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Epidemiology and Etiology of *Ganoderma* Upper and Basal Stem Rot in Oil Palm on Peat in Sarawak

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Assoc. Prof. Joseph Bong Choon-Fah (UPM)
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Knowledge Sharing at the 14th MPOB/Industry Meeting, 8 March 2017

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SOARING UPWARDS

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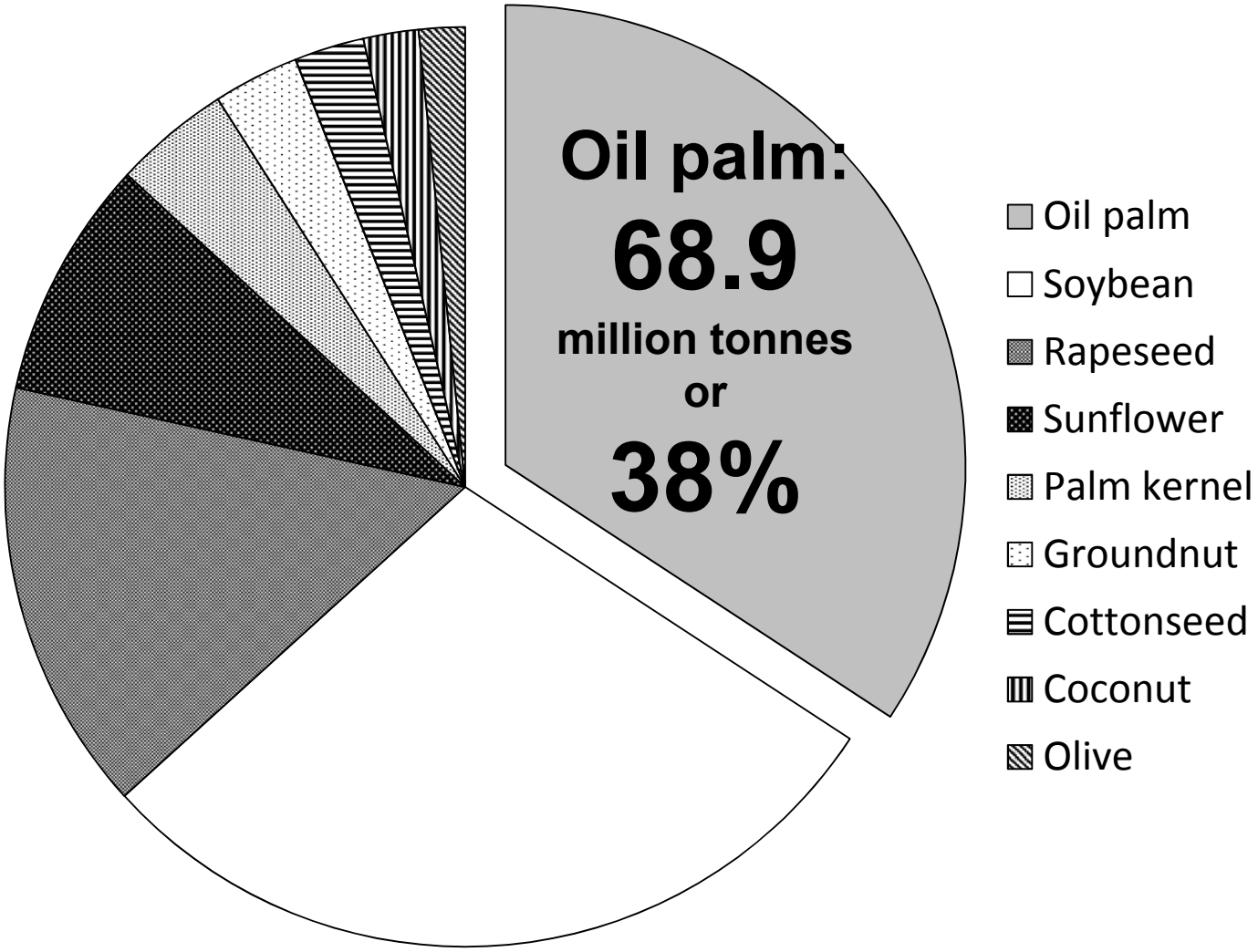
- **INTRODUCTION**
- **ASPECTS**
  1. **Genetic and morphological diversity**
  2. **Aggressiveness**
  3. **Symptoms, occurrence and spatio-temporal distribution**
  4. **Relationship between oil palm nutrients status and spatial distribution of *Ganoderma* species**
- **SUMMARY, GENERAL CONCLUSION AND RECOMMENDATION FOR FUTURE RESEARCH**

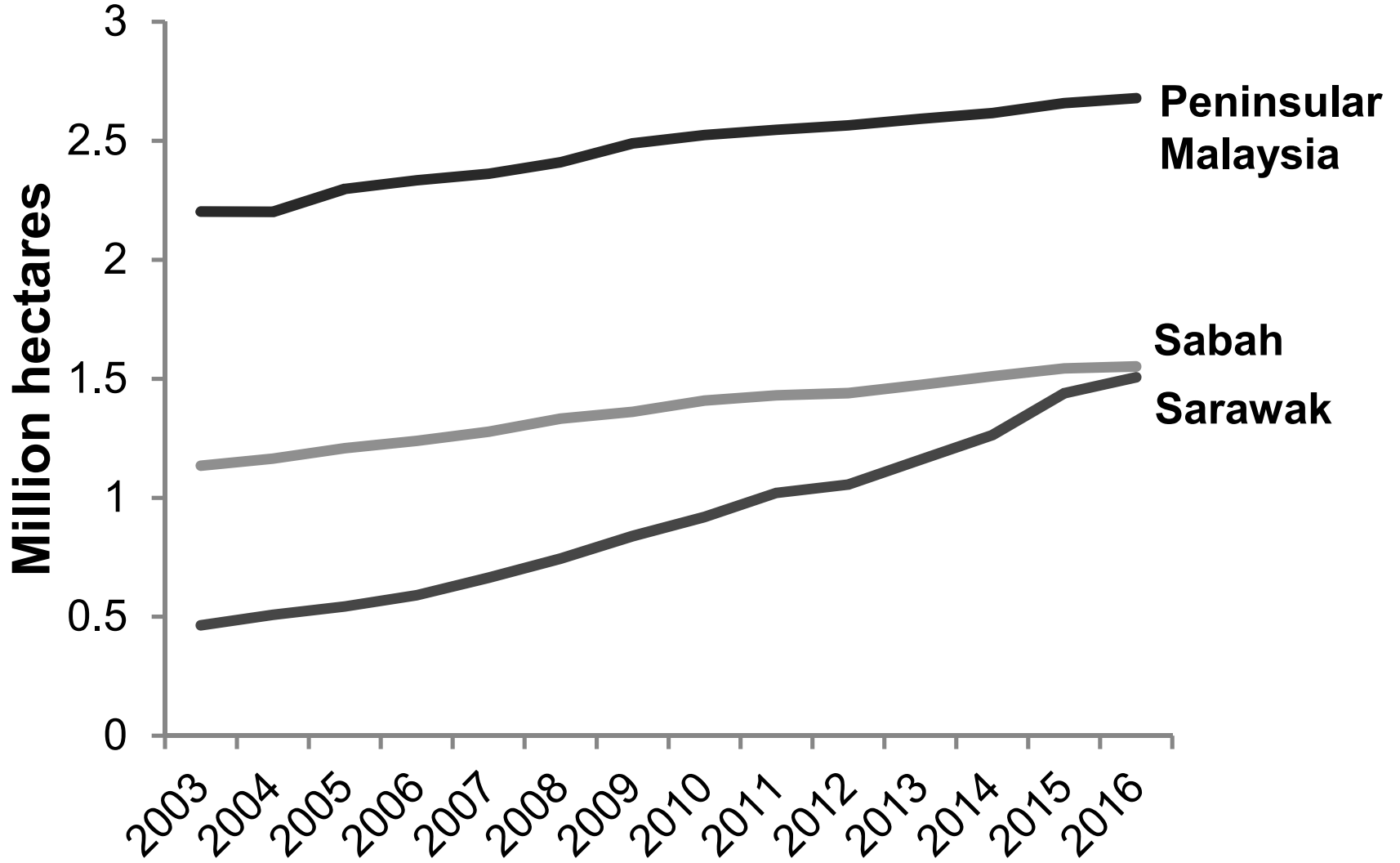
INTRODUCTION

World's major vegetable oil producing crops in 2015



(USDA, 2016)

Malaysia: Plantation area (hectare) over 13 years (2003 – 2016)



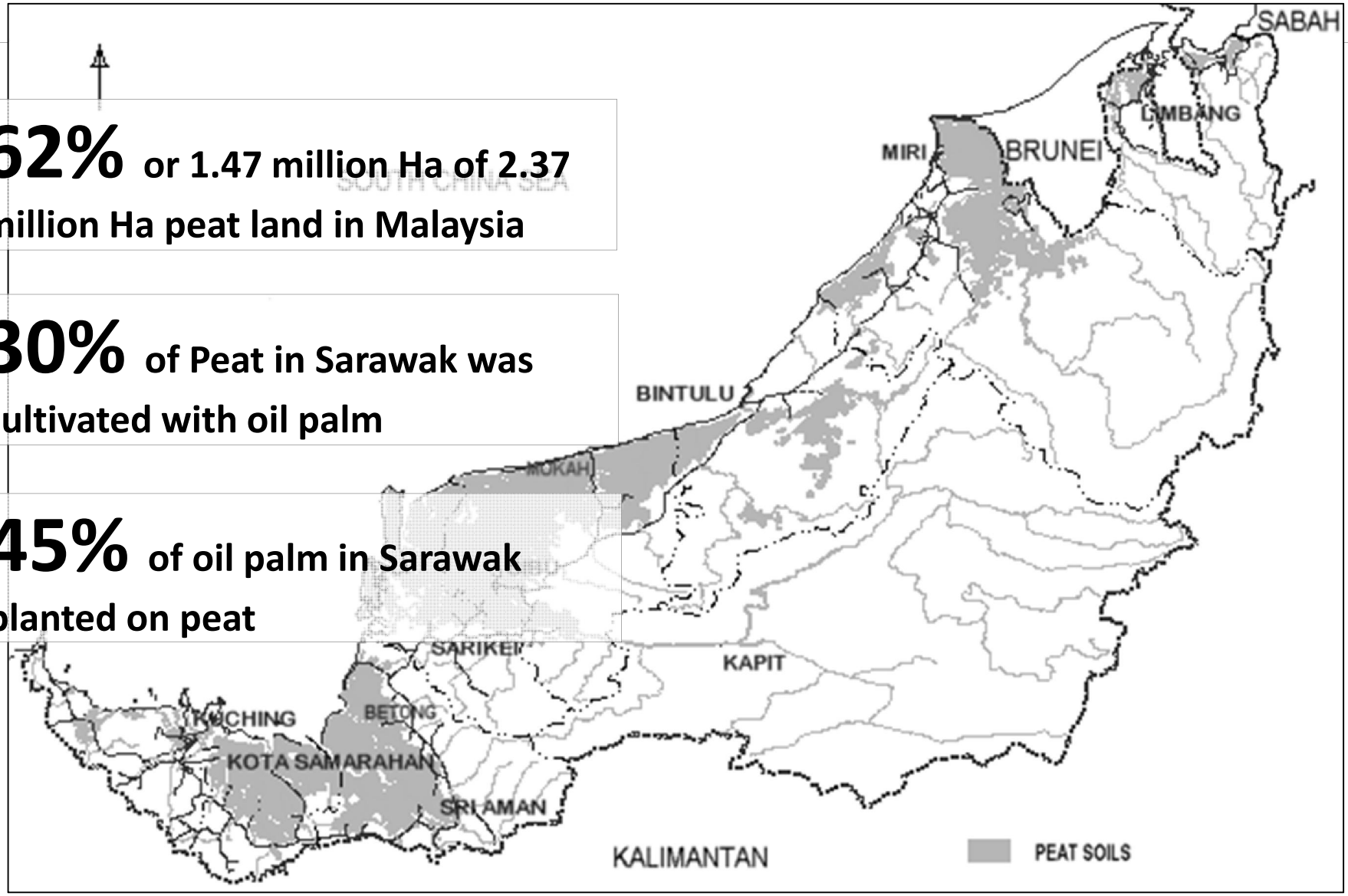
(MPOB, 2016)

Peat for oil palm cultivation in Sarawak

62% or 1.47 million Ha of 2.37 million Ha peat land in Malaysia

30% of Peat in Sarawak was cultivated with oil palm

45% of oil palm in Sarawak planted on peat



(Said *et al.*, 2009, Lim *et al.*, 2012)

Sustainability of oil palm

➢ Threats: Pests and diseases

- ➢ Reduction in terms of yield
- ➢ Shortening the economic life of an oil palm
- ➢ Maybe could wipe out the whole plantation

Disease of oil palm: Basal stem rot (BSR)

Disease:

Basal stem rot (BSR)

Pathogen:

Ganoderma boninense

Disease of oil palm: Upper stem rot (USR)

Combat against *Ganoderma* spp.

CULTURAL

- Resistant planting material
- Cover crops
- Fertilizers manipulation
- Estate sanitation

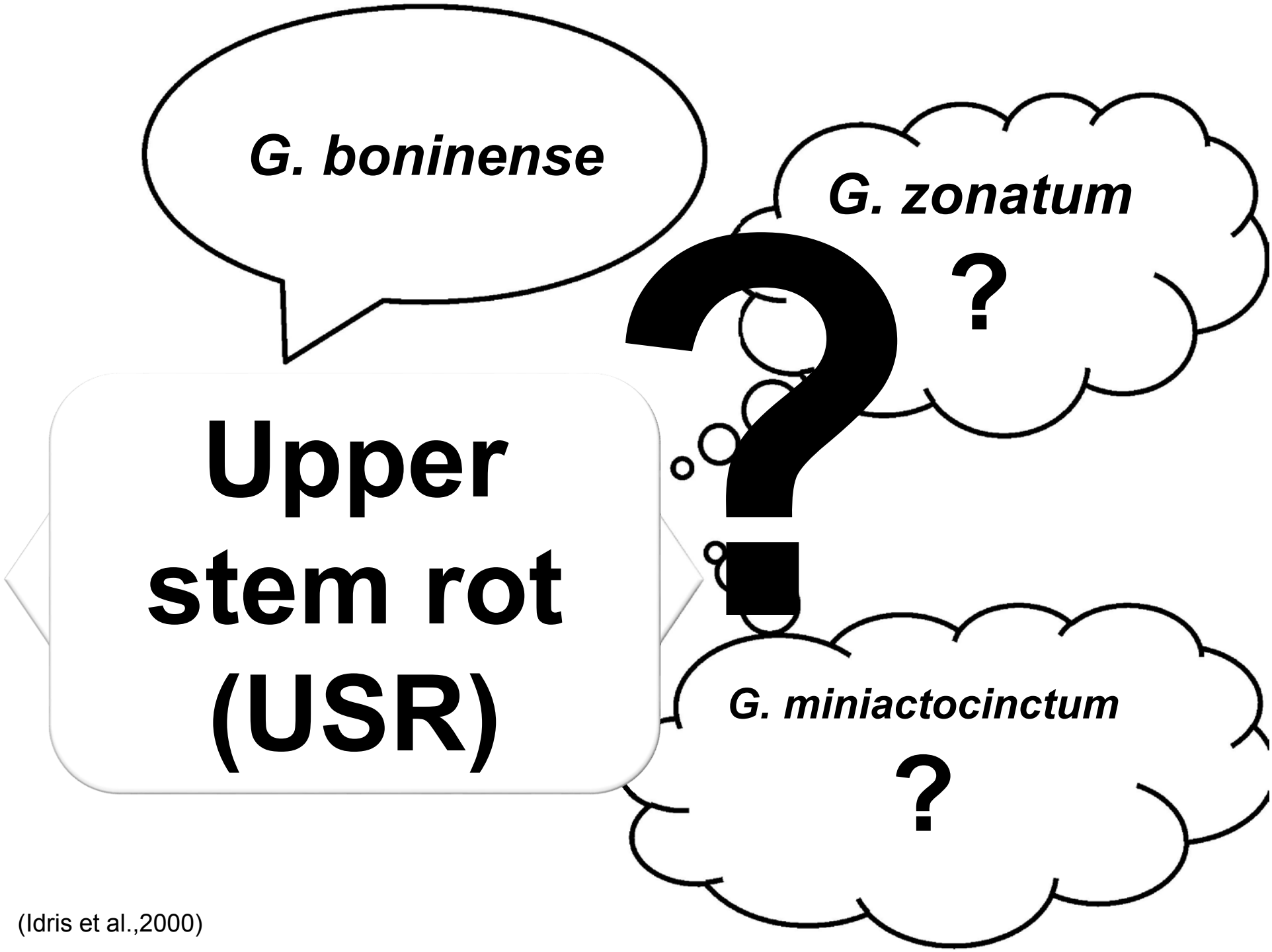
CHEMICAL

Fungicides
- Spray
- Trunk injection

BIOLOGICAL

Bio-control agents
- Bacteria
- Fungi

Ganoderma remains as the **major threats** to the **sustainability** of oil palm

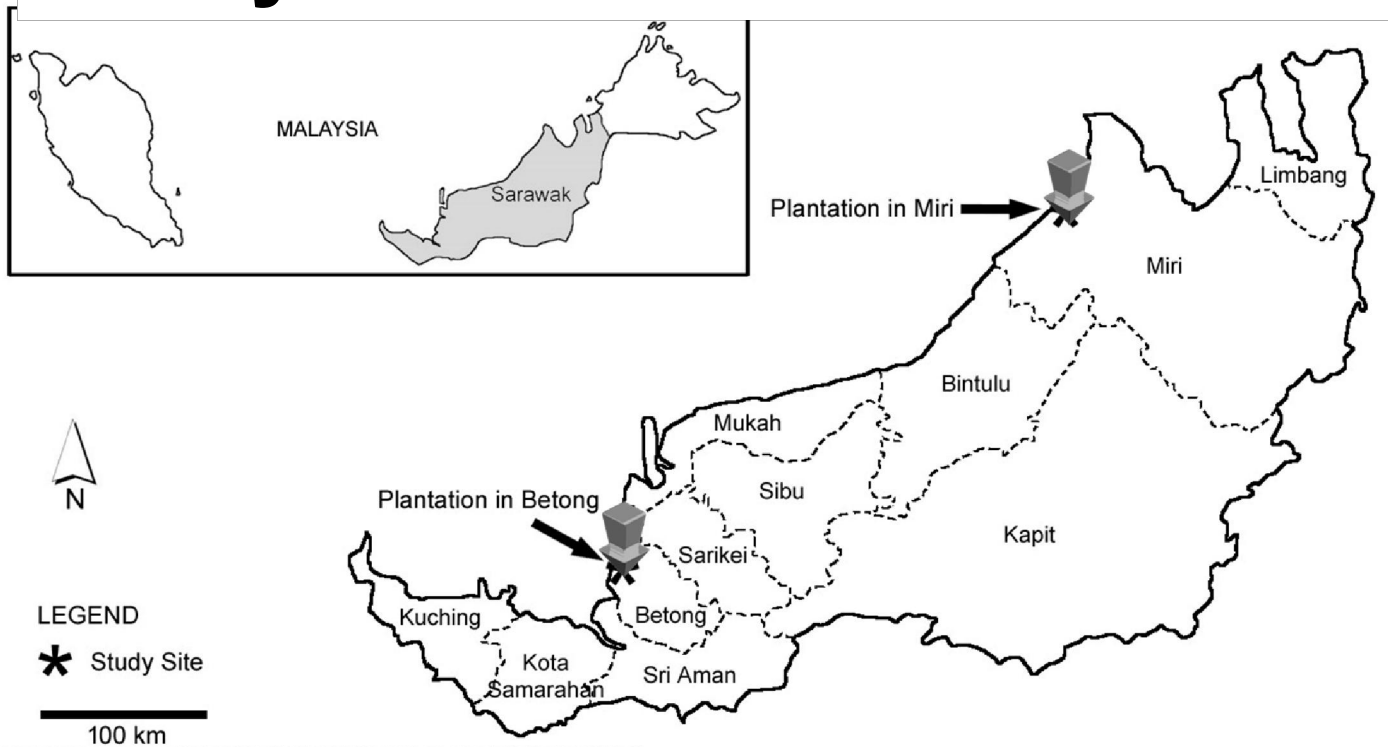


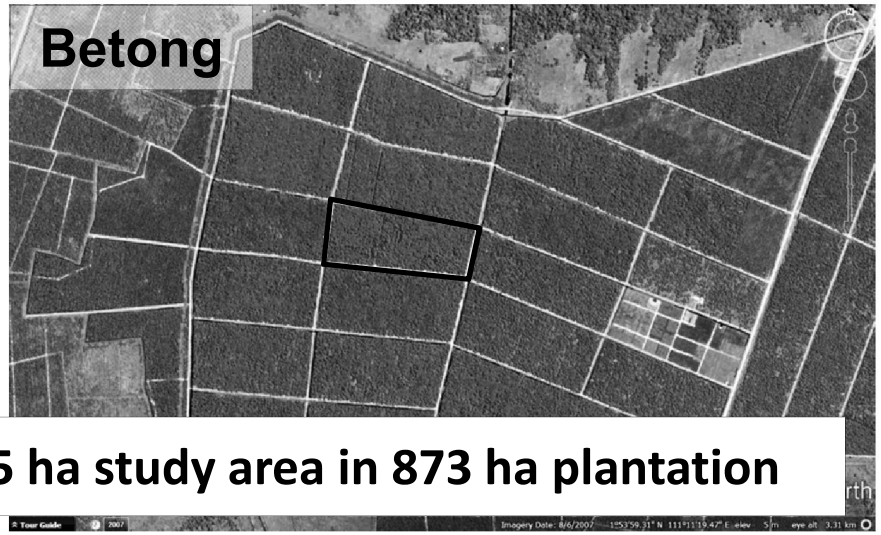
(Idris et al.,2000)

OBJECTIVE 1

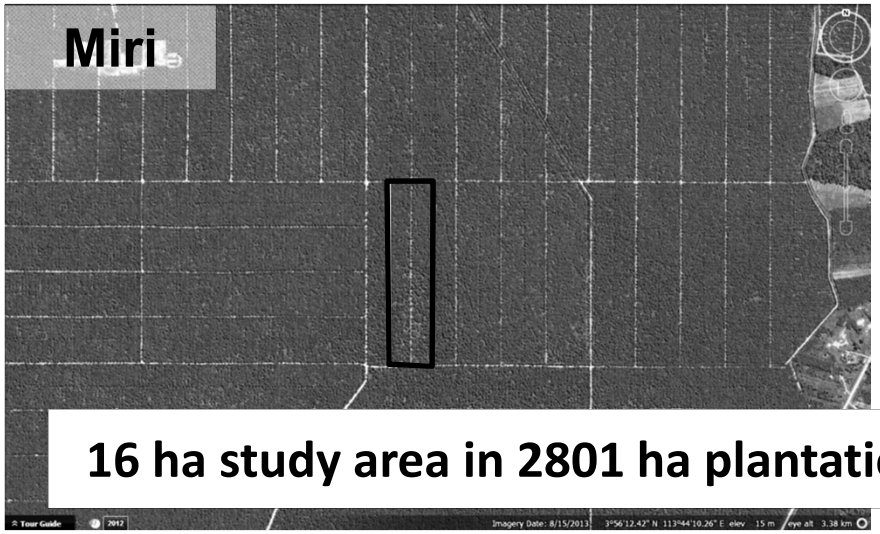
Investigate the genetic and morphological diversity of *Ganoderma* species associated with USR and BSR in oil palm

1 Study Sites





15 ha study area in 873 ha plantation

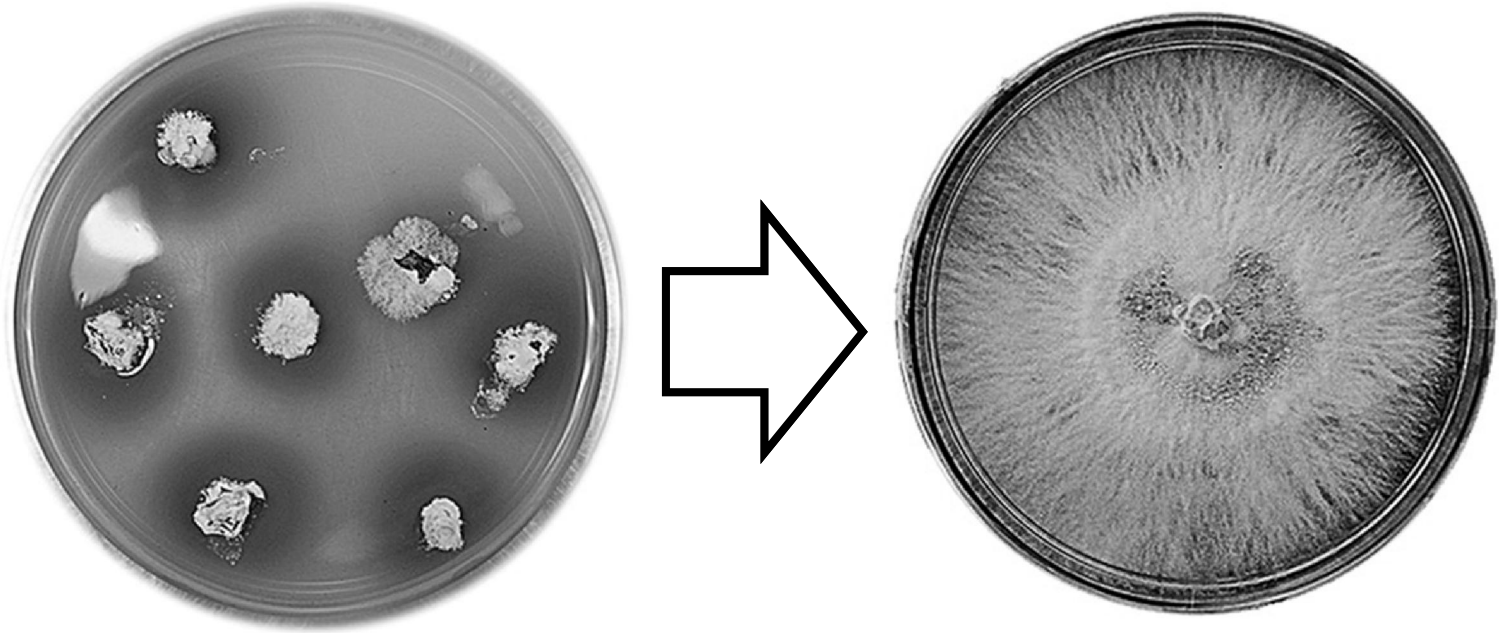


16 ha study area in 2801 ha plantation

2 Random sample collection of *Ganoderma* basidiomata from USR- and BSR infected palms

Site	Disease	Code of sample	No. of sample
Betong	USR	G1 to G14	14
	BSR	G15 tot G21	7
Miri	USR	G22 to G37	16
	BSR	G38 to G46	9
TOTAL SAMPLES			**46**

3 Isolation of *Ganoderma* on *Ganoderma* selective medium (GSM)



Isolation on GSM

Pure culture on PDA

(Ariffin and Idris, 1992; Rees *et al.*, 2007)

4 Identification of *Ganoderma* using multiplex PCR

Mycelia in PDB → DNA extraction → **Multiplex PCR** → Gel electrophoresis

(Idris *et al.*, 2010; Wong *et al.*, 2012)

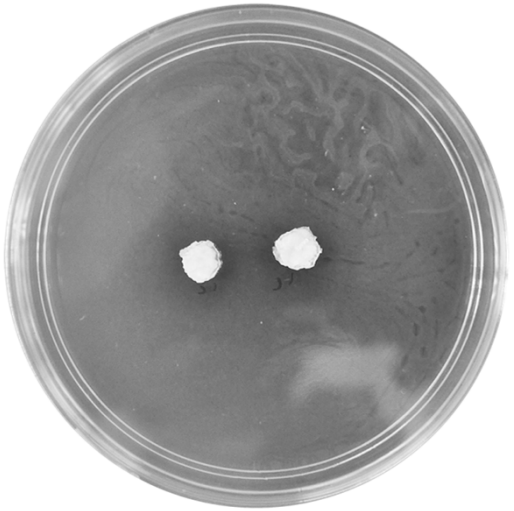
5 Somatic compatibility study of *Ganoderma*

Pairing of isolates in all combination on PDA

Incubation for 14 days

Assessments

Compatible	**Incompatible**
(Isolates merged into single colony)	(Formation of inhibition zone / barrage between isolates)



(Miller *et al.*, 1999)

6 *In-vitro* cultural characteristics of *Ganoderma*

Cultures of *Ganoderma* on PDA

Incubation for 14 days

Assessments

- **Colony diameter (daily)**
- **Days for full plate**
- **Colony's texture, appearance of zone, and surface and reverse colour (7th day)**

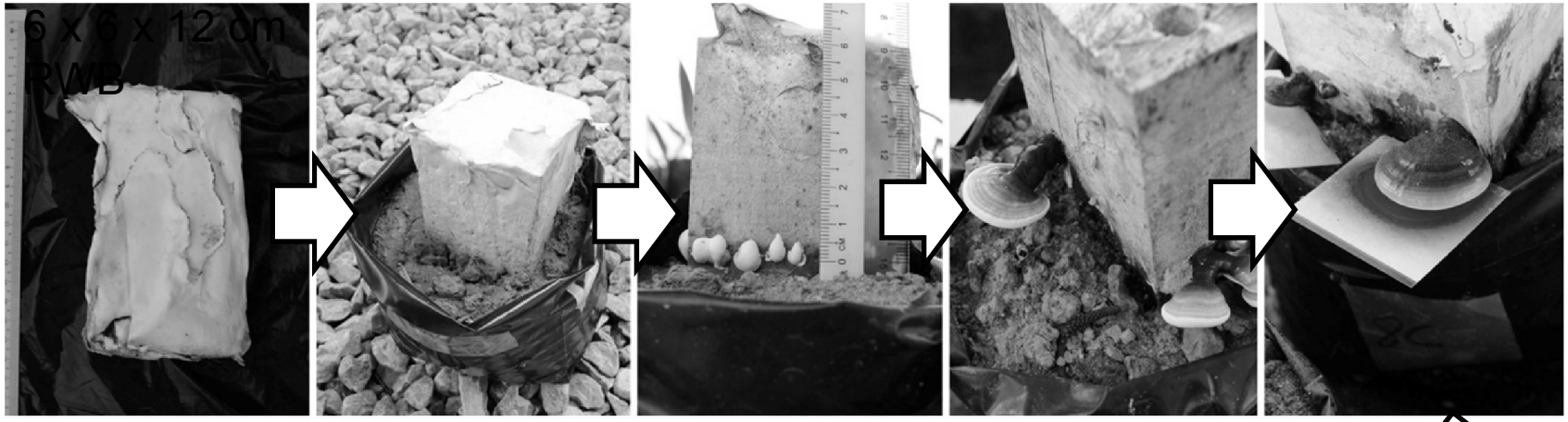
Qualitative data → Transformed to 25 codes → Binary matrix generated

Similarity matrices using simple matching coefficient

Dendrogram using UPGMA

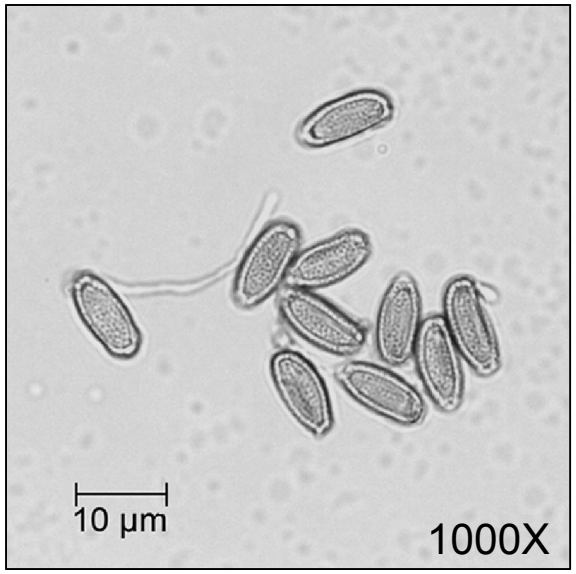
(Idris *et al.*, 2000)

7 Basidiospores characteristics of *Ganoderma*



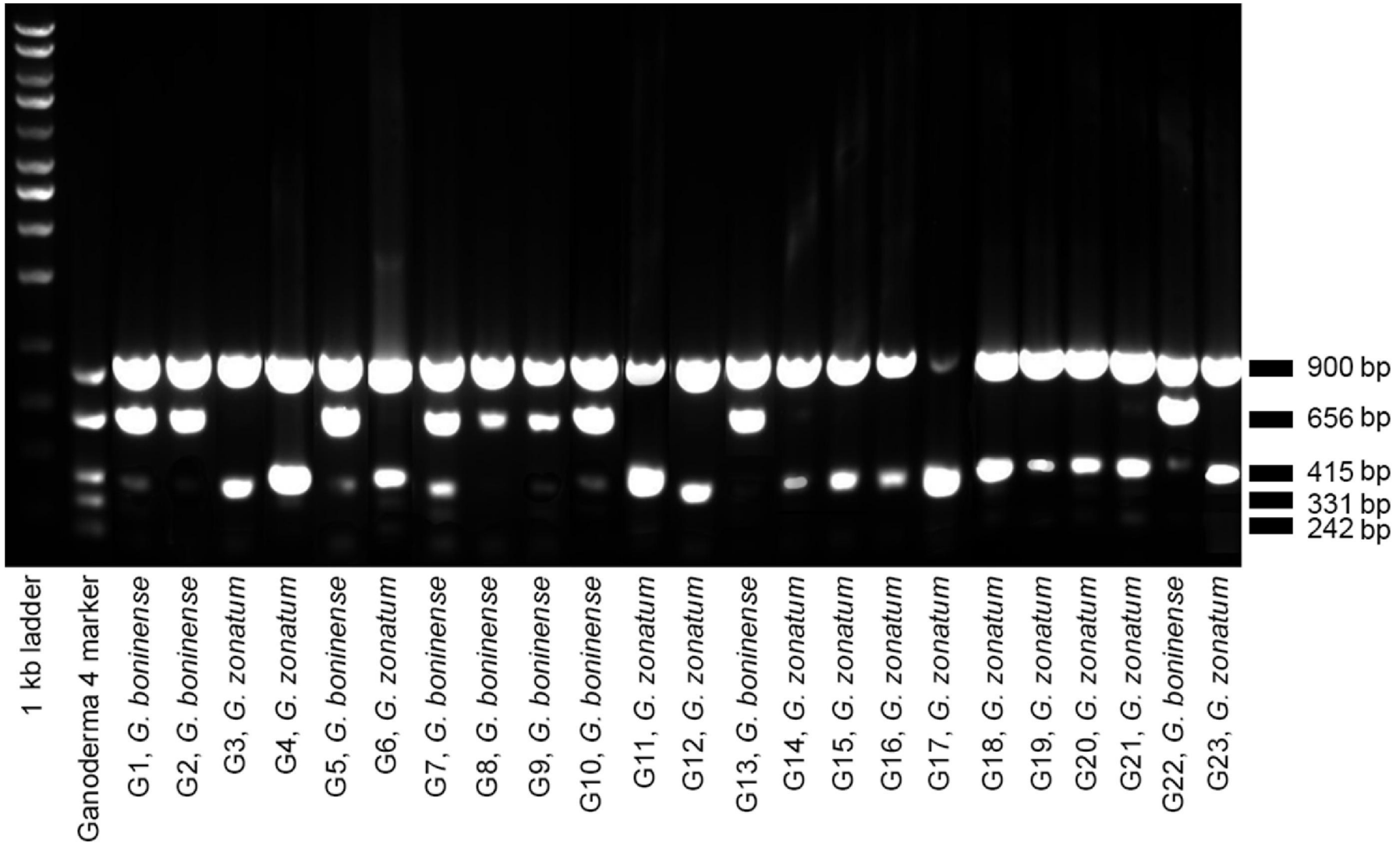
Assessments of basidiospores:

- Shape
- Length
- Diameter
- Spore shape index (SSI) = diameter / length x 100

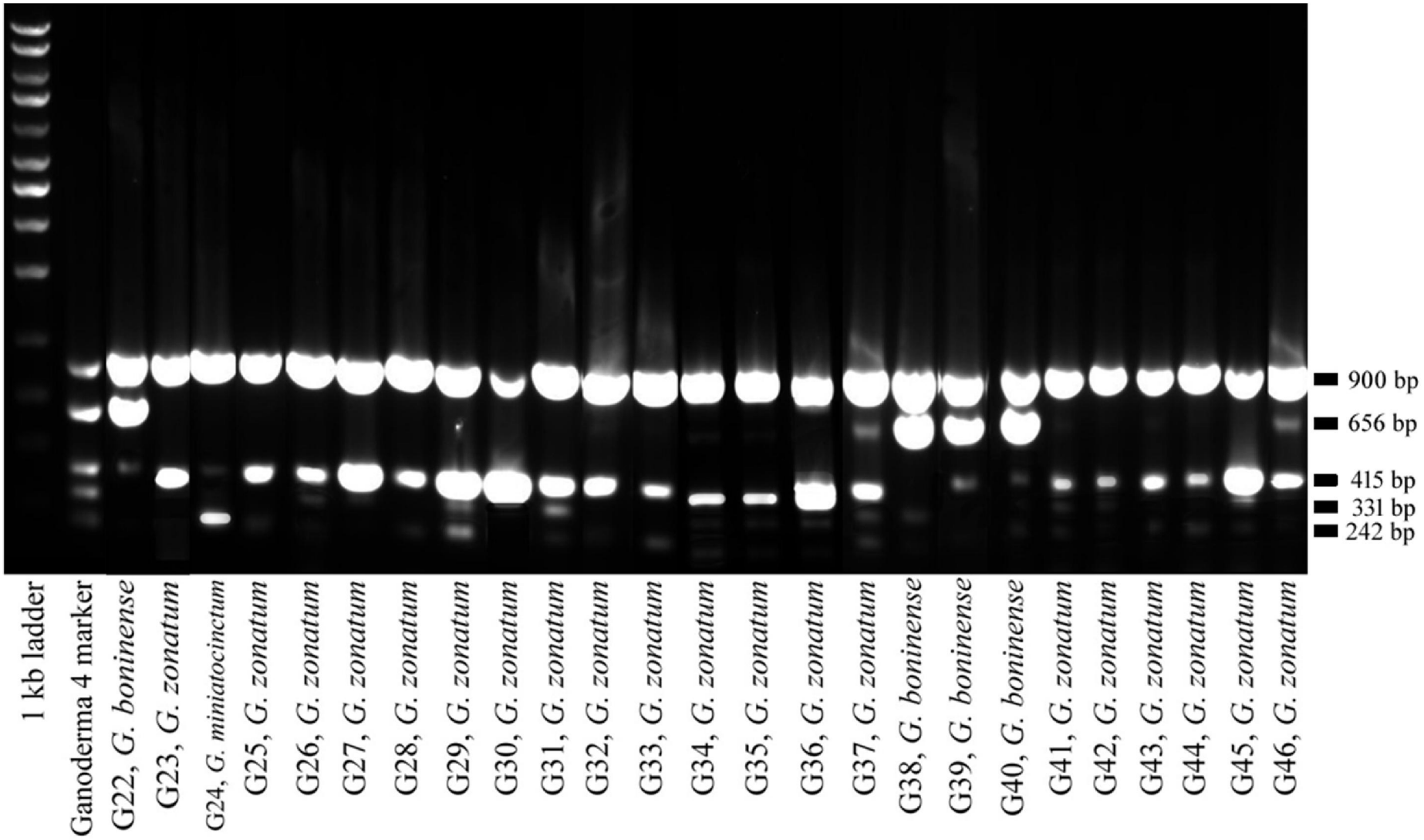


(Miller *et al.*, 1999)

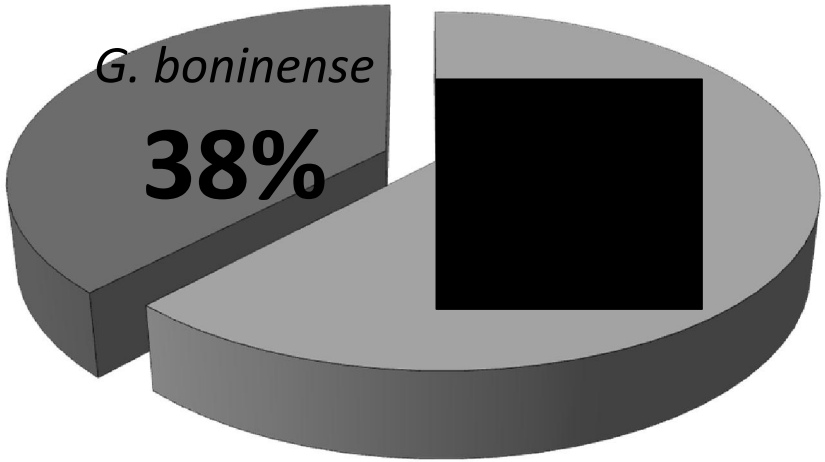
Identity of *Ganoderma* spp.
Gel electrophoresis of multiplex PCR



Identity of *Ganoderma* spp.
Gel electrophoresis of multiplex PCR

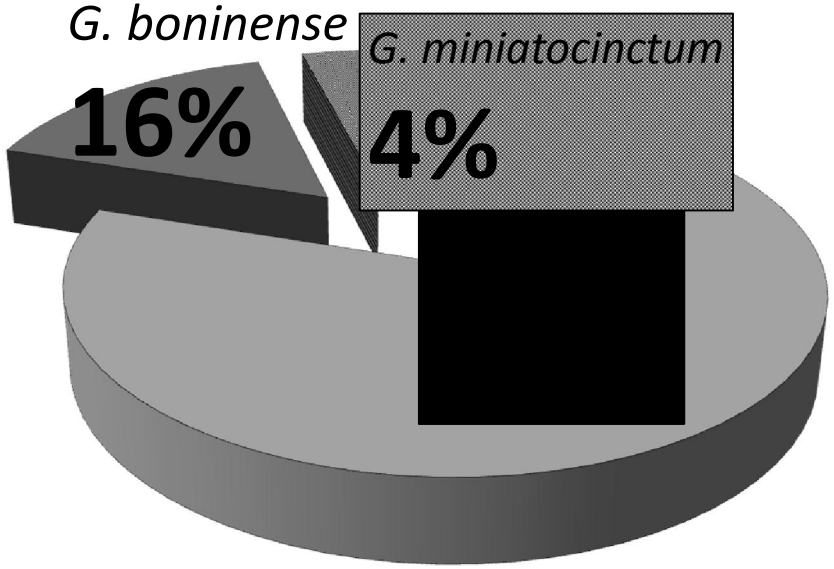


Betong and Miri: Identity of *Ganoderma* spp.

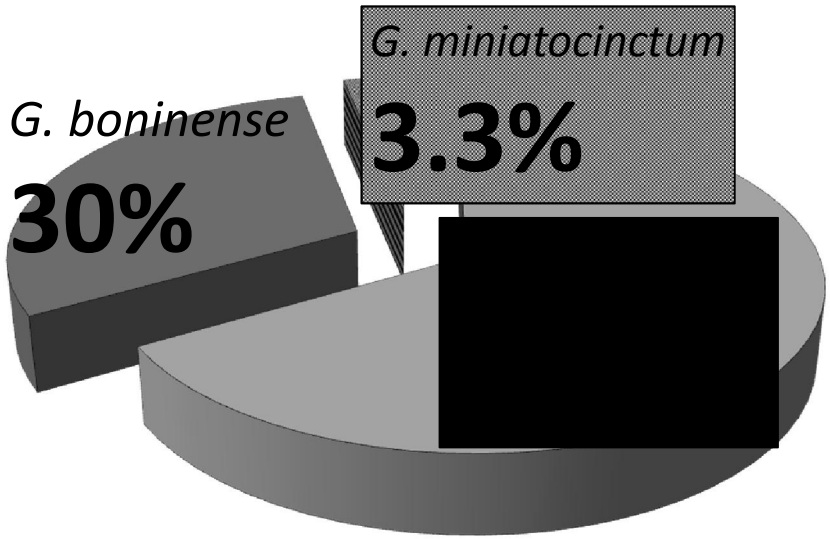


Betong

Miri

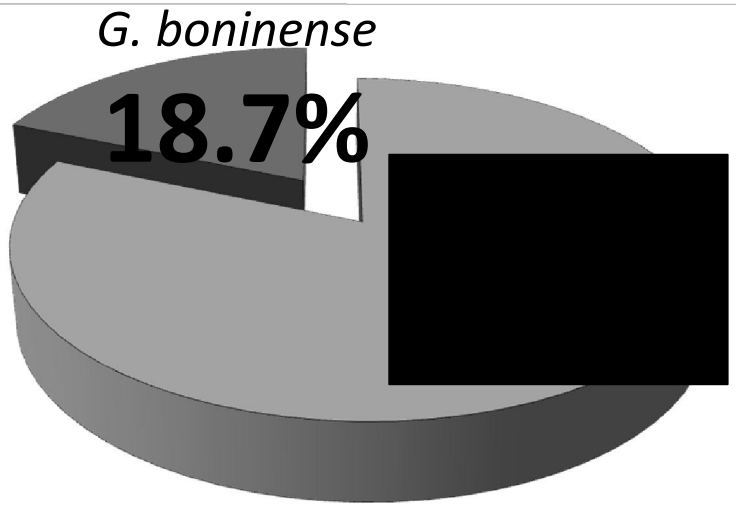


USR and BSR: Identity of *Ganoderma* spp.

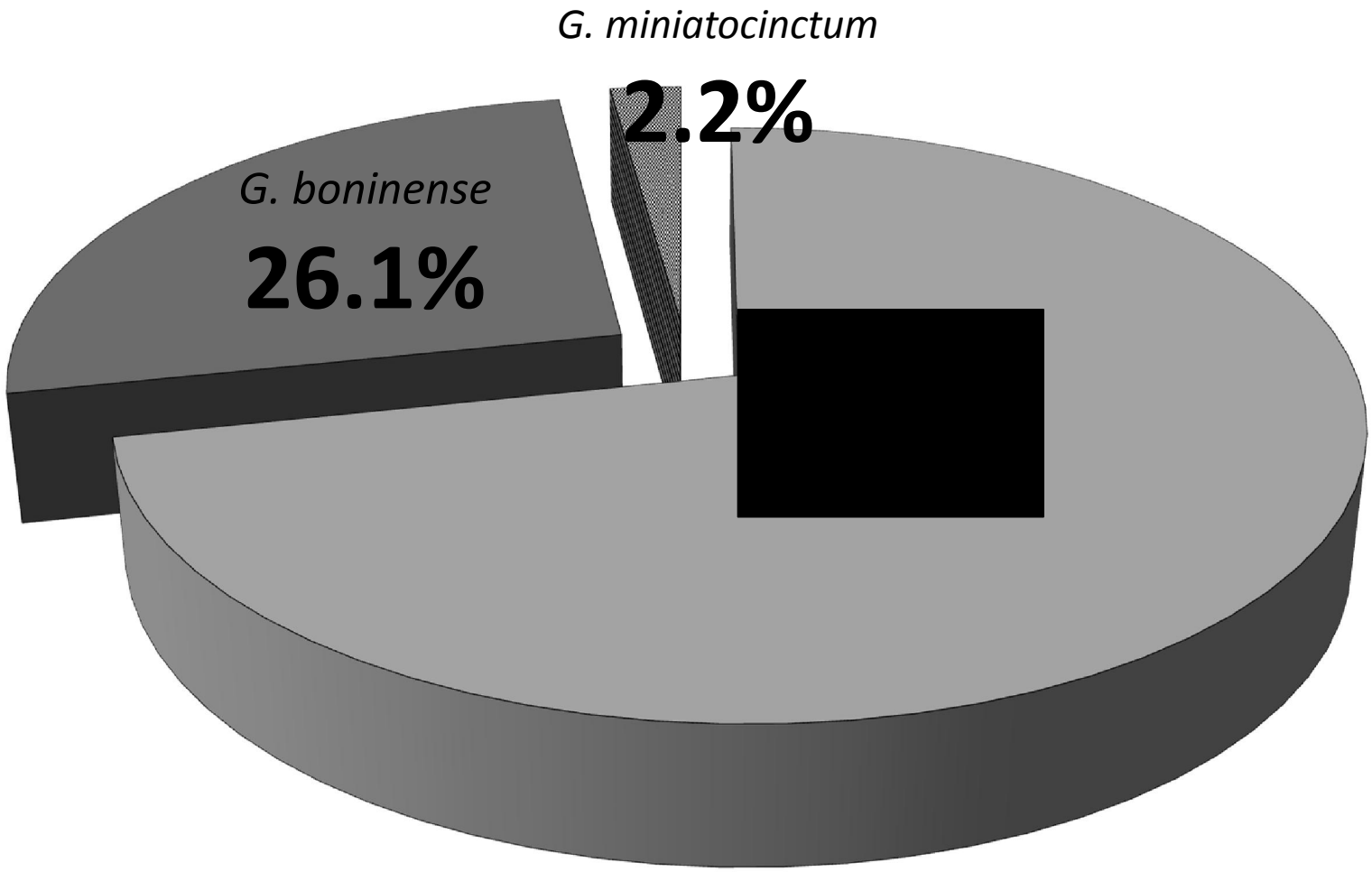


USR

BSR

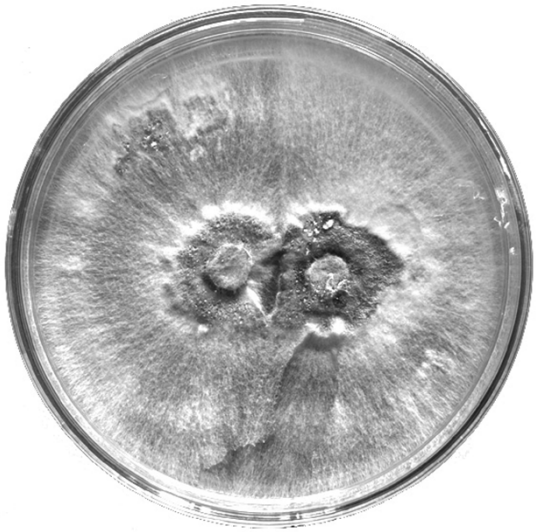


Overall: Identity of *Ganoderma* spp.



Genetic compatibility

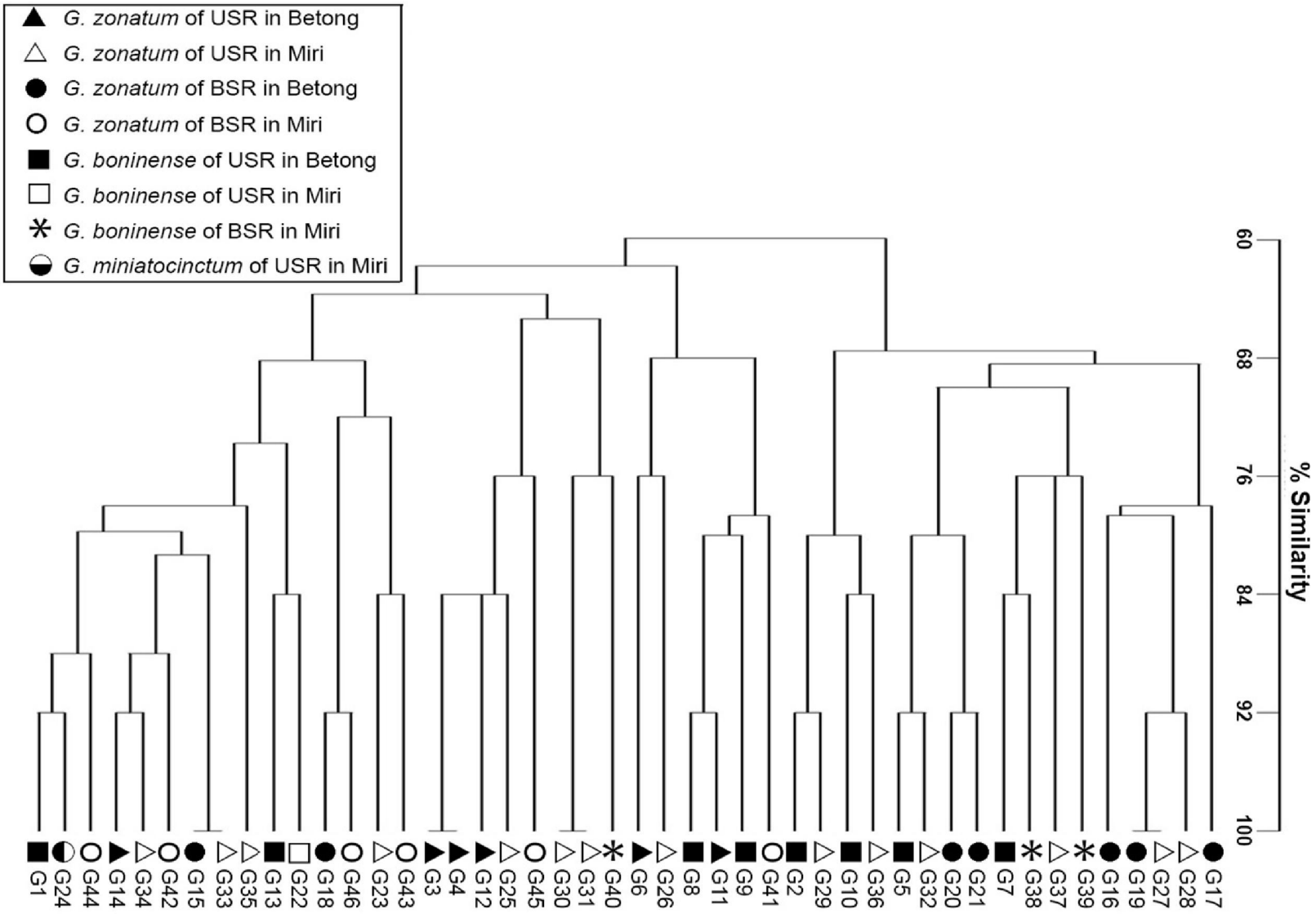
- None of the isolates were compatible, except in control
- Incompatible isolates formed inhibition zone / barrage line



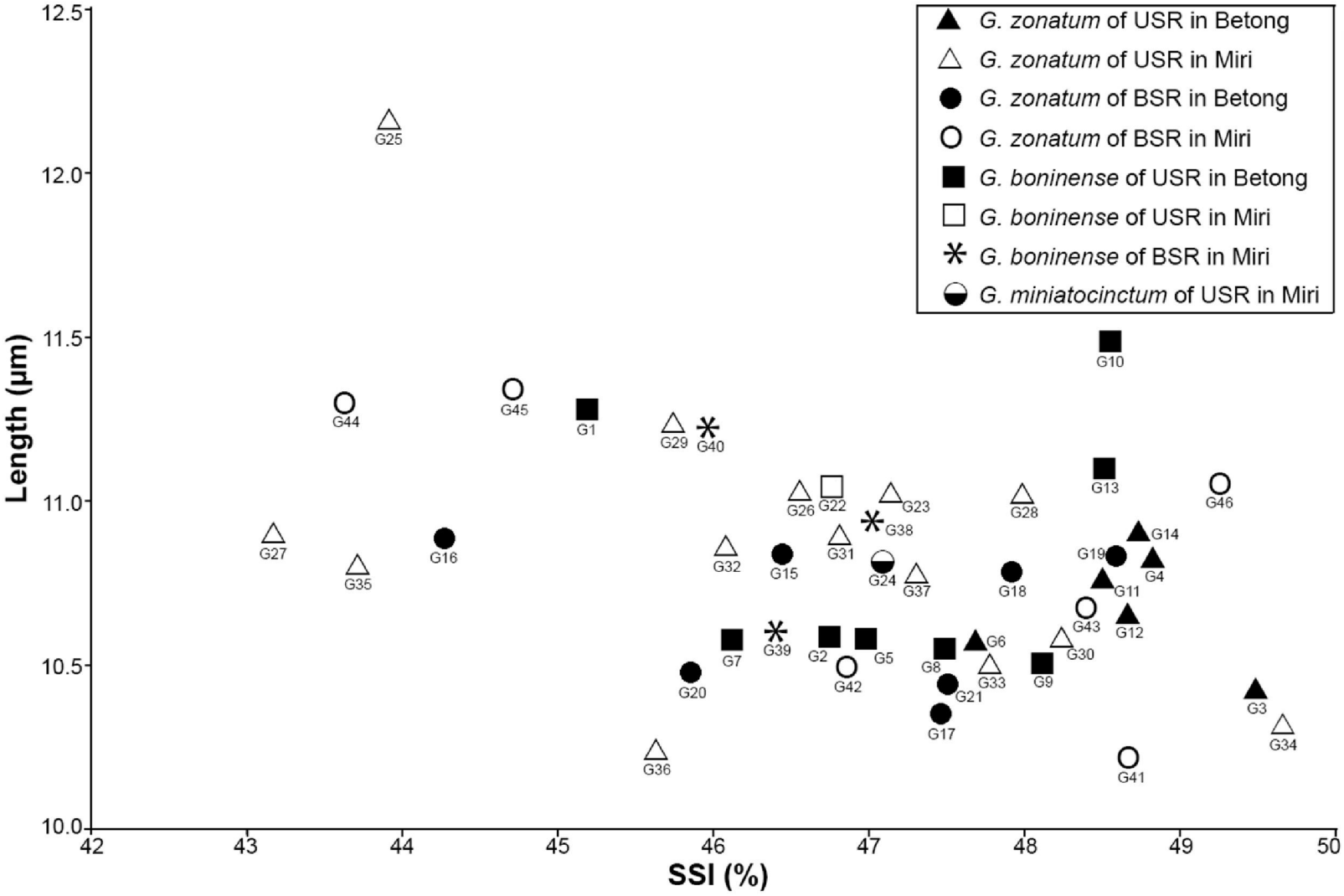
Indicates *Ganoderma* spp. were genetically heterogeneous

Suggested spread of *Ganoderma* spp. due to basidiospores spread

Similarity of cultural characteristics



Distribution of SSI on spore length



1.3 Conclusion

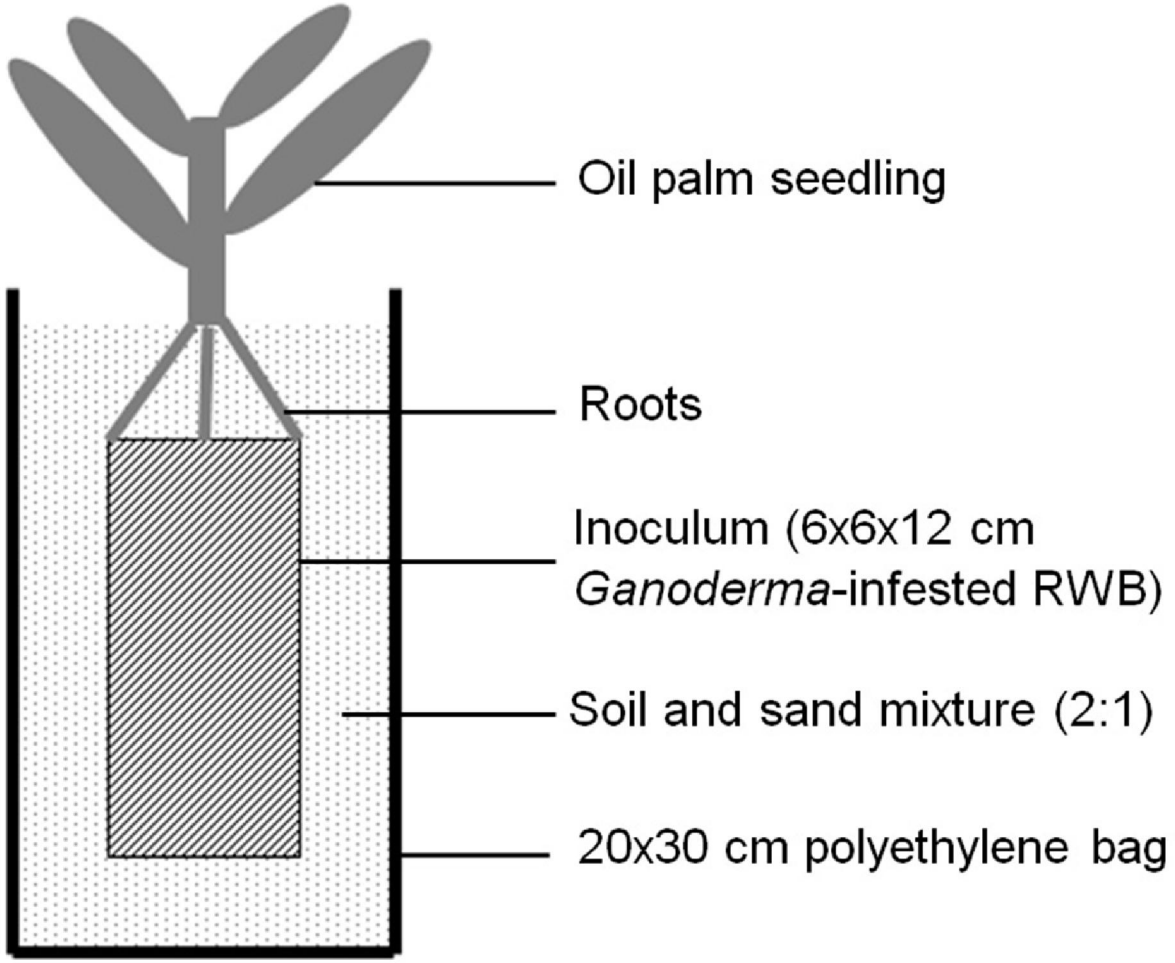
- ***Ganoderma*** **species were genetically and morphologically diverse**

- ***G. zonatum*** **(71.7%),** ***G. boninense*** **(26.1%) and** ***G. miniatocinctum*** **(2.2%) were associated with USR and BSR**

OBJECTIVE 2

Evaluate the aggressiveness of *Ganoderma* species isolated from USR- and BSR-infected oil palms

1 Artificial inoculation of *Ganoderma* on oil palm seedling



(Idris *et al.*, 2006; Breton *et al.*, 2006; Kok *et al.*, 2013)

2 Assessments for the artificial inoculation

Assessments for 24 weeks (6 months)

Assessments at 4 weeks interval

External symptoms

- Disease incidence (DI)
- Severity of foliar symptoms (SFS)
- Disease severity index (DSI)

24 weeks after inoculation (destructive sampling)

Internal symptoms

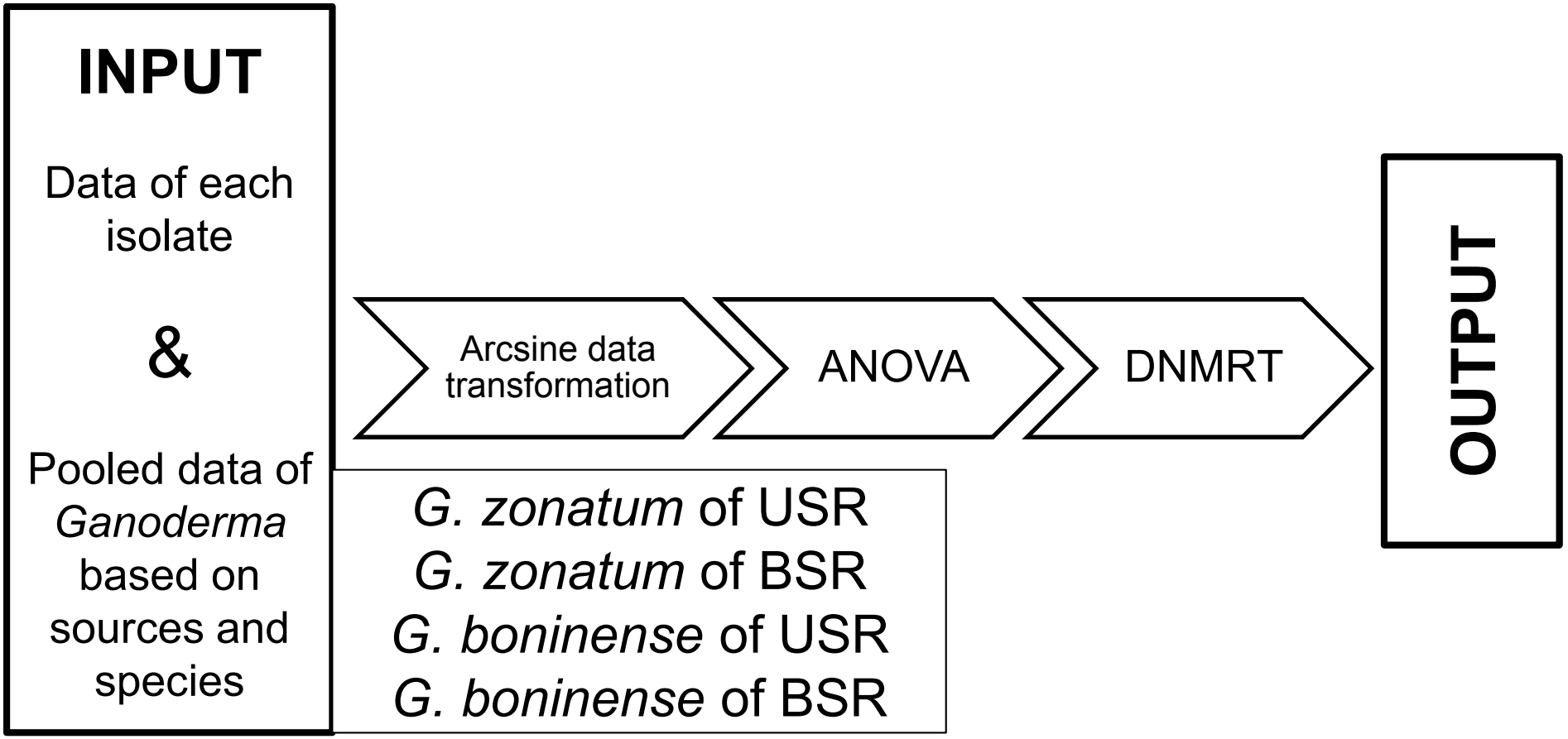
- % of necrotic primary root
- % of stem bole necrotic area

Presence of *Ganoderma*

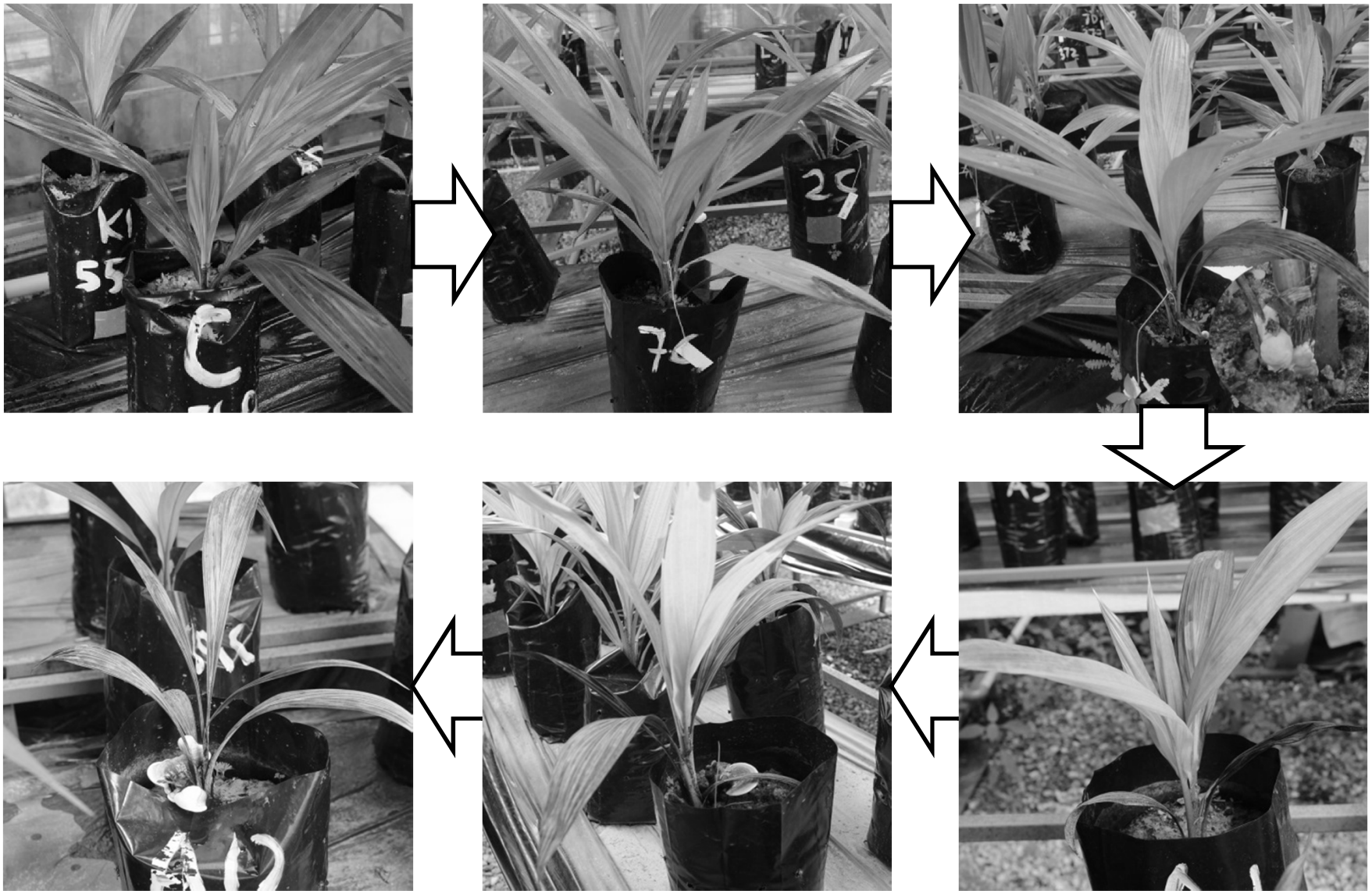
- Either verified visually or plating on GSM to complete Koch's postulate

(Idris *et al.*, 2004; Breton *et al.*, 2006; Shamala *et al.*, 2008; Rees *et al.*, 2009)

3 Data analysis

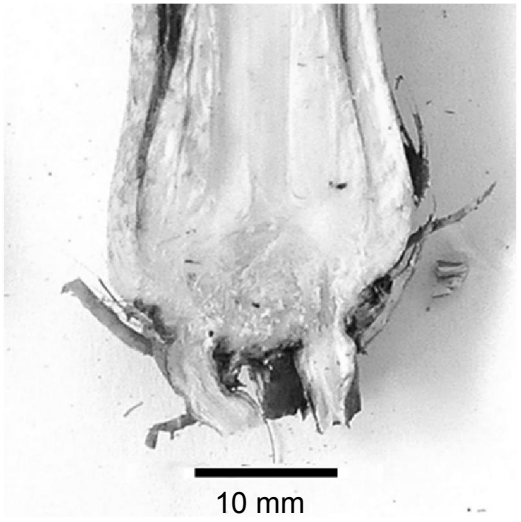


External infection symptoms

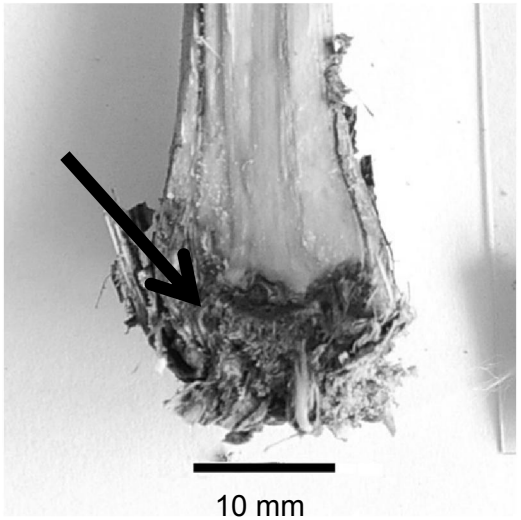


Internal infection symptoms

Healthy

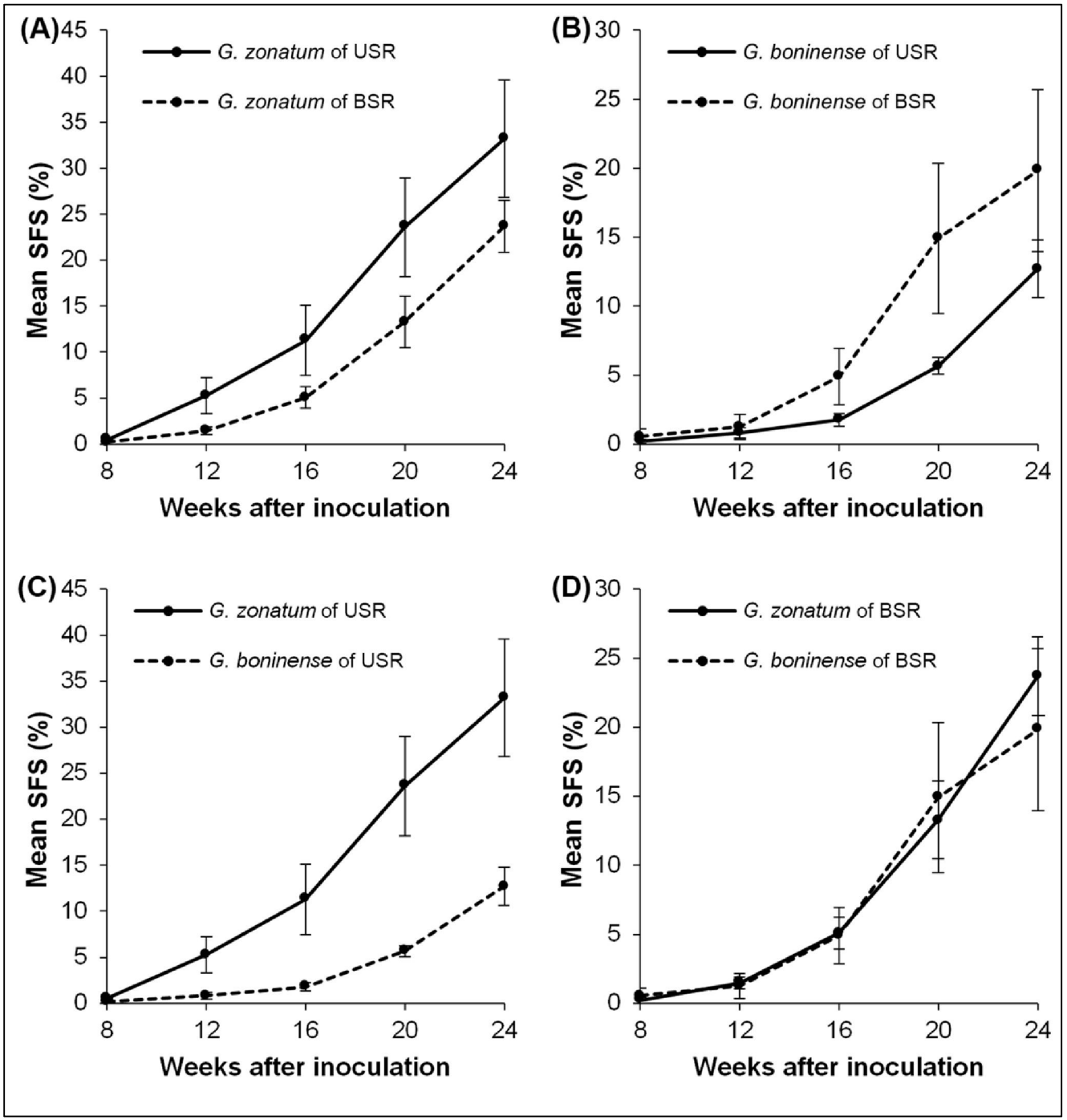


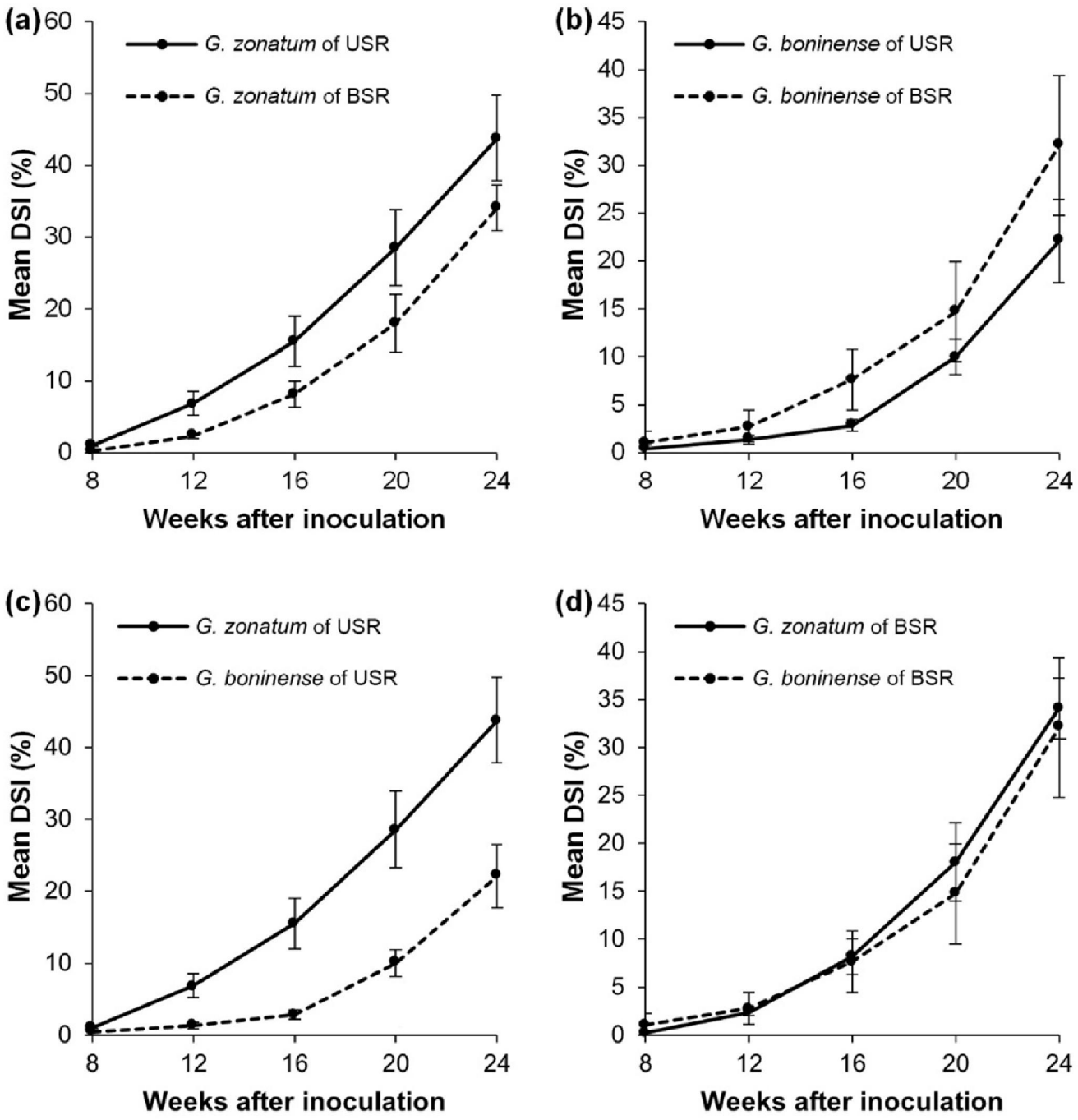
Infected



Isolate	Species	Disease severity index (weeks after inoculation) (%)					
		4	8	12	16	20	24
Site: Betong							
G1	*G. Boninense* of USR	0.00a	3.33a	5.00b	9.58bcd	14.58efg	17.08fg
G2		0.00a	1.66a	8.33b	11.66bcd	20.00cdefg	31.67bcdefg
G5		0.00a	0.00a	6.66b	9.16bcd	30.42abcdefg	53.33abcdef
G7		0.00a	1.66a	1.66b	6.67bcd	11.67fg	18.34fg
G8		0.00a	0.00a	0.00b	1.66d	8.34g	23.33efg
G9		0.00a	0.00a	0.00b	0.00d	18.33defg	45.00abcdefg
G10		0.00a	0.00a	0.00b	1.66d	5.00g	10.00g
G13		0.00a	0.00a	0.00b	5.00cd	11.67fg	35.00bcdefg
G3	*G. Zonatum* of USR	0.00a	3.33a	8.33b	25.00abc	43.34abcde	58.33abcde
G4		0.00a	0.00a	11.66ab	31.66a	48.34ab	65.00ab
G6		0.00a	0.00a	1.66b	3.33d	11.67fg	36.67bcdefg
G11		0.00a	1.66a	21.66a	33.33a	53.33a	68.33a
G12		0.00a	0.00a	10.00b	19.16abcd	46.67abc	53.33abcd
G14		0.00a	1.66a	3.33b	16.66abcd	31.67abcdefg	50.83abcdef

Means within column with different alphabets were significantly different at $p < 0.05$ by DNMRT


(A)
G. zonatum of USR
G. zonatum of BSR
Mean SFS (%)
Weeks after inoculation
(B)
G. boninense of USR
G. boninense of BSR
Mean SFS (%)
Weeks after inoculation
(C)
G. zonatum of USR
G. boninense of USR
Mean SFS (%)
Weeks after inoculation
(D)
G. zonatum of BSR
G. boninense of BSR
Mean SFS (%)
Weeks after inoculation


(a)
G. zonatum of USR
G. zonatum of BSR
Mean DSI (%)
Weeks after inoculation
(b)
G. boninense of USR
G. boninense of BSR
Mean DSI (%)
Weeks after inoculation
(c)
G. zonatum of USR
G. boninense of USR
Mean DSI (%)
Weeks after inoculation
(d)
G. zonatum of BSR
G. boninense of BSR
Mean DSI (%)
Weeks after inoculation

Factors attributed to difference in aggressiveness among *Ganoderma* spp.

Genetic variations due to:

- Isolates from different geographical origins
- Isolates from different disease source (USR or BSR)
  - Complex host-pathogen interactions
  - Different growing environment may alter the genetic traits

(Campanile *et al.*, 2004; Gasch, 2007; Kok *et al.*, 2013)

Difference in lignin degradation capability

(Wong, 2013)

2.3 Conclusion

- **All 46 *Ganoderma* isolates tested were confirmed to be pathogenic**

- **There were wide range of variations in terms of aggressiveness among the *Ganoderma* isolates tested**

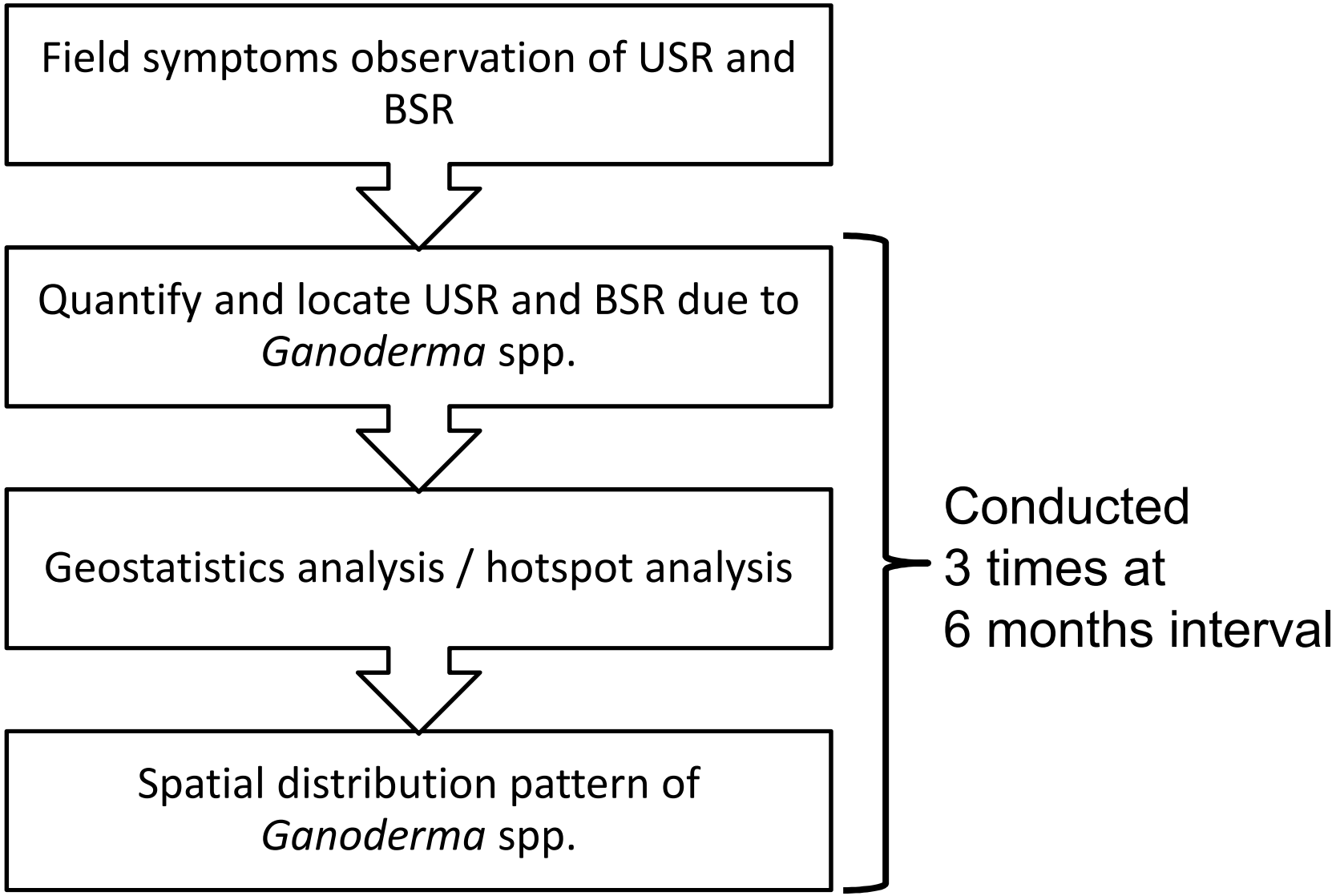
2.3 Conclusion

- **Level of aggressiveness:**

  ***G. zonatum* of USR > *G. zonatum* and *G. boninense* of BSR > *G. boninense* of USR**

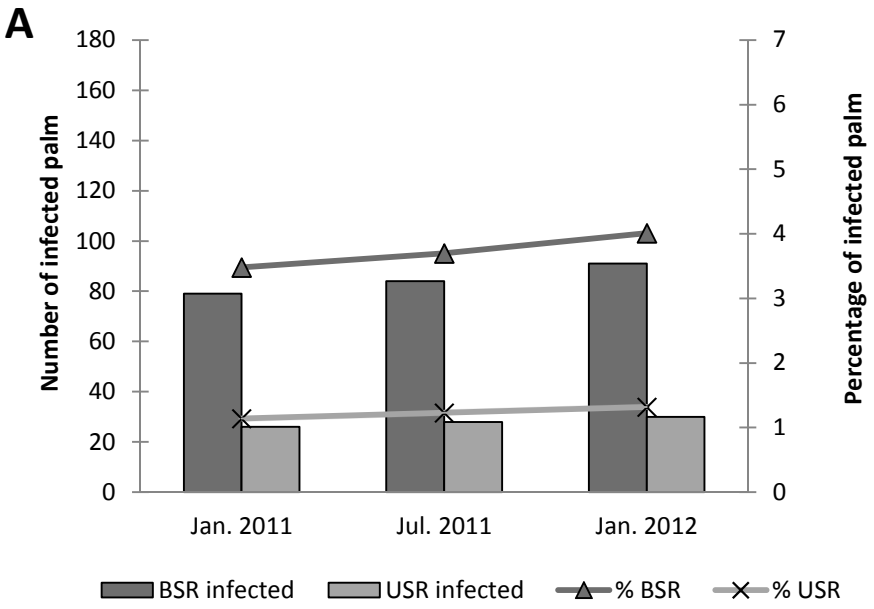
OBJECTIVE 3

Investigate disease symptoms, occurrence and spatio-temporal distribution of USR and BSR, and hotspot analysis of *Ganoderma* species of the diseases in oil palm

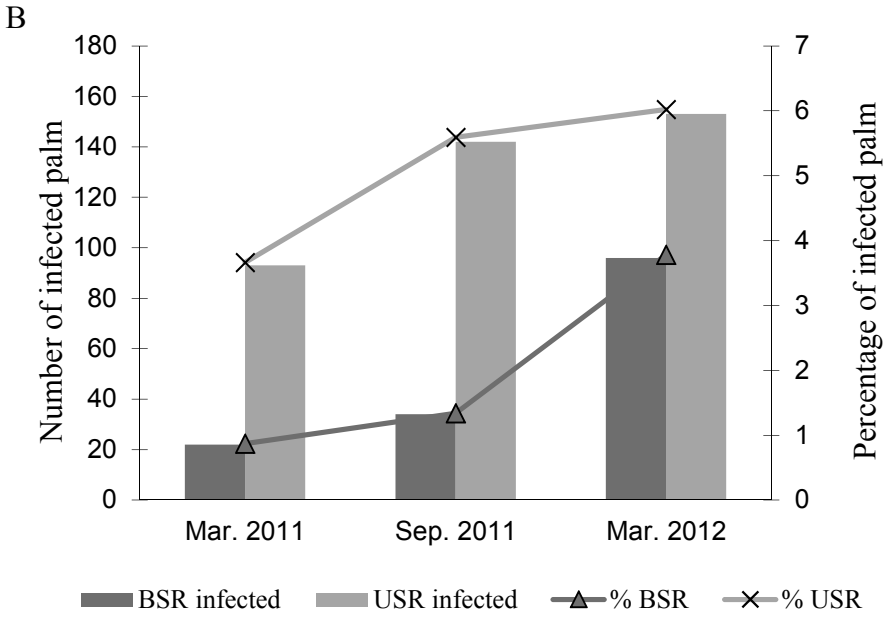

Field symptoms observation of USR and BSR
Quantify and locate USR and BSR due to *Ganoderma* spp.
Geostatistics analysis / hotspot analysis
Spatial distribution pattern of *Ganoderma* spp.
Conducted
3 times at
6 months interval

Disease symptoms: USR vs BSR

Occurrence of USR and BSR



Betong

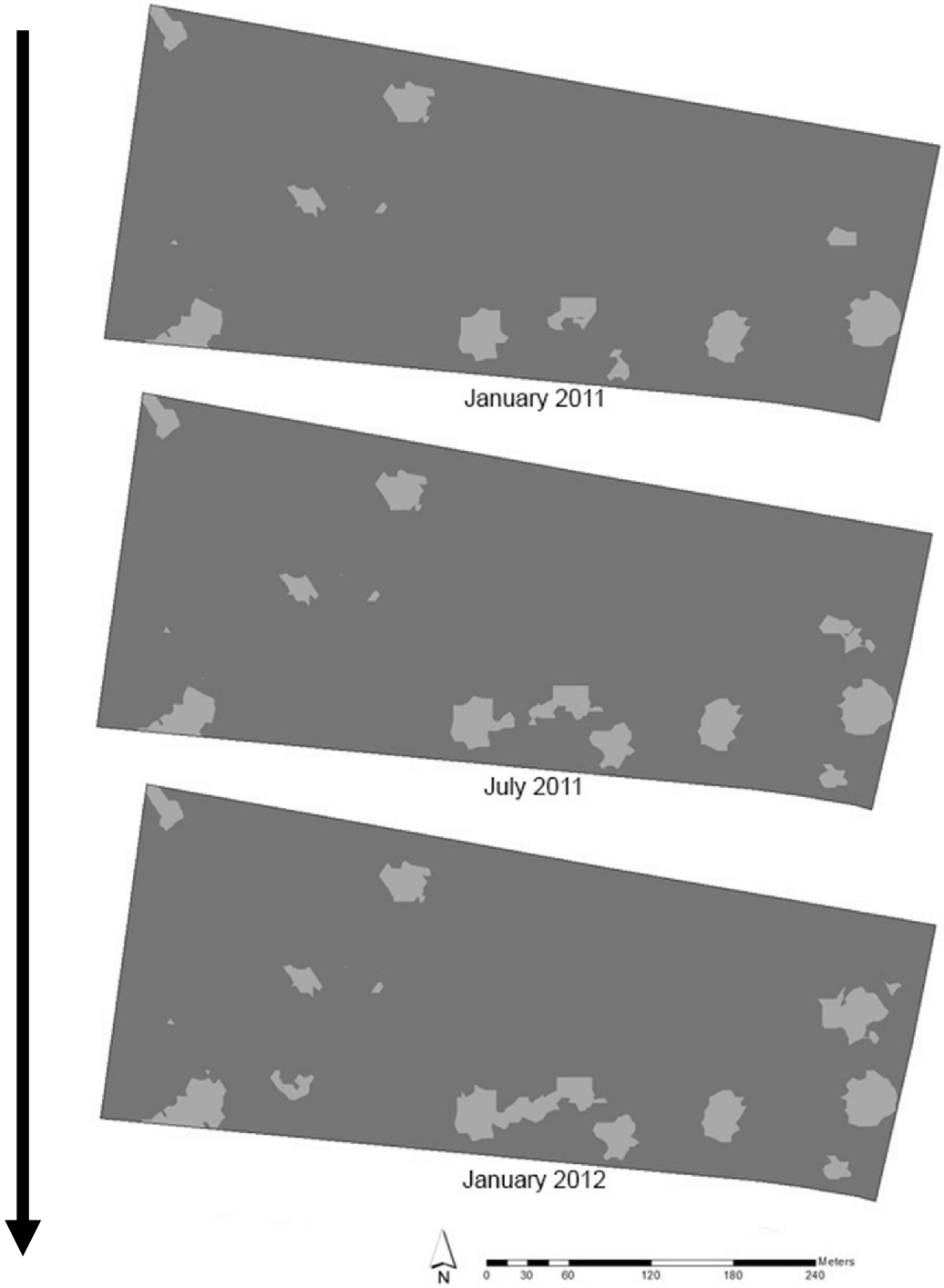


Miri

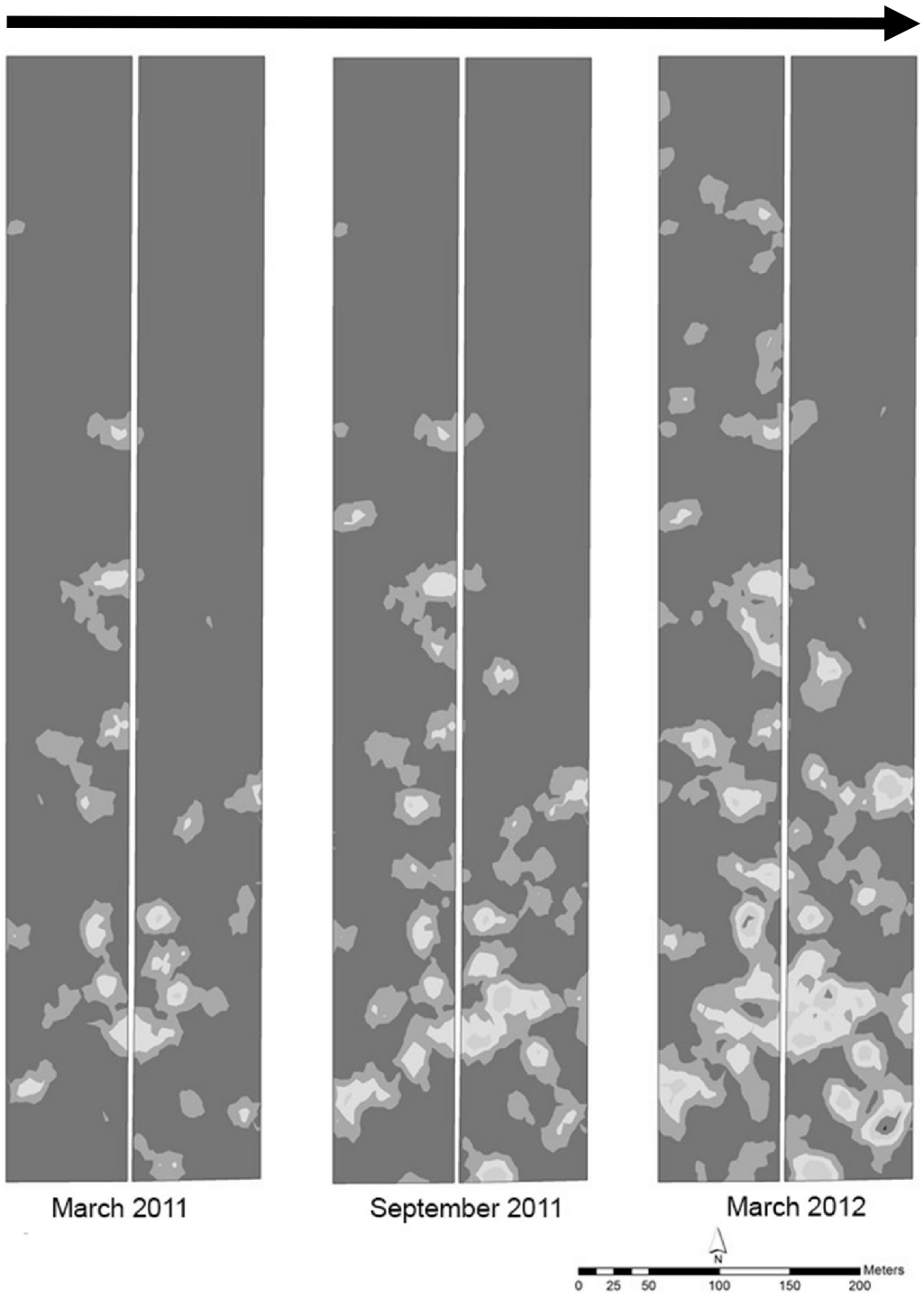
Summary of spherical semivariogram model of the hotspot analysis

Site	Census	Nugget (C_0)	Standard variance (C)	$C_0/(C_0+C)$ (%)	Range (m)	Spatial dependence level
Betong	Jan. 2011	0.04146	0.00893	82.28	705.667	**Weak**
	Jul. 2011	0.04351	0.01012	81.13	705.667	**Weak**
	Jan. 2012	0.04571	0.01339	77.34	705.667	**Weak**
Miri	Mar. 2011	0.01205	0.03122	27.85	11.663	**Moderate**
	Sep. 2011	0.00961	0.05496	14.88	11.663	**Strong**
	Mar. 2012	0.07530	0.00854	89.81	11.663	**Weak**

< 25% = Strong; **25-75% = Moderate;** **> 75% = Weak**


January 2011
July 2011
January 2012
N
0 30 60 120 180 240
Meters
LEGEND
Disease Density (%)
0 - 15.0
15.1 - 30.0
30.1 - 45.0
45.1 - 60.0
60.1 - 75.0
75.1 - 100

Betong



Miri

LEGEND

Disease Density (%)

- 0 - 15.0
- 15.1 - 30.0
- 30.1 - 45.0
- 45.1 - 60.0
- 60.1 - 75.0
- 75.1 - 100

3.3 Conclusion

- **USR and BSR infection was mainly identified based on appearance to *Ganoderma* on different portion of an oil palm stem**

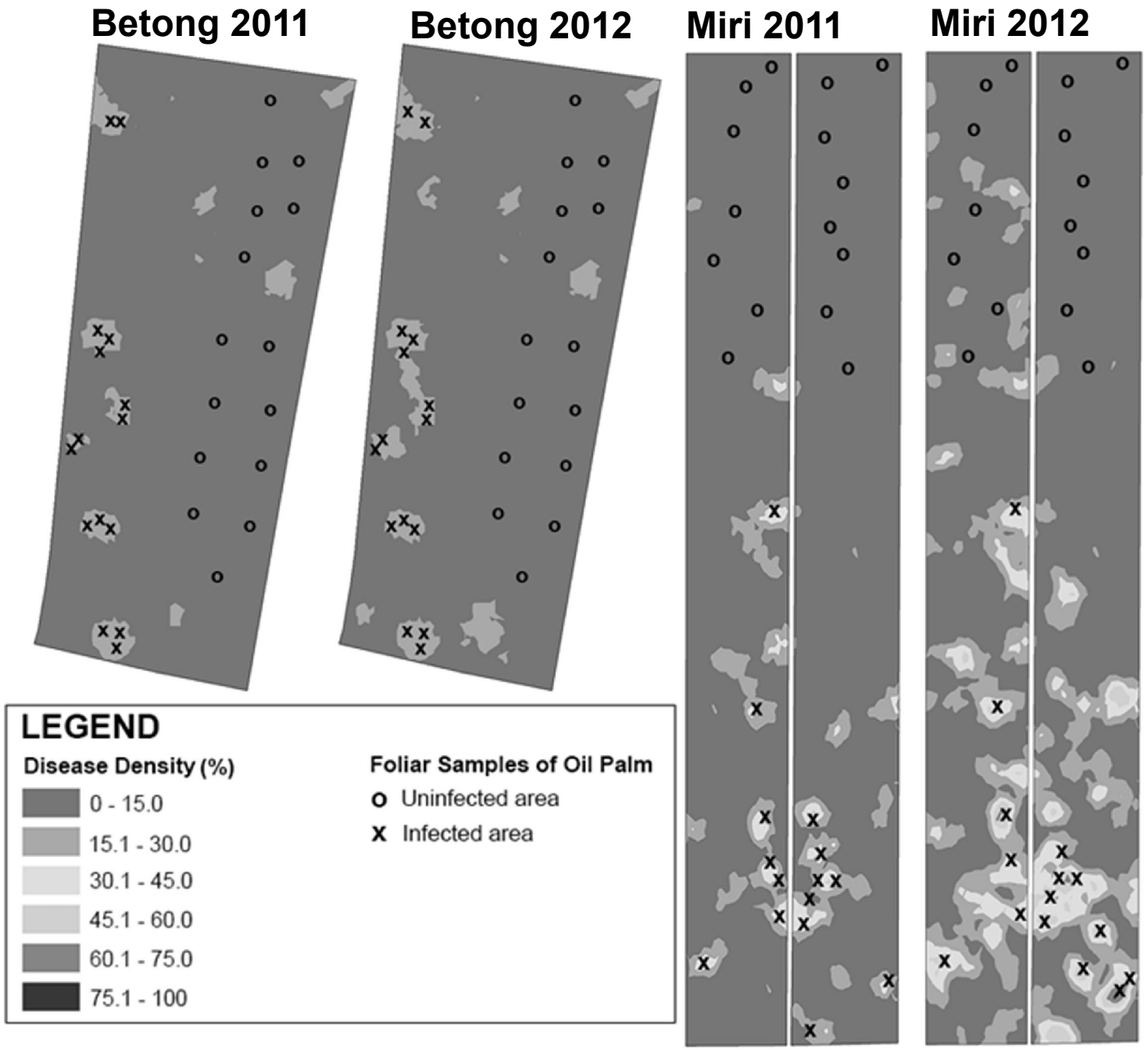
- **USR and BSR coexisted in both sites**

3.3 Conclusion

- **Occurrence of *Ganoderma* spp. was higher and rapid in Miri as compared with in Betong**
- **Spatial distribution of *Ganoderma* was generally random.**
- **Hotspot distribution patterns were generated**

OBJECTIVE 4

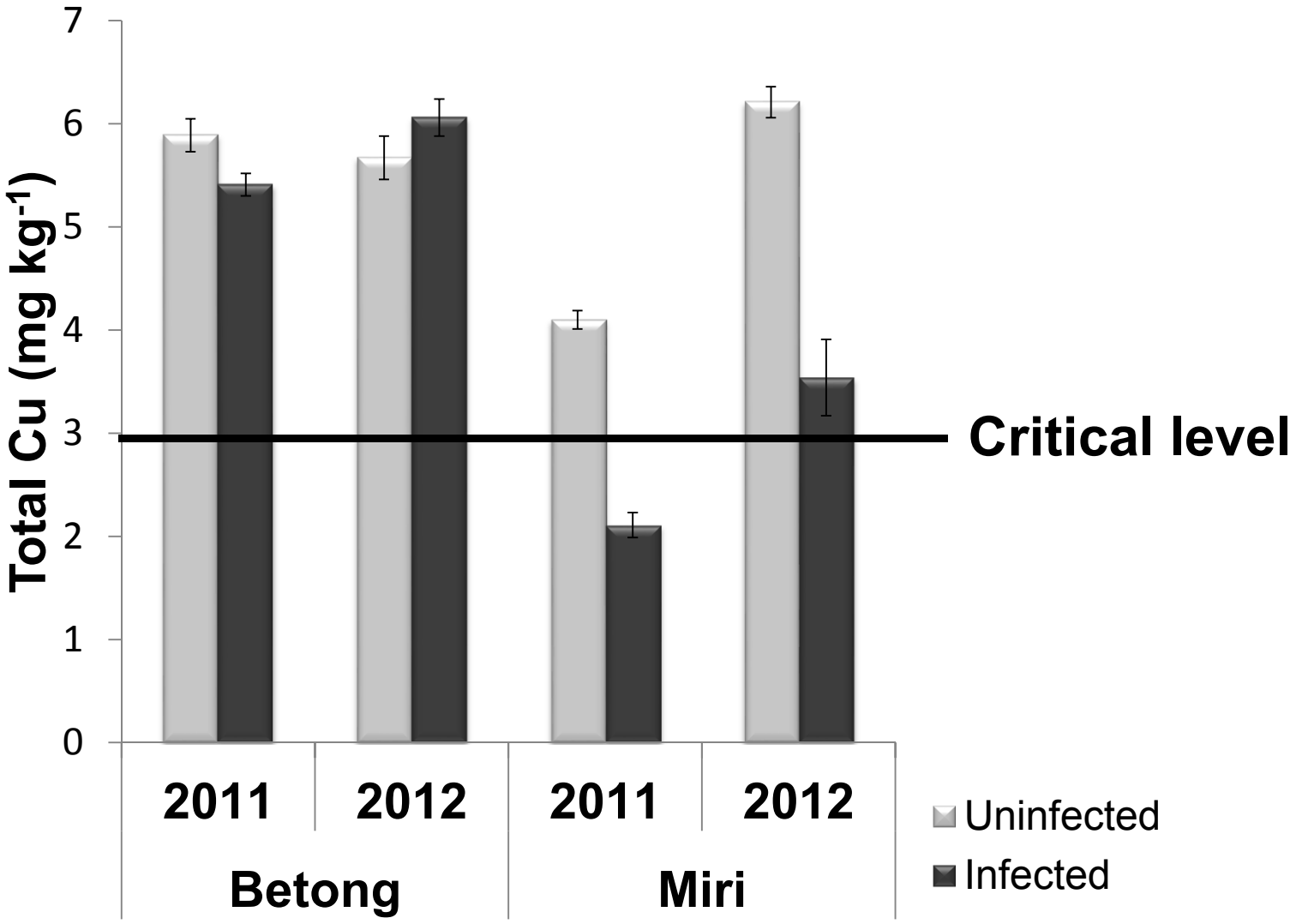
Investigate the relationship between oil palm nutrients status and spatial distribution of *Ganoderma* species in oil palm on peat


Betong 2011
Betong 2012
Miri 2011
Miri 2012
LEGEND
Disease Density (%)
0 - 15.0
15.1 - 30.0
30.1 - 45.0
45.1 - 60.0
60.1 - 75.0
75.1 - 100
Foliar Samples of Oil Palm
O Uninfected area
X Infected area

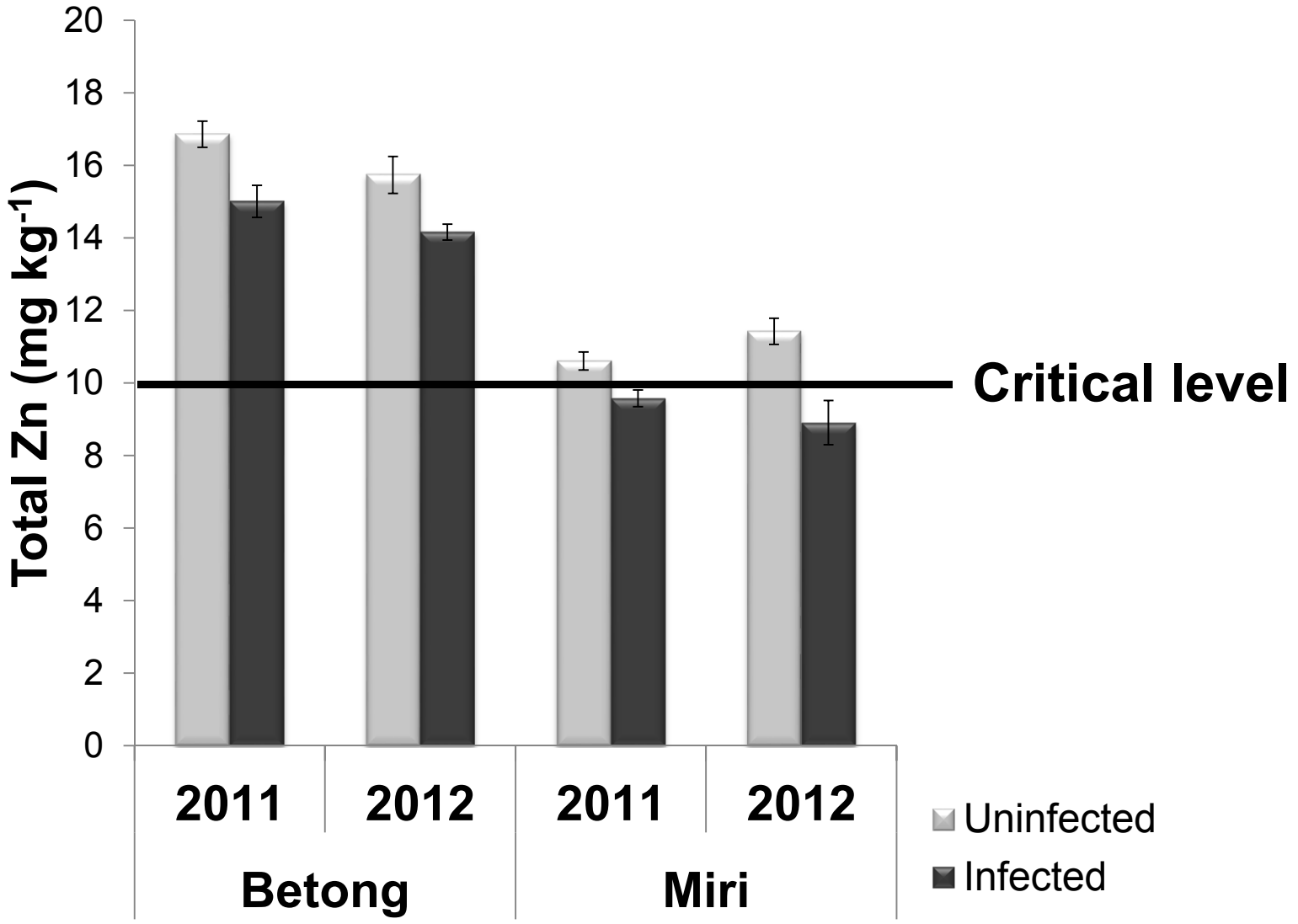
Foliar analysis

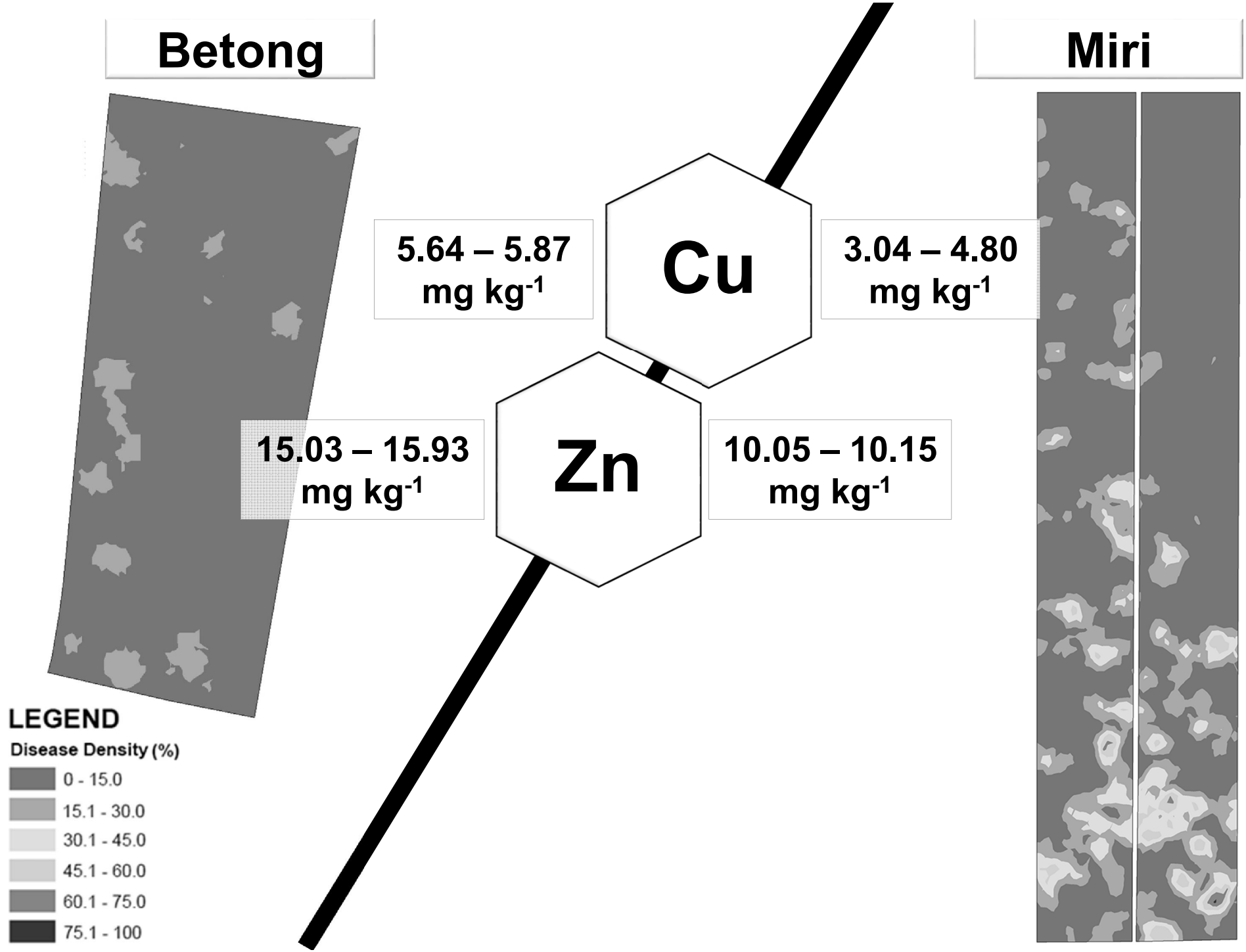
Properties	Unit	Method	Extractant	Equipment	Reference
Total nitrogen (N)	%	Combustion	NA	CNS analyzer (CNS-2000, LECO)	Campbell, 1992; Isaac and Johnson, 1992
Total phosphorus (P)		Dry ashing, followed by molybdate-blue complex colourmetric	Dry ashing: Concentrated HCl HNO_3 (20%) (Hue et al., 2000; Gupta, 2007; Korn et al., 2008)	Spectrophotometer (Lambda 25, Perkin Elmer)	Murphy and Riley, 1962; Gupta, 2007
Total potassium (K)		Dry ashing, followed by atomic absorption		Atomic absorption spectrometer (AAnalyst 400, Perkin Elmer)	NA
Total calcium (Ca)					
Total magnesium (Mg)					
Total copper (Cu)	mg kg^{-1}				
Total zinc (Zn)					
Total manganese (Mn)					
Total iron (Fe)					
Total boron (B)		Dry ashing, followed by azomethine-H colourmetric		Spectrophotometer (Lambda 25, Perkin Elmer)	Michio et al., 1989; Zenki et al., 1989; Sabbe, 1992

Leaf (frond 17) total Cu between uninfected and infected palms

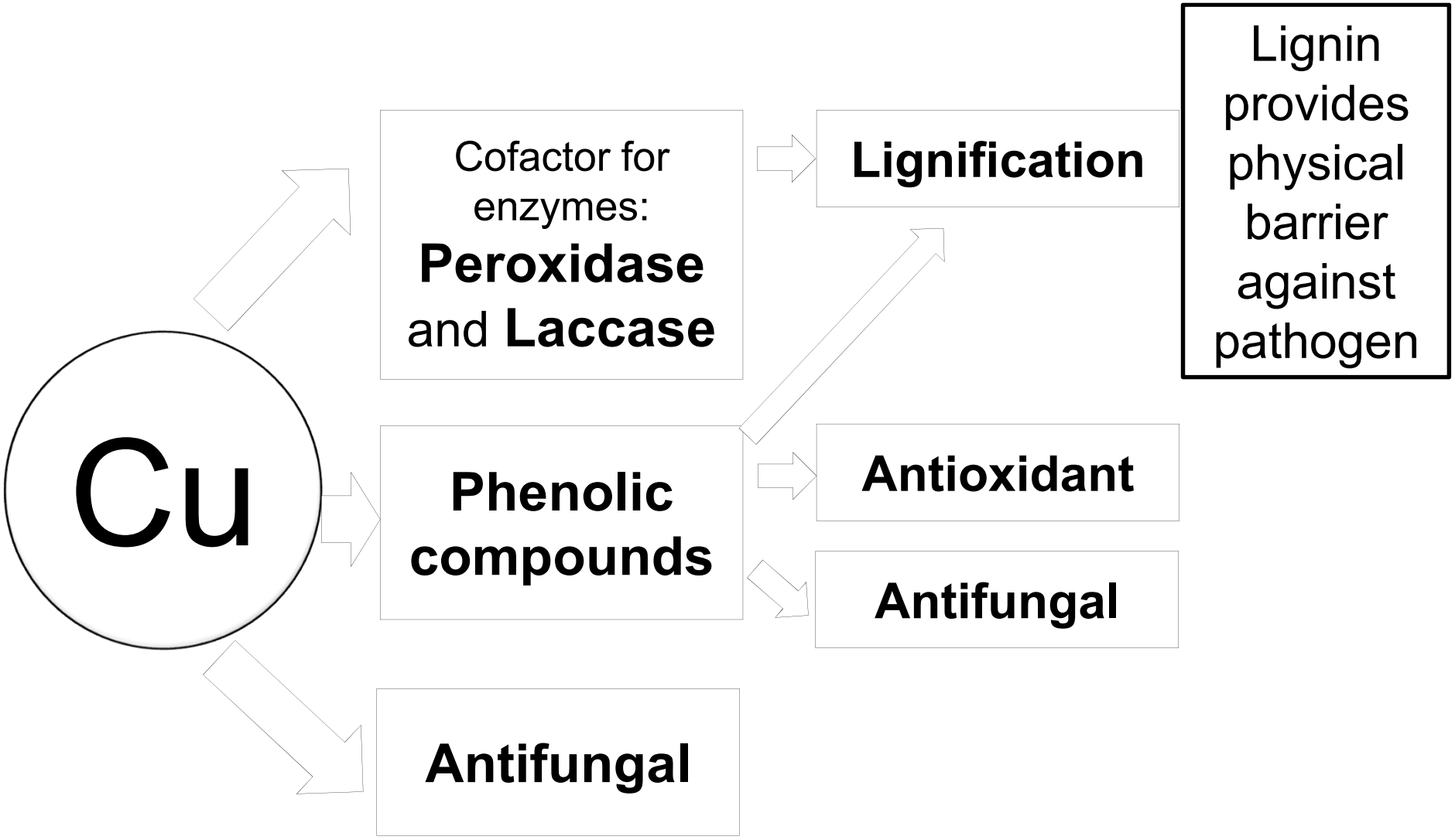


Leaf (frond 17) total Zn between uninfected and infected palms



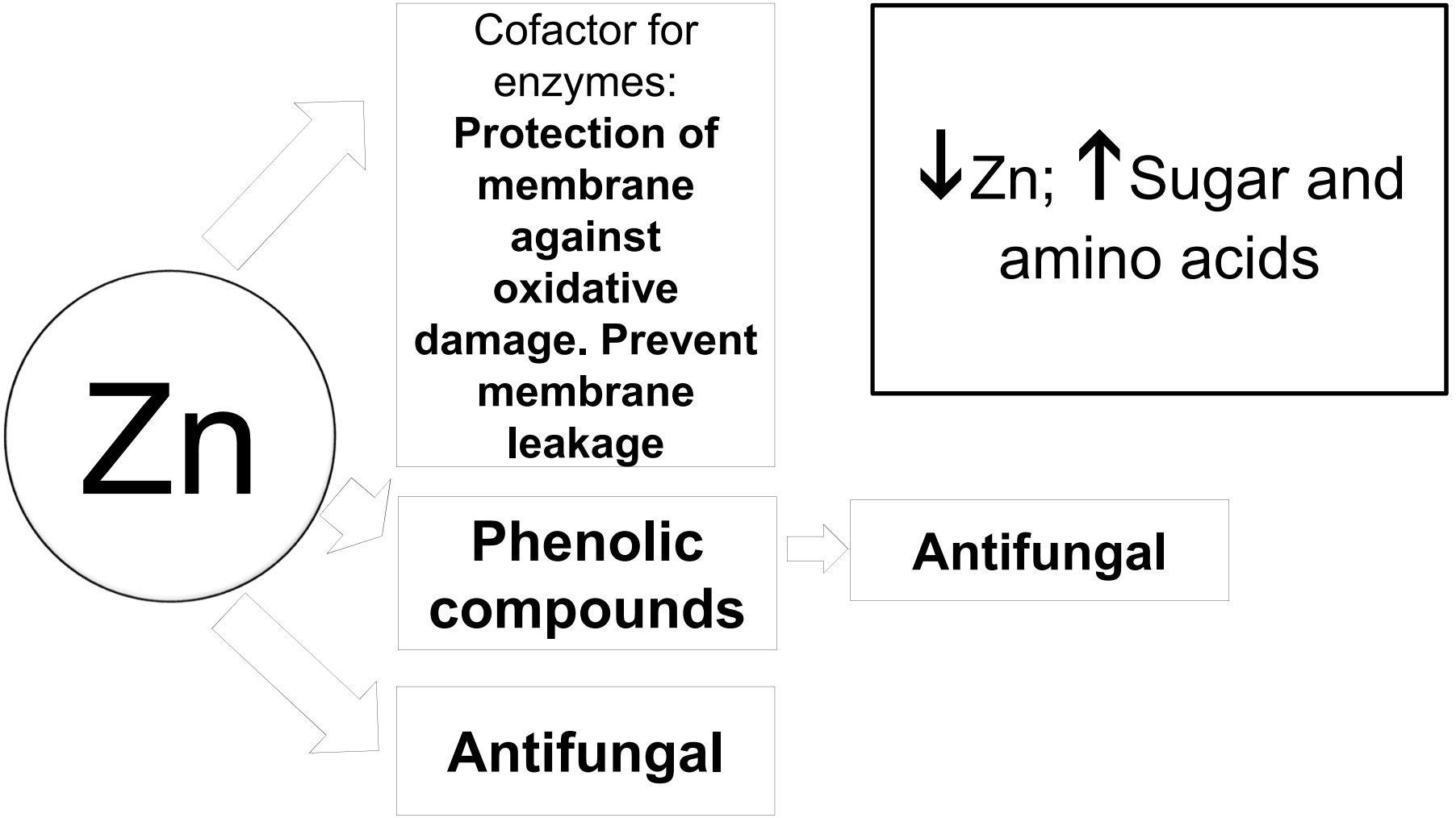

Betong
Miri
5.64 – 5.87 mg kg-1
Cu
3.04 – 4.80 mg kg-1
15.03 – 15.93 mg kg-1
Zn
10.05 – 10.15 mg kg-1
LEGEND
Disease Density (%)
0 - 15.0
15.1 - 30.0
30.1 - 45.0
45.1 - 60.0
60.1 - 75.0
75.1 - 100

Cu: Plant's defence mechanism against disease



Source: Marschner (1995); Chen *et al.* (2002); Rengel *et al.* (1994); Lattanzio *et al.* (2006)

Zn: Plant's defence mechanism against disease



Source: Marschner (1995); Michalak (2006)

3.3 Conclusion

- **Low, as well as deficiencies in foliar concentration of Cu and Zn were associated with higher distribution of *Ganoderma* species**

SUMMARY, GENERAL CONCLUSION AND RECOMMENDATION FOR FUTURE RESEARCH

- USR and BSR were associated with similar pathogens. The distinctive characteristic was the elevation of infection on the stem of oil palm

- Random spatial distribution and heterogeneity of the *Ganoderma* species suggested that the spread of *Ganoderma* in oil palm plantation was mainly related to spread of basidiospores

Factors that attributed to higher occurrence of disease:

- Dominant occurrence of *G. zonatum*
- Higher aggressiveness of *G. zonatum* of USR-infected palm
- Low concentration, as well as deficiency of foliar Cu and Zn in oil palm

- USR emerged as a major important disease in an oil palm plantation besides the prominent BSR caused by *G. boninense*

- *G. zonatum* which was previously overlooked was also found to be one of the major important pathogen that may affect oil palm industry seriously

Recommendations:

- More specific pest and disease control strategies for USR and *G. zonatum* should be taken in consideration
- Use of advance tools such as the GIS for better understanding of the disease distribution and help in better site-specific disease management as in precision agriculture
- Further investigations are required to distinguish between USR and BSR

Publications

Journal articles:

1. Rakib, M.R.M., C.F.J. Bong, A. Khairulmazmi, A.S. Idris, M.B. Jalloh and O.H. Ahmed. (In press) Association of copper and zinc levels in oil palm (Elaeis guineensis) to the spatial distribution of Ganoderma species in the plantations on peat. Journal of Phytopathology.
2. Rakib, M.R.M., C.F.J. Bong, A. Khairulmazmi and A.S. Idris. 2015. Aggressiveness of Ganoderma boninense and G. zonatum isolated from upper- and basal stem rot of oil palm (Elaeis guineensis) in Malaysia. Journal of Oil Palm Research 27: 229-240.
3. Rakib, M.R.M., C.F.J. Bong, A. Khairulmazmi and A.S. Idris. 2014. Occurrence and spatial distribution of Ganoderma species causing upper and basal stem rot in oil palm. Journal of Food, Agriculture and Environment 12: 360-364.
4. Rakib, M.R.M., C.F.J. Bong, A. Khairulmazmi and A.S. Idris. 2014. Genetic and morphological diversity of Ganoderma species isolated from infected oil palms (Elaeis guineensis). International Journal of Agriculture and Biology 16: 691-699.

Publications

Thesis:

1. Rakib, M.R.M. 2015. Epidemiology and Etiology of Ganoderma Upper and Basal Stem Rot in Oil Palm (Elaeis guineensis Jacq.) on Peat in Sarawak, Malaysia. PhD Thesis, Universiti Putra Malaysia, Selangor, Malaysia.

Proceedings:

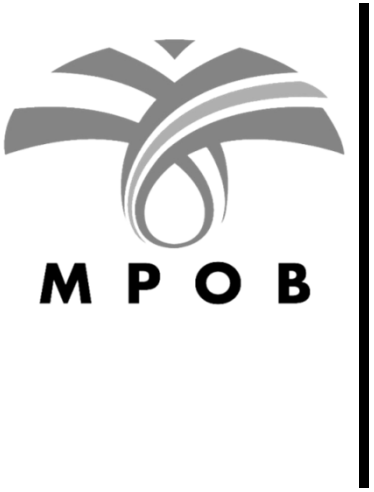
1. Rakib, M.R.M., C.F.J. Bong, A. Khairulmazmi and A.S. Idris. 2016. Application of GIS for mapping the spatial distribution of Ganoderma stem rot in oil palm plantations. In: eds. Lassim et al., Regional Conference on Sustainable Agriculture, 24-26 October 2016. Universiti Malaysia Sabah (UMS) Sandakan Campus, Sabah, Malaysia, pp. 73-75.
2. Rakib, M.R.M., C.F.J. Bong, A. Khairulmazmi and A.S. Idris. 2014. Association of copper and zinc to Ganoderma spp. spatial distribution in oil palm (Elaeis guineensis) plantations on peat. In: eds. Roseli et al., International Conference on Plant Physiology, 26-28 August 2014. Kuta, Bali, Indonesia, pp. 275-279.
3. Rakib, M.R.M. C.F.J. Bong, A. Khairulmazmi and A.S. Idris. 2013. Genetic and morphological diversity of Ganoderma sp. from upper and basal stem rot infected oil palms. In: eds. Nadarajah et al., International Congress of the Malaysian Society for Microbiology, 12-15 December 2013. Langkawi, Kedah, Malaysia, pp. 80-84.

Publications

Papers presented in conference (without proceedings)

1. Rakib, M.R.M., C.F.J. Bong, A. Khairulmazmi and A.S. Idris. 2014. Upper stem rot: another threat to oil palm (Elaeis guineensis) plantations based on a study in Sarawak. In: 2nd National Postgraduate Symposium on Sustainable Agriculture, 8-9 October 2014. Universiti Malaysia Sabah (UMS) Sandakan Campus, Sabah, Malaysia.
2. Bong, C.F.J, M.R.M. Rakib and L.C. Wong. 2014. Ganoderma diseases in Sarawak. In: Workshop on Integrated Management of Ganoderma Disease in Oil Palm, 3-4 December 2014. Kota Kinabalu, Sabah, Malaysia.

Acknowledgements



SARAWAK OIL PALMS BERHAD
GROUP OF COMPANIES

“Bertekad Cemerlang”