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AGGRESSIVENESS OF *Ganoderma boninense* ISOLATES ON THE VEGETATIVE GROWTH OF OIL PALM (*Elaeis guineensis*) SEEDLINGS AT DIFFERENT AGES

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ABSTRACT

Basal stem rot (BSR) disease caused by *Ganoderma boninense* is rapidly spreading in both coastal and inland soils planted with oil palms (*Elaeis guineensis*) across Indonesia and Malaysia. A study was conducted to evaluate the effects of different *G. boninense* isolates collected from different regions of Peninsular Malaysia on oil palm seedlings. *Ganoderma boninense* isolates from 12 different estates located in 7 states were collected and used for assessing the effects of pathogenic *Ganoderma* isolates on 2- and 5-month-old oil palm seedlings. These seedlings were challenged artificially with *Ganoderma*-inoculated rubber wood blocks as the inoculum source. Based on the disease severity index (DSI), *G. boninense* isolates from Bt Lintang (Kedah) (T10) (63.3%) were the most aggressive, whereas, Fraser (Johor) (T4) (8.3%) and Pinji (Perak) (T8) (3.7%) were the least aggressive. Vegetative growth measurements (VGM), namely leaf area (cm^2), diameter of the bole girth (cm) and height (cm), for both 2- and 5-month-old seedlings challenged with the most aggressive T10 isolate were significantly lower compared to both of the least aggressive isolates – T4 and T8, as well as the controls at 4 months-after-treatment (MAT). The linear correlation between DSI with VGM at 16-weeks after treatment was negative, ranging from 0.64 to 0.88 for 5-month-old seedlings. Two-month-old seedlings are more preferable in the future nursery experiments compared to 5-month-old seedlings, as the former seedlings were more responsive in terms of DSI and all the VGM parameters measured.

Key words: aggressiveness, disease incidence, disease severity, *Ganoderma,* vegetative growth

INTRODUCTION

The oil palm industry, especially in Indonesia and Malaysia which are the major palm oil producers in the world, is being increasingly threatened by the white-rot fungus *Ganoderma boninense*, the causal agent of basal stem rot (BSR) (Rees *et al.,* 2012). This lethal disease can pose substantial yield and palm losses through unproductive diseased palms and casualty (Azahar *et al.,* 2011; Rees *et al.,* 2012; Singh, 1991).

The common *Ganoderma* species in oil palm plantations are *G. boninense*, *G. zonatum*, and *G. miniatotinctum* (Idris, 1999; Wong *et al.,* 2012). Among them, *G. boninense* is the most common and aggressive causal agent of BSR. Furthermore, BSR due to *G. boninense* has become more alarming in East of Malaysia as well, especially in Sabah (Chong *et al.*, 2011). In Malaysia, the spread rate of *Ganoderma* disease can vary from one estate to another, which implies differences in *G. boninense* aggressiveness among isolates from different regions. Variation in the degree of aggressiveness among different *Ganoderma boninense* isolates and the age of oil palm seedling were reportedly to be the crucial factors that can affect disease severity in treated seedlings (Breton *et al.,* 2006; Kok *et al.,* 2013). Therefore, the objectives of this study were 1) to evaluate the level of disease severity in 5-month-old seedlings separately inoculated with twelve different *G. boninense* isolates, 2) to study the effects of twelve *G. boninense* isolates on vegetative growth of 2- and 5-month-old seedlings, and 3) to study the relationship between disease severity and seedling growth.

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MATERIALS AND METHODS

Fungal isolates and *in-vitro* culture conditions

Twelve different *Ganoderma* isolates used in this study were collected from 12 separate sites spanning 7 states in Peninsular Malaysia. The twelve *Ganoderma* isolates were sequenced and identified as *Ganoderma boninense* (Kok *et al.,* 2013). All the isolates were maintained on oil palm extract medium (OPEM) for 5 days prior to inoculation onto rubber wood block (RWB).

Preparation of rubber wood blocks and artificial inoculation of oil palm seedlings

Blocks of rubber wood (*Hevea brasiliensis*) measuring 6 x 6 x 12 cm^3 were drilled in the centre with 1 cm diameter holes. The blocks were then treated and each of them was inoculated with one of the twelve *G. boninense* isolates according to the protocols outlined in Kok *et al.* (2013). Inoculated blocks were then incubated at room temperature (27pC) in darkness for 2 months. The *Ganoderma*-infected RWBs were used as fungal inoculum sources.

Germinated oil palm seeds (Dumpy Yangambi Avros DxP) were used in the experiment. The seeds were planted, maintained and fertilized as described in Kok *et al.* (2013) for 5 months. Seedlings with relatively similar uniformity were then selected and artificially inoculated with *G. boninense*-infected RWBs; by placing the primary oil palm roots in close proximity to the inoculated RWBs (Kok *et al.,* 2013). Two types of control, treatment without RWB and with uninoculated RWB, were incorporated into this experiment (Table 1). Seedlings were then transferred and grown in 38 x 51 cm black polythene bags filled with Bungor series soil (*Typic Paleudult*) taken from the top 45 cm. Control and treated seedlings were watered, fertilized and maintained in the nursery according to Kok *et al.* (2013).

The degree of aggressiveness among the 12 *G. boninense* isolates on 5-month-old seedlings was evaluated using disease severity index (DSI). DSI was calculated based on the formula proposed by Sapak *et al.* (2008), with slight modifications (Kok *et al.,* 2013): DSI = (number of seedlings in the rating x rating number)/(total number of seedlings assessed x highest rating). Descriptions on the rating and appearance/symptom were highlighted in Kok *et al.* (2013). Disease incidence (DI) was determined based on: DI = Number of seedlings identified as diseased or potentially infected / number of seedlings per treatment set) x 100% (Campbell and Madden, 1990). The experiment was set up in a randomized complete block design (RCBD) with ten replicates.

Infection in both the stem bole and roots was confirmed by re-isolating *G. boninense* from the infected palm tissues on GSM (Rees *et al.,* 2007).

Vegetative Growth Measurements

Measurements of the vegetative growth for both the 2- and 5-month-old oil palm seedlings were taken at five different time points after being challenged with *Ganoderma* isolates i.e. at 0, 1, 2, 3, 4, and 5 months after treatment (MAT). Measurements, calculations and recording on the vegetative growth for all the treatments were performed according to the non-destructive approaches described by Corley and Tinker (2003), Corley *et al.* (1971) and Hardon *et al.* (1969). The measurements were height of the seedlings (cm), girth diameter of the bole (cm) and leaf area (cm^2). Height of the seedling was measured from the base of stem bole to the tip of longest leaf and the girth was measured with a calliper at right angle to the stem bole. Leaf area was estimated and calculated using the following equation: Leaf Area (cm^2) = b*(nlw), where b is the correction factor (0.57), n is the number of leaflets per leaf and lw is the mean of length x mid-width (cm) (Corley *et al.,* 1971).

Statistical analysis

Means for disease severity index (DSI) of 5-month-old oil palm seedlings treated with 12 different *G. boninense* isolates and two controls at 8, 12 and 16 weeks after artificial inoculations were not normally distributed. Therefore, differences in DSI means among the 14 treatments at 5 different time points were analyzed with the Wilcoxon signed-rank test at $P = 0.05$ (SAS, 2010). Differences in means for the vegetative growth parameters (height, girth diameter and leaf area) at five separate time points were analyzed separately using analysis of variance (ANOVA) and least significance difference (LSD) test at $P = 0.05$ (SAS, 2010). Linear relationships between DI and DSI for 5-month-old seedlings at the five different time points after treatments were analyzed using linear regression test. Furthermore, linear relationships amongst vegetative growth measurements (VGM) of 2- and 5-month-old seedlings and between DI and DSI with VGM (5-month-old seedlings) at 16-week after treatment were determined (SAS, 2010).

RESULTS AND DISCUSSION

Disease incidence (DI) and disease severity index (DSI)

Signs of *Ganoderma* infection (leaf symptoms or presence of fungal mass/fruiting bodies) in 5-month-old seedlings were noticed as early as six weeks after treatments for T10 (Bt Lintang G10) and T12 (Sg. Jernih G3), although disease severity index (DSI) was not significantly different (Data not shown). By the 8^{th} week after inoculation, DSI value for T10 was significantly higher than all other

Table 1. Disease incidence (DI) and disease severity index (DSI) of 5-month-old oil palm seedlings at 8, 12 and 16 weeks after artificial inoculation for 14 different treatments (with 12 separate *Ganoderma boninense* isolates and 2 controls)

Treatment (Description)		Disease Incidence (after weeks) (%)‡			Disease Severity Index (after weeks) (%)† §		
		8	12	16	8	12	16
T1	Control 1*	0	0	0	0 b	0 d	0 d
T2	Control 2*	0	0	0	0 b	0 d	0 d
T3	Paloh G14	10	20	70	3.3 (3.3) b	11.7 (8.6) bcd	51.7 (13.0) ab
T4	Fraser G8	0	10	10	0 b	3.3 (3.3) cd	8.3 (8.3) cd
T5	UluPedas G6	0	30	40	0 b	10.0 (5.1) bcd	26.7 (12.5) bcd
T6	GBA G12	10	30	60	1.7 (1.7) b	10.0 (5.1) bcd	43.3 (12.7) ab
T7	Jeram G5	0	22.2	44.4	0 b	7.4 (4.9) cd	35.2 (14.0) abc
T8	Pinji G1	0	0	11.1	0 b	0 d	3.7 (3.7) d
T9	Pelam G11	10	20	60	1.7 (1.7) b	11.7 (8.6) bcd	41.7 (13.0) ab
T10	BtLintang G10	40	50	70	20.0 (11.1) a	40.0 (15.4) a	63.3 (14.0) a
T11	Bebar G4	0	10	30	0 b	3.3 (3.3) cd	25.0 (12.7) bcd
T12	Sg. Jernih G3	30	50	70	10.0 (5.1) ab	28.3 (11.7) ab	50.0 (12.9) ab
T13	Sg. Sokor G9	10	40	40	1.7 (1.7) b	20.0 (9.2) bc	33.3 (13.6) bc
T14	Pasir Gajah G2	10	10	50	3.3 (3.3) b	3.3 (3.3) cd	41.7 (13.9) ab

* Control 1 and 2 represent treatment without rubber wood block (RWB) and with uninoculated RWB, respectively.
‡ Disease incidence (DI) was determined using this formula:
DI = (Number of seedlings identified as diseased / number of seedlings per treatment set) x 100%
† Disease severity index (DSI) was calculated using following formula:
DSI = (number of seedlings in the rating x rating number)/(total number of seedlings assessed x highest rating) (Sapak *et al.,* 2008) (Kok *et al.,* 2012).
§ DSI for respective treatments at 8, 12 and 16 weeks after artificial inoculation presented in mean of 10 replications and the numbers in the bracket were standard error. DSI at different weeks after inoculation were analyzed separately. Means within each column of weeks after inoculation followed by the same letter are not significantly different at *P* = 0.05 after Wilcoxon signed-rank test.

treatments, except T12 (Table 1). At 12 weeks after inoculation, DSI for T10 remained significantly higher than other treatments, except T12 (Table 1).

Categories for benchmarking the aggressiveness in *G. boninense* was based on DSI (%) as proposed by Kok *et al.* (2013) with slight modifications were: a) high level of aggressiveness – more than 81%; b) moderately high level of aggressiveness – 61-80%; c) moderate level of aggressiveness – 41-60%; less aggressiveness – 21-40%, and least aggressive – 0-20%. Moreover, DI (% of diseased seedlings) with similar benchmarks as DSI was incorporated in the study as well. By 16-weeks after inoculation, none of the *G. boninense* isolates fell into the category with high level of aggressiveness in terms of DSI values (Table 1). Based on the DSI values, BtLintang (T10) isolate was categorized under moderately high level of aggressiveness. Paloh (T3), GBA (T6), Pelam (T9), Sg. Jernih (T12), and Pasir Gajah (T14) isolates were moderately aggressive based on DSI values. On the other hand, Jeram (T7), Ulu Pedas (T5), Bebar (T11), and Sg. Sokor (T13) isolates were less aggressive followed by Fraser (T4) and Pinji (T8) isolates (Table 1).

Ganoderma boninense isolates from Paloh (T3), Bt Lintang (T10), and Sg. Jernih (T12) were under the category of moderately high level of disease incidence (DI). GBA (T6), Jeram (T7), Pelam (T9), and Pasir Gajah (T14) isolates were with moderate incidence. Pinji (T8) and Fraser (T4) isolates were with the lowest disease incidence values (Table 1).

The various isolates showed greater variation in the onset symptoms and signs of infection, the severity of disease development and also the number of palms that was infected at the beginning. Variations in severity of disease development and disease incidence diminished among some isolates (Paloh, Bt Lintang, Pelam, and Sg. Jernih isolates – T3, T10, T9, and T12) as time progressed. This suggests the importance of recording DSI and DI over a period of time rather than determining infection at the end of a screening exercise. Perhaps there may be similar differences among different oil palm genotypes in response to the same *G. boninense* isolates. If so, the DSI and DI values would be able to detect the differences.

DI and DSI versus Vegetative Growth Measurement (VGM)

Vegetative growth measurements based on leaf area (LA), diameter of the bole girth (G) and height of the seedlings (H) for 2- and 5-month-old oil palm seedlings challenged with the 12 different *G. boninense* isolates and 2 controls at three separate time points, namely 0, 2 and 4 month after treatment (MAT) are summarized in Tables 2 and 3. At the end of the experiment (4 MAT), leaf area, diameter of the bole girth and plant height of the seedlings

Table 2. Vegetative growth measurements (VGM) of 2-month-old oil palm seedlings treated with 12 different *G. boninense* isolates and 2 controls based on leaf area (LA), diameter of the bole girth (G) and height of the seedlings (H) at five separate time points – 0, 2 and 4 month-after-treatment (MAT)

	0 MAT			2 MAT			4 MAT		
	LA (cm²)	G (cm)*	H (cm)	LA (cm²)	G (cm)	H (cm)	LA (cm²)	G (cm)	H (cm)
T1	19.5 (1.3)ab	0.33 (0.0)ab	12.9 (0.4)a	59.6 (3.0)d	0.69 (0.0)ab	25.3 (0.6)cde	159.0 (9.0)f	1.36 (0.1)d	39.88 (1.0)e
T2	20.6 (1.8)b	0.34 (0.0)ab	13.7 (0.6)a	48.0 (3.9)abc	0.61 (0.0)a	22.5 (1.1)abcd	131.9 (8.6)def	1.29 (0.0)d	38.33 (1.4)e
T3	19.9 (1.4)ab	0.34(0.0)ab	13.6 (0.6)a	51.1 (4.4)abcd	0.71 (0.0)ab	24.6 (1.1)bcde	44.9 (15.6)ab	0.59 (0.2)ab	16.38 (5.5)abc
T4	18.5 (0.8)ab	0.33(0.0)ab	12.6 (0.2)a	57.9 (3.3)cd	0.70 (0.0)ab	25.7 (0.6)e	137.1 (5.2)ef	1.30 (0.0)d	37.89 (0.8) de
T5	19.4 (1.1)ab	0.33 (0.0)ab	13.3 (0.5)a	47.6 (3.3)abc	0.60 (0.1)a	23.5 (0.9)abcde	41.9 (12.1)ab	0.62 (0.1)ab	17.2 (4.8) abc
T6	18.3 (0.8)ab	0.32 (0.0)ab	12.9 (0.4)a	47.2 (4.2)abc	0.67 (0.0)ab	23.1 (1.1)abcde	34.7 (14.5)ab	0.44 (0.2)ab	13.1 (5.4)ab
T7	18.4 (1.3)ab	0.34 (0.0)ab	13.2 (0.5)a	54.7 (4.2)bcd	0.74 (0.0)b	25.3 (1.3)de	117.4 (15.5)de	1.26 (0.2)d	34.0 (4.0)de
T8	18.2 (1.0)ab	0.31 (0.0)a	13.0 (0.6)a	55.3 (1.8)cd	0.74 (0.0)b	25.1 (0.9)cde	118.9 (6.7)de	1.34 (0.1)d	36.8 (1.3)de
T9	18.7 (1.0)ab	0.33 (0.0)ab	13.0 (0.3)a	47.8 (3.6)abc	0.66 (0.0)ab	23.4 (1.0)abcde	59.0 (17)bc	0.76 (0.2)bc	20.9 (4.9)bc
T10	16.7 (0.7)a	0.31(0.0)a	12.5 (0.4)a	41.9 (5.0)a	0.60 (0.1)a	21.2 (1.2)a	17.4 (9.5)a	0.32 (0.2)a	8.1 (4.2)a
T11	19.2(1.3)ab	0.34 (0.0)ab	13.0 (0.5)a	54.2 (4.8)bcd	0.67 (0.0)ab	24.3 (1.2)bcde	61.7 (20.4)bc	0.73 (0.2)abc	19.5 (5.6) abc
T12	18.2 (0.7)ab	0.37 (0.0)b	12.6 (0.5)a	43.6 (3.9)ab	0.62 (0.1)ab	22.0 (1.4)ab	46.4 (20.3)ab	0.50 (0.2)ab	13.9 (5.8)ab
T13	18.8 (1.5)ab	0.30 (0.0)a	12.7 (0.5)a	47.5 (5.1)abc	0.64 (0.0)ab	23.1 (1.3)abcde	95.2 (17.1)cd	1.07 (0.2)cd	26.5 (4.7)cd
T14	18.5 (1.3)ab	0.33(0.0)ab	12.7 (0.6)a	46.7 (3.1)abc	0.63 (0.0)ab	22.5 (0.6)abc	35.6 (13.0)ab	0.61 (0.2)ab	14.2 (4.8)ab

* Means with same letters within each column of respective VGM parameters were not significantly different with LSD at $P = 0.05$. VGM for respective treatments were presented in mean of 10 replications and the numbers in the bracket were standard error. * No variations in the diameter of bole girth were noted at 0 MAT because all the seedlings were relatively even and uniform during the first three months.

Table 3. Vegetative growth measurements (VGM) of 5-month-old oil palm seedlings treated with 12 different *G. boninense* isolates and 2 controls based on leaf area (LA), diameter of the bole girth (G) and height of the seedlings (H) at five separate time points – 0, 2 and 4 month-after-treatment (MAT)

	0 MAT			2 MAT			4 MAT		
	LA (cm²)	G (cm)*	H (cm)	LA (cm²)	G (cm)	H (cm)	LA (cm²)	G (cm)	H (cm)
T1	94.3(7.2)a	0.73 (0.03)abc	32.5 (1.2)cd	134.9 (4.4)a	1.13 (0.05)c	39.7 (0.7)ab	203.0(11.0) ab	1.66 (0.06)abc	45.7 (1.0)ab
T2	89.8 (6.5)ab	0.72 (0.03)bc	33.6 (1.0)bcd	123.8 (9.9)a	1.22 (0.03)abc	39.1 (1.1)ab	189.2(14.4)abc	1.91 (0.15)a	48.8 (1.4)a
T3	85.0 (5.9)ab	0.81 (0.05)ab	32.7 (0.8)bcd	130.1 (12.5)a	1.28 (0.08)abc	38.3 (1.3)ab	117.6 (28.4)de	1.21 (0.29)bcd	31.0(7.0)cd
T4	84.0 (2.9)ab	0.77 (0.03)abc	33.9 (1.2)bcd	118.6(8.5)a	1.23 (0.04)abc	39.7 (1.3)ab	169.2(18.1)abcd	1.86 (0.14)ab	47.3 (1.8)a
T5	91.2 (4.4)ab	0.74 (0.03)abc	34.2 (0.8)abc	123.7 (12.6)a	1.22 (0.04)abc	39.0 (1.2)ab	174.7(18.8)abcd	1.9 (0.15)ab	47.1 (2.3)a
T6	88.1 (6.1)ab	0.75 (0.02)abc	34.9 (1.0)ab	129.8 (7.6)a	1.28 (0.05)abc	40.2 (1.2)ab	146.2 (21.6)abcd	1.78 (0.22)abc	41.8 (4.9)abc
T7	89.0 (9.7)ab	0.73 (0.09) a	30.0 (3.6)bcd	113.4 (14.4)a	1.17 (0.15)ab	35.8 (4.1)ab	153.5 (26.9)abcd	1.61 (0.28)abc	37.5 (6.3)abc
T8	80.5 (9.2)b	0.63 (0.08)c	28.7 (3.3)d	115.3 (16.1)a	1.02 (0.13)c	33.4(3.8)bc	186.0 (27.1)a	1.67 (0.22)ab	43.3 (5.2) a
T9	89.1 (3.9)ab	0.79 (0.03)ab	34.6 (0.9)abc	139.3 (10.0)a	1.31 (0.08)a	41.5 (1.1)a	135.3 (32.5)bcde	1.39 (0.34)abcd	34.6 (7.8)bcd
T10	84.1 (5.7)ab	0.78 (0.04)abc	33.6 (1.0)bcd	93.0(11.7)b	1.18 (0.15)abc	33.1 (3.8)c	72.9 (24.4)e	0.84 (0.28)d	20.6 (6.9)d
T11	86.8(5.2)ab	0.72 (0.03)bc	34.1 (0.8)abcd	129.7 (10.7)a	1.19 (0.06)abc	39.7 (1.0)ab	129.6 (35.2)cde	1.33 (0.32)abcd	33.5 (7.5)bcd
T12	94.9 (3.7)ab	0.78 (0.03)abc	36.3 (0.8)a	127.2 (7.0)a	1.25 (0.05)abc	41.5 (1.1)a	116.6 (34.5)de	1.16 (0.32)cd	29.6 (8.1)cd
T13	92.2 (5.3)ab	0.76 (0.06)abc	34.8 (1.1)ab	118.8 (6.8)a	1.15 (0.06)bc	39.3 (1.0)ab	137.5(25.6)bcde	1.51 (0.28)abcd	36.9 (6.3)abc
T14	86.1(5.0)ab	0.76 (0.03)abc	34.3 (1.1)abc	119.9 (6.7)a	1.24 (0.06)abc	39.5 (1.1)ab	157.6 (20.3)abcd	1.83 (0.23)abc	41.7 (4.9)abc

* Means with same letters within each column of respective VGM parameters were not significantly different with LSD at $P = 0.05$. VGM for respective treatments were presented in mean of 10 replicationsand the numbers in the bracket were standard error.

artificially inoculated with moderately high and moderately aggressive *G. boninense* isolates reported by Kok *et al.* (2013) - Paloh (T3), Ulu Pedas (T5), GBA (T6), Pelam (T9), Bt Lintang (T10), Bebar (T11), Sg. Jernih (T12) and Pasir Gajah (T14) were significantly poorer than both the controls and other treatments (Table 2).

Four months after treatments, the leaf area and plant height of 5-month-old seedlings treated with *Ganoderma* isolates with moderately high level of aggressiveness (highly aggressive in 2-month-old seedlings) (Kok *et al.,* 2013), especially Bt Lintang (T10), were significantly lower than both the controls and most of the *G. boninense* isolates tested (Table 3). This agreed with Sapak *et al.* (2008) who also found that *Ganoderma*-infected seedlings were also significantly lower in height, diameter of the stem and root biomass compared to the control. Based on vegetative growth, *Ganoderma*-inoculated 5-month-old seedlings were less responsive compared with 2-month-old seedlings in relation to the degree of aggressiveness among the different *G. boninense* isolates (Tables 2 and 3). The age of oil palm seedlings at the onset of the experiment affected the DSI and DI in current and other previous studies (Breton *et al.,* 2006 and Kok *et al.,* 2013). Germinated seeds being inoculated with *Ganoderma* isolates were more susceptible to infection compared to the 3-leaf (approximately 2 to 3 months old) seedlings (Breton *et al.,* 2006). The degree of susceptibility towards fungal pathogens may decrease with age as reported for tobacco and castor (Mamza *et al.,* 2008; Reuveni *et al.,* 1986; Shibata *et al.*, 2010). Reduction in disease incidence and severity in older seedlings can be attributed to their higher lignin content or other mechanisms to counter *Ganoderma* infection (Cooper *et al.,* 2011; Idris *et al.,* 2004; Paterson *et al.,* 2009). In addition, the production and accumulation of antifungal phenolic acids, such as syringic acid by oil palm seedlings, were proposed to be relatively important mechanism to counter attacks by *G. boninense* (Chong *et al.,* 2012). However, more detailed studies are needed to verify this, e.g., whether older seedlings have higher level of phenolic acids and other metabolites.

Furthermore, it has been reported that leaves of infected seedlings progressively become chlorotic, browning and desiccated from older to the youngest, and stunted in growth, followed by stem decay as well as bole (Alizadeh *et al.,* 2011; Rees *et al.,* 2009; Sariah *et al.,* 1994). However, results showed that the leaf symptoms and formation of *Ganoderma* fruiting bodies appeared to manifest at different rates for different *G. boninense* isolates. This accounted for the large variation in vegetative growth among the treatments (12 different *G. boninense* isolates and 2 controls). Variations in the rate of infection or manifestation of the symptoms could also be partly due to the changes of infection developmental stages, for instance, biotrophic, more aggressive necrotrophic and resistance resting pseudo-sclerotia phases (Rees *et al.*, 2009). Cooper *et al.* (2011) also proposed that during the fungal penetration and colonization stages, a wide range of cell wall- and lignin-degrading enzymes were produced as well to aid with the infection process.

Hasan and Turner, (1998) reported that *Ganoderma*-infected palms could remain symptomless until internal infection becomes severe. This may explain the sudden increase in DI and DSI or a decline in vegetative growth, especially for T3 (Paloh) isolate after week 16^{th} although the seedlings were healthy prior to that (Table 1, 2 and 3).

Based on the observations performed using the method proposed by Rees *et al.,* (2007) for re-isolation of *G. boninense* from infected tissues on GSM, *Ganoderma* fungal mass was mainly concentrated at the stem bole and roots that were located near the bole (approximately 0 to 6 cm from the bole) in current experiment for both 2- and 5-month-old seedlings (Data not shown).

The correlation between 2- and 5-month-old seedlings in vegetative growth showed $R^2 = 0.71$ for height, 0.59 for diameter of girth and 0.75 for leaf area. Removal of two treatments, T5 (Ulu Pedas) and T14 (Pasir Gajah), had improved the above correlations ($R^2 = 0.87$ for height, 0.97 for diameter of bole girth and 0.89 for leaf area) (Table 4). Linear relationships between DI or DSI and VGM (height, diameter of bole girth and leaf area) at 16-weeks after inoculation for 5-month-old seedlings were negatively correlated as outlined in Table 4. Comparisons between DSI and DI with the three different vegetative growth parameters – leaf area, diameter of bole girth and height are illustrated in Fig. 1. In general, regression coefficient values were higher in the relationships between DI or DSI with VGM for 2-month-old seedlings (R^2 = -0.89 to -0.97) (Kok *et al.*, 2013) compared with DI or DSI with VGM parameters taken for 5-month-old seedlings (R^2 = -0.64 to -0.88) (Table 4). Disease severity in 5-month-old seedlings increased slower compared to 2-month-old seedlings (Kok *et al.*, 2013).

Oil palm seedlings at 2-month-old (approximately 3-4 leaf stage) challenged with *Ganoderma* inoculum (Kok *et al.,* 2013) were observed to be more responsive in terms of DI, DSI and VGM towards *G. boninense* infection compared to oil palm seedlings being inoculated at 5-month-old (approximately 5-6 leaf stage). Therefore, two-month-old seedlings are more favourable compared to 5-month-old seedlings for future nursery studies. In addition, more than one *G. boninense* isolates

Table 4. Linear relationships between different parameters – Disease incidence (DI), Disease severity index (DSI) and three vegetative growth measurements (VGM) (height, diameter of bole girth and leaf area) against different treatments (12 *G. boninense* isolates and 2 controls) for 2- and 5-month-old seedlings recorded during the experiment

Linear Relationship		Regression Coefficient
VGM (2 mth) x VGM (5 mth) at 16-weeks after treatment	Height	0.713
	Height (fitted)	0.871 (with T5 & T14 – removed)
	Diameter of bole girth	0.591
	Diameter of bole girth (fitted)	0.965 (with T5 & T14 – removed)
	Leaf Area	0.753
	Leaf Area (fitted)	0.886 (with T5 & T14 – removed)
DI (5 mth) x VGM (5 mth) at 16-weeks after treatment	Height	-0.772
	Diameter of bole girth	-0.641
	Leaf Area	-0.818
DSI (5 mth) x VGM (5 mth) at 16-weeks after treatment	Height	-0.830
	Diameter of bole girth	-0.692
	Leaf Area	-0.877

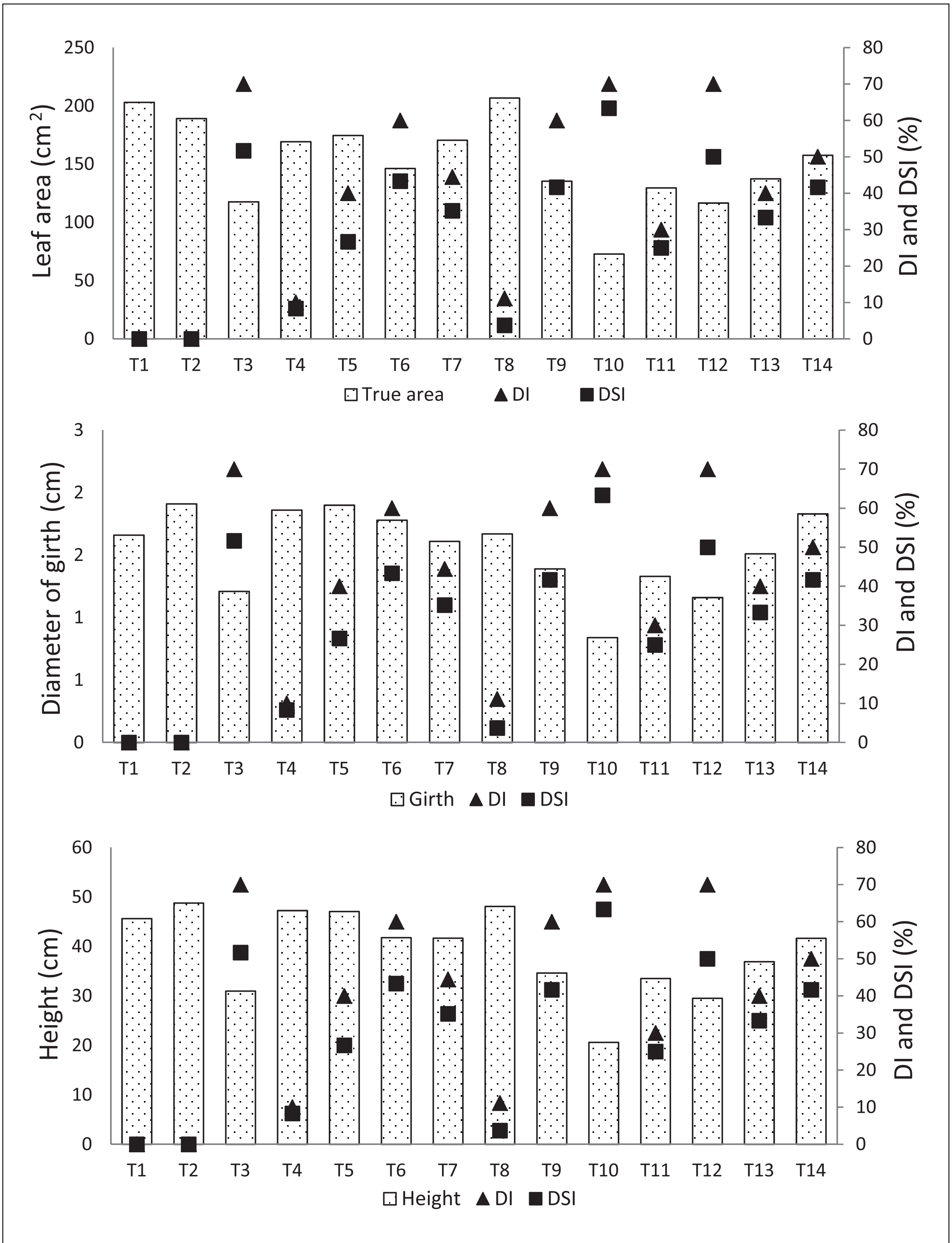

Fig. 1. Comparisons between both DI and DSI with three different vegetative growth measurements – leaf area, diameter of bole girth and height for 5 months old oil palm seedlings on week 16.

should be incorporated to screen for *Ganoderma*-tolerant planting materials, potential biocontrol agents for controlling *Ganoderma* pathogens, plant-pathogen (bi-trophic) or plant-pathogen-beneficial microbe (tri-trophic) interactions, and other nursery experiments on *G. boninense*.

CONCLUSIONS

Among 12 different *Ganoderma boninense* isolates, the isolates from BtLintang (Kedah) (T10) (63.3%) were the most aggressive, whereas, Fraser (Johor) (T4) (8.3%) and Pinji (Perak) (T8) (3.7%) were the least aggressive based on disease severity index (DSI) (Table 1). Based on the results obtained from vegetative growth measurements (VGM), namely leaf area (cm^2), diameter of the bole girth (cm) and height (cm), for both 2- and 5-month-old seedlings challenged with the T10 isolate were significantly lower compared to both of the T4 and T8 isolates, as well as the controls at 4 months-after-treatment (MAT) (Table 2 and 3). The linear correlation between DI or DSI with VGM at 16-weeks after treatment was negative, ranged from 0.64 to 0.88 for 5-month-old seedlings. In addition, the linear relationships between VGM readings for all three parameters obtained from 2- and 5-month-old seedlings at 4 MAT showed relatively high positive correlations (Table 4). Oil palm seedlings at the age of 2-month-old are more preferable in future nursery trials compared to 5-month-old seedlings, as the former seedlings were more responsive in terms of DSI with VGM parameters measured.

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