{7} = 39.07$$

Coefficient a and b of the regression line ($Y = a + bX$) for the growth in length:

$$b = \frac{\sum X \cdot Y - \frac{\sum X \cdot \sum Y}{n}}{\sum X^2 - \frac{(\sum X)^2}{n}} = \frac{50445 - \frac{1181 \times 273.5}{7}}{218391 - \frac{1394761}{7}} = 0.225$$

$$a = \bar{Y} - b \bar{X} = 39.07 - 0.225 \times 168.71 = 1.11$$

$$Y = 1.11 + 0.225X$$

Correlation coefficient (r):

$$r = \frac{\sum X \cdot Y - \frac{\sum X \cdot \sum Y}{n}}{\sqrt{\left[\sum X^2 - \frac{(\sum X)^2}{n} \right] \times \left[\sum Y^2 - \frac{(\sum Y)^2}{n} \right]}}$$

$$r = \frac{50445 - \frac{1181 \times 273.5}{7}}{\sqrt{\left[218391 - \frac{1394761}{7} \right] \times \left[11657.25 - \frac{74802.25}{7} \right]}} = 0.998$$

$$r = 0.998$$

Table 2 Statistical data and correlation equation between growth in width (l) and time of sporocarp of *Ganoderma resinaceum* growing on natural substrate

X (time in days)	Y (growth in width)	X ²	Y ²	X.Y
92	10,5	8464	110,25	966
120	12,5	14400	156,25	1500
143	17	20449	289	2431
163	18	26569	324	2934
190	20	36100	400	3800
220	23	48400	529	5060
253	24	64009	576	6072

Each value of X and Y represents the mean of 6 replicates (6 different sporocarps monitored at different sites)

$$n = 7$$

$$\sum X = 1181; \quad \sum X^2 = 218391; \quad (\sum X)^2 = 1394761$$

$$\sum Y = 125; \quad \sum Y^2 = 2384.50; \quad (\sum Y)^2 = 15625$$

$$\sum X \cdot Y = 22763$$

Means of X and Y from Table 2:

$$\bar{X} = \frac{\sum X}{n} = \frac{1181}{7} = 168.71 \quad \text{et} \quad \bar{Y} = \frac{\sum Y}{n} = \frac{125}{7} = 17.86$$

Coefficients a and b of the regression line (Y= a+ bX) for the growth in width:

$$b = \frac{\sum X \cdot Y - \frac{\sum X \cdot \sum Y}{n}}{\sum X^2 - \frac{(\sum X)^2}{n}} = \frac{22763 - \frac{1181 \times 125}{7}}{218391 - \frac{1394761}{7}} = 0.087$$

$$a = \bar{Y} - b \bar{X} = 17.86 - 0.0874 \times 168.71 = 3.1$$

$$Y = 3.1 + 0.087X$$

Correlation coefficient (r) for the growth in width:

$$r = \frac{\sum X \cdot Y - \frac{\sum X \cdot \sum Y}{n}}{\sqrt{\left[\sum X^2 - \frac{(\sum X)^2}{n} \right] \times \left[\sum Y^2 - \frac{(\sum Y)^2}{n} \right]}}$$
$$r = \frac{22763 - \frac{1181 \times 125}{7}}{\sqrt{\left[218391 - \frac{1394761}{7} \right] \times \left[2384.5 - \frac{15625}{7} \right]}} = 0.98$$

$$r = 0.98$$

Discussion and conclusion

Having set for the first time the correlation equation $Y = a + bX$ in order to estimate or anticipate parameters such as time of growth, life span and optimal period of collection of spore-prints on actively growing sporocarps of *G. resinaceum* on the field, this equation could be considered as a major progress in field mycology, since it henceforth enables to collect these spore-prints at the right time on mature sporocarps spotted before on the field just at their primordium or very young stage of growth.

The equation obtained here ($Y = 1.11 + 0.225X$ for the growth in length, and $Y = 3.1 + 0.0874X$ for the growth in width) with our field data in the growth conditions (Table 3) indicated, could be considered as a model applicable in most tropical parts of the world with comparable climates and this model could be used to work out other equations from data recorded in totally different conditions of growth. In fact, considering that *G. resinaceum* is a cosmopolitan species growing as well in tropical as in temperate climates, a correlation equation ($Y = a + bX$) could be worked out by monitoring sporocarps evolving in the northern hemisphere with relatively cold climates, and this will enable to assess whether these correlation equations are comparable and could apply in any part of the world, no matter the climate conditions in which the sporocarp grows.

As far as mycological studies including investigations or cultures of isolated single or multispores of *Ganoderma* in general and *G. resinaceum* in particular are concerned, the correlation equation could be considered as a major progress since it henceforth enables to avoid misjudgements of the real stage of growth and presumed period of spore-prints production by sporocarps on natural substrate. Using this correlation equation now offers the possibility of collecting these spore-prints at the right time on the field since sporocarps of this species and most other *Ganoderma* cease to produce spore-prints once they are collected from their natural substrate.

Data recorded on field show that when growing on dead wood (Table 3) which is their most suitable substrate, sporocarps of *G. resinaceum* of the same age or so, generally show no significant difference, neither in their size and other external features in direct relationship with the substrate species, nor in the quantity, density and regularity in the production of spore-prints.

Also, field observations show that climatic parameters such as rains, wind etc. had no significant effect on monitoring the optimal period of spore-prints production, since once the

Table 3 Geographical coordinates and climatic parameters of growth of the 6 replicates (6 sporocarps) of *Ganoderma resinaceum* monitored to compute the correlation equation

Replicate number	Herbarium number of sporocarps monitored on the field	Period of start and end of sporocarps monitoring	Growth area of sporocarps monitored	GPS coordinates of the growth area	Substrate (mostly dead wood)	Some geographical and climatic parameters of the growth and monitoring area of sporocarps
1	HUY1-DM 887	Nov. 2012 to Oct. 2013	Campus of the University of Yaoundé1/Cameroon	N 03° 51' 31,6" E 11° 29' 59"	Stump of angiosperm	<ul style="list-style-type: none"> • Average yearly temperature: 24.72°C.
2	HUY1-DM 888	Nov. 2012 to Oct. 2013	Yaoundé/Cameroon	N 03° 52' 21" E 11° 31' 03"	Stump of oil palm tree (<i>Elaeis guineensis</i>)	<ul style="list-style-type: none"> • Average annual rainfall: 1537.2 mm. • Average monthly rainfall: 128.1 mm.
3	HUY1-DM 889	Nov. 2012 to Oct. 2013	Yaoundé/Cameroon	N 03° 51' 38" E 11° 30' 07"	Stump of angiosperm	<ul style="list-style-type: none"> • Average yearly air moisture: RH = 77.1%
4	HUY1-DM 890	Nov. 2012 to Oct. 2013	Yaoundé/Cameroon	N 03° 52' 28" E 11° 31' 04"	Stump of oil palm tree (<i>Elaeis guineensis</i>)	<ul style="list-style-type: none"> • Average altitude: 750 m
5	HUY1-DM 891	Nov. 2012 to Oct. 2013	Yaoundé/Cameroon	N 03° 51' 33" E 11° 31' 02"	Tree trunk	<ul style="list-style-type: none"> • Photoperiodicity: about equally 12 hours of daylight and 12 hours of darkness over the 12 months of the year.
6	HUY1-DM 892	Nov. 2012 to Oct. 2013	Yaoundé/Cameroon	N 03° 52' 26" E 11° 32' 06"	Stump of angiosperm	

sporocarps of *G. resinaceum* have reached maturity, they start producing spore-prints continuously and mostly overnight, so that even just few hours or days after a rainfall, new dust of spore-prints appears on the pileus.

About the status of *Ganoderma* species in general which are said to be annual to perennial according to several authors (Ryvarden & Johansen 1980; Breitenbach & Kränzlin 1986, Giltbertson and Ryvarden 1986; Ryvarden & Giltbertson 1993; Ryvarden 2000, 2004), and as far as *G. resinaceum* is concerned, we can here affirm on the basis of our field observations (Mossebo et al. 2014a, Mossebo & Ayissi 2014b) over several years on numerous specimens of this species and also the sporocarps specially monitored in the framework of this study, that in tropical conditions (Table 3), sporocarps of *G. resinaceum* tend to be more annual than perennial, except for some rare specimens found underwood under the wide canopy of the green tropical forest where they undoubtedly benefit from cooler biotopes and lower temperatures that preserve them better and for longer periods and this explains why perennial specimens are found much more in cold climates of the northern hemisphere. Otherwise, most sporocarps of *G. resinaceum* monitored in savannahs or in urban areas and growing in tropical conditions close to those mentioned earlier (Table 3) rarely exceed a life span of 10 to 12 months as mentioned in our results, this presumably due to heat, high temperatures and high air moisture that do not at all favour conservation for long periods of time, but rather favour development of various types of moulds and insects which contribute to a much more rapid deterioration of sporocarps on their natural substrate and thereby reduce their life span and potential period of collection of spore-prints on the field in tropical conditions.

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