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SELECTION CRITERIA OF *Graptophyllum pictum* RESISTANCE TO *Doleschallia bisaltide* CRAMER (LEP: NYMPHALIDAE) ATTACK BASED ON INSECT FEEDING PREFERENCE

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SUMMARY

The damage caused by *Doleschallia bisaltide* (Lepidoptera: Nymphalidae) larval phase attack is a serious problem on caricature plant development. One solution for overcoming larval attack is the use of resistant varieties. This study was conducted to determine the resistance level of 13 caricature plant accessions and identify selection criteria for the resistance trait based on *D. bisaltide* larval feeding preference. Phytochemical substances were analyzed to determine selection criteria of plant resistance. The experiment used nonfactorial randomized complete block design, using 13 accessions in the non-choice experiment. Data were analyzed by ANOVA and multiple comparisons performed with Tukey test. Correlation analysis between traits was also done. Several putative caricature plant accessions were found to be resistant to *D. bisaltide* larval attack. All the resistant accessions influenced the growth of *D. bisaltide* larvae lightly**.** Alkaloids, cellulose, and c organic contents influenced the feeding preference of *D. bisaltide* larvae. Cellulose content can be used as a selection criterion for resistance trait based on *D. bisaltide* feeding preferences.

Key words: Caricature plant, phytochemical substances, resistance trait, selection criteria, feeding preference, *Doleschallia bisaltide*

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INTRODUCTION

The caricature plant (*Graptophyllum pictum*) is a traditional medicinal plant that has been used by local communities in West Java, Mollucas, and the Papua Islands (Khumaida *et al.*, 2008b). Its leaves are used to treat wounds, swelling, ulcers, skin diseases, liver, ears, and cough (Wibowo, 2000). Khumaida *et al.* (2008a) found 38 caricature plant accessions from several Indonesian islands, 13 of which had high phytochemical substances, such as alkaloids, flavonoids, steroids, glycosides, and terpenoids. These accessions can be used as parental lines in a breeding program to develop new varieties.

The important parts of caricature plants are the leaves. Therefore, managing herbivore attack on leaves is important to improve production. The development of resistant varieties is a high priority in caricature plant breeding programs.

Related to biotic stress breeding in caricature plant are several important pests. Baringbing and Mardiningsih (2000) mentioned that *Valanga* sp. and *Doleschallia* sp. attack caricature plant leaves. Mardiningsih *et al.* (2012) enumerated other pests associated with the caricature plant: *Rastrococcus viridarii* Williams (Hemiptera: Pseudococcidae), *Saissetia neglecta* De Lotto (Hemiptera: Coccidae), *Insignorthezia insignis* Browne (Hemiptera: Ortheziidae), *Aphis gossypii* Glover (Hemiptera: Aphididae), and *Astrothrips tumiceps* Karny (Thysanoptera: Thripidae).

Previous research hasidentified a pecies in genus *Doleschallia* that attacks *Graptophyllum pictum.* Mardiningsih *et al.* (2008) reported that *D. bisaltide* attacked caricature plants in West Java while Sartiami *et al.* (2009) mentioned 2 other species, *D. nacar* and *D. hexoptalmos* specifically attacking caricature plants in Mollucas and Papua region, respectively.

Among the species that attack caricature plant leaves, *D. bisaltide* caused the highest yield loss, up to 70% in only several days. Abang (2006) reported some species in subfamily Nymphalinae having gregarious larvae. Many publications confirm that caricature plants and some others in Acanthaceae are primary hosts for this species (Edwards *et al.,* 2001; Vane-Wright and De Jong, 2003; Winanti, 2010; Anonymous, 2013). The literature points to *D. bisaltide* having a preference for *Graptophyllum* but no research mentions how *Graptophyllum* responds to *D. bisaltide* and how breeding for resistance traits can help.

Plant resistance breeding requires knowledge of plant defense mechanisms. Rosenthal and Kotanen (1994) mentioned 2 categories of plant defense, resistance and tolerance. Furthermore, Van Emden (2002) divided resistance into antixenosis and antibiosis. Villarino and Ravetta (2007) defined resistance as a mechanism to prevent the arrival of herbivores (antixenosis) or prevent herbivores from continuously feeding on the plant (antibiosis), whereas tolerance is described as a mechanism for maintaining fitness and productivity at the level of specific attacks.

Related to plant defense mechanisms, Fraenkel (1969) and Honda (1995) found that secondary metabolites constitute the main defense system in plants. Schoonhoven *et al.* (2005) said that morphology and plant nutrient content also influence growth and development of herbivores. Therefore, the feeding preferences of *D. bisaltide* larvae for each accession of caricature plant can be used as a parameter for determining resistance in caricature plant germplasm accessions against *D. bisaltide*. It also can be used as a selection criterion for screening segregating populations. Furthermore, research on secondary metabolites of caricature plant leaves can provide information about phytochemical substances that can be used as selection criteria to determine its resistance to biotic stresses. This study aimed to identify the resistance levels of 13 caricature plant accessions and to identify selection criteria based on the feeding preferences of *D. bisaltide* larvae.

MATERIALS AND METHODS

Preparation of plant materials

All plant materials came from the Indonesian Medicinal and Aromatic Crop Research Institute (IMACRI) collection. The origin of the 13 accessions studied is shown in Table 1. Cuttings of the 13 caricature plant accessions were planted in a sandbox containing soil: manure: husk medium (at 1:1:1 ratio), and maintained on pads with 55% shade for 8 months. The plants were grown in the IMACRI screen house. Plants were at the vegetative phase until the experiment was completed.

To measure phytochemical content, the plants in the screen house were divided into 2 groups. The first group was harvested without any treatment. They were used as control samples (normal condition if the plant is not attached). All plants group 2 were infested by larvae for a minimum of 12 h, and then harvested. This method was based on a protocol described by Rosenthal and Jansen (1979) that assumes that plants will produce secondary defense metabolites within 12 h after being attached. All leaf samples were then dried at 40 ^{o}C and extracted for analysis.

Table 1. The origin of 13 caricature plant accessions.

Accession number	Origin
1	Clone 1, West Java
2	Clone 2, West Java
3	Clone 7, East Java
4	Clone 8, Middle Borneo
5	Clone 9, South Borneo
6	Clone 11, Mollucas
7	Clone 12, Mollucas
8	Clone 14, Mollucas
9	Clone 17, Papua
10	Clone 18, Papua
11	Clone 19, Papua
12	Clone 20, West Java
13	Clone 38, West Java

Insect rearing and infestation

The insects used in this experiment were the second-generation larvae reared in gauze cages. The first-generation eggs were collected from the caricature plant garden that forms part of the IMACRI genetic collection. First-instar larvae until second-instar (II0 larvae were reared in the nursery box; then instar III larvae were transferred into a gauze cage until their pupal stage. When the butterflies emerged, they were transferred to a different cage. For maintenance, the larvae were fed with caricature plant leaf and the butterflies were fed with flowers of *Ixora coccinea* (Rubiaceae), *Hydrangea macrophylla* (Hydrangeaceae), and 10% pure honey solution. The next-generation larvae that emerged from the eggs were used in this experiment.

First-instar larva was used to infest one plant using a brush, and the plant was then isolated using plastic mica. The top of the plastic was covered with gauze pads so that the larvae do not migrate to other plants. To minimize larval stress due to lack of food, caricature plants were replaced before all the leaves were consumed. The larvae were observed every day until they turned into pupae.

Experimental design

The experiment used a randomized complete block design non-factorial with non-choice method. Each treatment had 5 replications involving 3 plants per replication. Data recorded included leaf area consumed per instar larva and plant phytochemical contents. We also determined the plant accession's influence on insect preference by observing insect growth and behavior, including larval behavior, adult oviposition preference, long life per larval stadium, pupal weight, and lifetime of the butterflies.

Leaf area calculation

Leaf area consumed by the larvae was determined by comparing total leaf area before and after eating by the larvae. Leaf area was calculated using the gravimetric method (Sitompul and Guritno, 1995, as cited in Fahrudin 2009).

The whole leaves of the caricature plant were drawn on A4 size paper (50 g). Those leaf scathes were cut and weighed. As control, 1 cm^2, 100 cm^2, and 580 cm^2 were weighed. The weights of the leaf images were compared with

those of the control using the following equation:

$$\text{Leaf area} = \frac{\text{Weight of leaf slices}}{\text{Weight of paper}} \times \text{paper area}$$

Total leaf area consumed was calculated by this equation:

Leaf area consumed = leaf area before eating – leaf area after eating

Percentage leaf area consumed by intact leaf areas was calculated using the following equation:

Consumed leaf area (%) = (Leaf area consumed/ Intact leaf area) x paper area

Phytochemical analysis

The test included several compounds that are related to the plant defense mechanisms against herbivores — e.g. anthocyanin, chlorophyll, carotenoids, alkaloids, terpenoids, phenols, fiber, saponins, flavonoids, nitrogen, organic carbon, and calcium elements. These phytochemical substances were correlated with larva-consumption area to determine the compounds' effects on larval preference. The analytical method is explained in the following sections.

Pigment analysis

The method used was reported by Sims and Gamon (2002). Each pigment was analyzed at different wavelengths: 663 nm for chlorophyll a (Chl a), 647 nm for chlorophyll b (Chl b), 537 nm for anthocyanin, and 470 nm for carotenoids. The data from the spectrophotometer were converted into mol/m^2 using the following equations:

Anthocyanin = 0.01373*A537 – 0.00697*A647 – 0.002228*A663

Chl a = 0.01373*A663 – 0.000897*A537 – 0.003046*A663

Chl b = 0.02405*A647 – 0.004305*A537 – 0.005507*A663

Carotenoid = (A470-(1.71*(Chl a+ Chl b) - 9.479*anthocyanin))/119.26

Total chlorophyll was estimated by the equation:

Total chlorophyll = 7.15*A663 – 18.71*A647

The other phytochemical substances were analyzed in the IMACRI laboratory by using the following methods: nitrogen was analyzed by the Kjeldahl method (Baker and Thompson, 1992); calcium was detected by the AAS method (Hanlon, 1992); and organic carbon was detected by spectrophotometry (Balittanah, 2009). Fiber was detected using gravimetric method (SNI 01-2891-1992), and the other phytochemicals were detected by screening (Depkes, 1995). Some data were scored from 1 to 4, based on IMACRI laboratory scoring.

Data analysis

Data analysis using SAS software version 9.0. We used ANOVA at 5% level of error with Tukey method (Gomez and Gomez, 1995). The Pearson correlation test (Gomez and Gomez, 1995) was also used to analyze feeding preferences with morphological and phytochemical characteristics of the caricature plant. According to Singh and Chaudhary (1979), path analysis was used to determine the role of each caricature plant character on larval feeding preference, both direct and indirect. Larva consumption area was used for determining the resistance level of each accession.

RESULTS AND DISCUSSION

Caricature plant phytochemical substances

Secondary metabolites play a major role in the interaction between the plant and the surrounding environment, including the relationship between plants with herbivores. Whittaker and Feeny (1971) reported the use of plant secondary metabolites as a defensive strategy against herbivores or competitors. On the other hand, Panda and Khush (1995) explained that secondary metabolites act as a fingerprint compound for herbivores to find their host.

The results show some variation within the 13 caricature plant accessions based on phytochemical substances. The data indicated

that all accessions contained alkaloids, tannins, steroids, and glycosides in high concentrations, while flavonoid, saponin, and triterpenoid contents varied among accessions. It was interesting to note that accession number 12 had the lowest saponin content (Table 2). The literature states that steroids, saponins, and triterpenoids are a deterrent to insects (Vickery and Vickery, 1981; Schoonhoven *et al.*, 2005; Brielman *et al*., 2006). In contrast, flavonoids may attract insects (Panda and Khush, 1995; Wink, 2006; Kristina and Mardiningsih, 2008).

In terms of pigment analysis, Table 2 indicates accessions rated from 1 and 9 for anthocyanin concentration. Accessions 10, 12, and 13 contained high concentration in chlorophyll, whereas accessions 9, 10, and 13 have high concentrations in carotenoids. Furthermore, the C-N ratio in 13 accessions ranged between 9.56 and 11.50, with accessions 1 and 12 having the lowest values. In other words, both accessions contain more nitrogen than organic carbon. Calcium values ranged from 0.59 to 1%. Fiber content ranged between 13.78 and 19.51%. The 13 accessions came from various regions in Indonesia. As to character similarities based on some morphological and phytochemical traits, it was found that accessions 1 and 3 and also accessions 4 and 5 have levels of similarity up to 85% (Figure 1).

Larval preference on caricature plant accessions

While in larval stadia , insects consume food and store it in their bodies. This food will be used as energy in the pupal stage. Insects will become pupae when they have developed sufficient food reserves in their bodies (Bernays 2001; Bjornson and Schutte 2003). Each instar larval stadium of *D. bisaltide* has a different feeding preference. The first instars generally consume shoots and a few pairs of leaves underneath, whereas the advanced instar stadia tend to eat mature leaves. The instar IV and the beginning of instar V stadia had the biggest consumed leaf area of the plant (Table 3). They eat all the leaves, leaf veins, petioles, and flowers.

During the larval phase, *D. bisaltide* could consume 300.28−745.86 cm^2 leaves (purple leafy accession). This insect was observed to consume 369.37 cm^2 in greenish chimera leafy caricature plant accession (accession 12). Furthermore, these data were used to assemble resistance categories by the box plot method (Table 4).

The feeding preferences of *D. bisaltide* on the 13 caricature plant accessions (n = 65, α = 0036) were different. Accessions 7 and 13 were the least consumed and differed from accession 2 based on Tukey's test. Furthermore, the grouping results showed accessions 7, 8, and 13 having putative resistance, while accessions 2, 5, and 9 were putatively susceptible (Table 5). The other accessions tend to be moderate. Assessment of 3 resistant accessions' effects on larval metabolism was done by analyzing the growth variables of the larvae.

When the insect larvae have reached a certain weight, they will molt. It is assumed that body weight is related to the amount of food that enters the larva. Hence, the time required for each larva to molt varies, depending on the increase in body weight. The data showed that the larvae of *D. bisaltide* lived for 14−16 days (Figure 2). Furthermore, the pupae need 4−7 days to emerge and become a butterfly. Larvae that consumed accessions 4, 9, and 12 have the shortest larval period, while larvae on accessions 2, 11, and 13 have the longest. Moreover, data on pupal weight showed larvae consuming more leaves (accessions 2, 5, and 9) and they did not necessarily have a greater weight than larvae that consumed fewer leaves (accessions 7, 8, and 12) (Figure 3). The data confirm that each caricature plant accession influences larval metabolism lightly.

The role of each caricature plant character on larval feeding preference

Correlation tests were used to explain the relationship between each caricature plant leaf character and *D. bisaltide* larval feeding preferences through the leaf area consumed. Roy (2008) states that a correlation value close to -1 or 1 means that the 2 characters are closely correlated. It means, the characters that correlate with consumed leaf area, either directly or indirectly, can be used in path analysis to determine the role of each character in establishing larval feeding preference.

Table 2.Phytochemical substances in 13 caricature plant accessions.

Accession	Alkaloids	Saponins	Tannins	Flavonoids	Terpenoids	Steroids	Glycosides	Anthocyanin	Chlorophyll	Carotenoids	C org	N	C/N	Ca	Fiber
	(Score)							(umol/ml)			(%)				
1	4	3	4	3	1	3	4	0.62	0.34	0.22	39.43	3.00	13.14	1.05	15.18
2	4	3	4	3	1	4	4	-	-	-	40.18	3.00	13.39	1.09	15.58
3	4	4	4	3	2	3	4	0.47	0.39	0.22	41.91	2.73	15.35	1.29	14.38
4	4	3	4	2	1	4	4	0.39	0.39	0.22	38.12	2.86	13.33	1.33	15.09
5	4	3	4	2	2	3	4	0.26	0.24	0.15	38.66	2.95	13.11	1.24	15.21
6	4	3	4	2	2	3	4	0.37	0.41	0.22	38.21	3.16	12.09	1.15	14.21
7	4	4	4	2	2	3	4	0.31	0.35	0.20	42.36	2.72	15.57	1.19	16.02
8	4	4	4	3	2	3	4	0.31	0.31	0.20	40.73	2.84	14.34	1.06	14.49
9	3	4	4	2	2	3	4	0.60	0.50	0.30	41.42	2.94	14.09	1.05	13.58
10	3	4	4	2	1	4	4	0.45	0.51	0.28	38.45	2.92	13.17	1.19	14.19
11	3	4	4	1	2	3	4	0.37	0.44	0.24	42.66	3.03	14.08	1.06	15.15
12	4	2	4	2	1	4	4	0.13	0.57	0.25	40.97	3.05	13.43	1.30	15.49
13	4	3	4	1	1	4	4	0.46	0.52	0.28	40.87	2.70	15.14	1.37	12.69

Table 3. Leaf area consumed by each instar larva *D. bisaltide* on 13 caricature plant accessions.

Accession	Leaf area before consumption		Consumed leaf area (cm^2)										Total consumed (cm^2)
			Instar I		Instar II		Instar III		Instar IV		Instar V		
1	1677.76	±567.85	13.99	±10.50	32.14	±28.06	68.50	±26.67	100.54	±87.95	199.47	±133.95	414.64 ab
2	1868.87	±234.44	42.01	±13.03	96.60	±57.63	162.87	±117.95	203.05	±109.48	241.32	±77.09	745.86 a
3	2156.52	±474.58	28.34	±16.38	52.56	±20.82	82.19	±25.14	71.65	±43.36	293.57	±120.64	528.30 ab
4	1963.35	±903.33	14.39	±12.20	32.32	±17.64	81.90	±61.28	123.23	±112.59	172.09	±158.57	423.93 ab
5	2193.24	±157.45	38.55	±23.86	66.67	±27.04	117.76	±56.09	115.66	±43.06	231.69	±64.18	570.33 ab
6	1754.15	±639.03	17.49	±8.70	80.14	±56.01	90.26	±72.43	69.59	±37.56	249.40	±184.86	506.89 ab
7	1310.25	±393.02	11.21	±5.33	28.47	±14.20	92.50	±56.66	77.01	±36.37	91.09	±55.07	300.28 b
8	1459.35	±194.67	30.08	±15.60	46.53	±28.93	33.72	±13.30	65.74	±41.37	130.03	±83.45	306.10 ab
9	1752.10	±380.70	30.87	±6.50	52.42	±15.06	94.58	±22.97	124.92	±29.63	240.43	±52.71	543.23 ab
10	1613.21	±415.66	26.16	±10.49	38.83	±24.83	97.47	±36.83	80.24	±36.06	200.16	±78.28	442.86 ab
11	1527.68	±395.52	26.86	±8.01	42.74	±28.53	83.18	±35.07	79.37	±37.89	222.71	±103.34	454.86 ab
12	1234.76	±159.31	16.76	±6.12	38.82	±26.07	71.72	±29.65	41.02	±14.01	201.05	±41.85	369.37 ab
13	1312.22	±431.53	8.66	±3.85	36.61	±6.62	29.98	±14.68	73.15	±9.85	122.40	±23.11	270.80 b

Note: Data mean±stdev

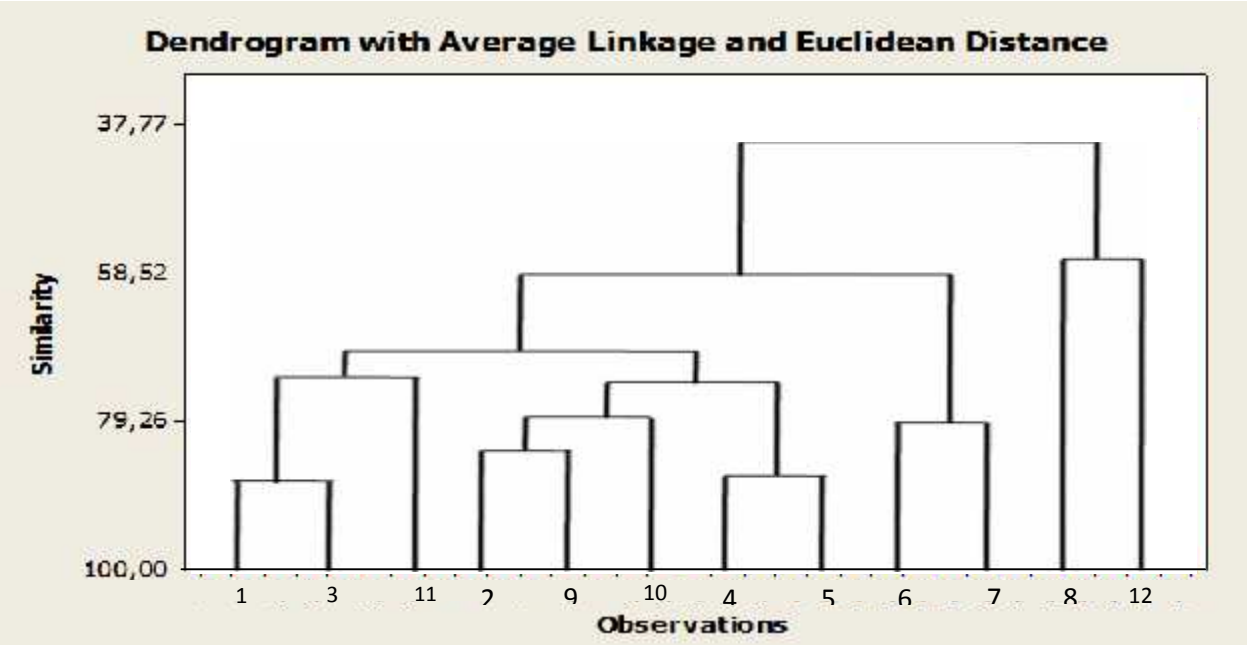

Figure 1. Genetic similarity among caricature plant accessions.

Table 4. Plant groupings based on resistance criteria (percentage of damage).

Damaged area (cm^2)	Relative damage criterion	Relative resistance criterion
x = 0	No damage	Immune
0 < x ≤ 40.69	Light damage	Very resistant
40.69 < x ≤ 337.735	Moderate damage	Resistant
337.735 < x ≤ 535.765	Slightly heavy damage	Moderate
535.765 < x ≤ 832.81	Heavy damage	Susceptible
832.81 < x	Very heavy damage	Very susceptible

Table 5. Feeding area, relative damage Criteria, and resistance category of 13 caricature plant accessions to *D. bisaltide* larvae.

Accession	Feeding area (cm^2)	Relative damage criterion	Relative resistance criterion
1	414.64 ab	Slightly heavy	Moderate
2	745.86 a	Heavy	Susceptible
3	528.30 ab	Slightly heavy	Moderate
4	423.93 ab	Slightly heavy	Moderate
5	570.33 ab	Heavy	Susceptible
6	506.89 ab	Slightly heavy	Moderate
7	300.28 b	Moderate	Resistant
8	306.10 ab	Moderate	Resistant
9	543.23 ab	Heavy	Susceptible
10	442.86 ab	Slightly heavy	Moderate
11	454.86 ab	Slightly heavy	Moderate
12	369.37 ab	Slightly heavy	Moderate
13	270.80 b	Moderate	Resistant

Note: The same letter on the same column indicates no significant difference based on Tukey at α = 5%.

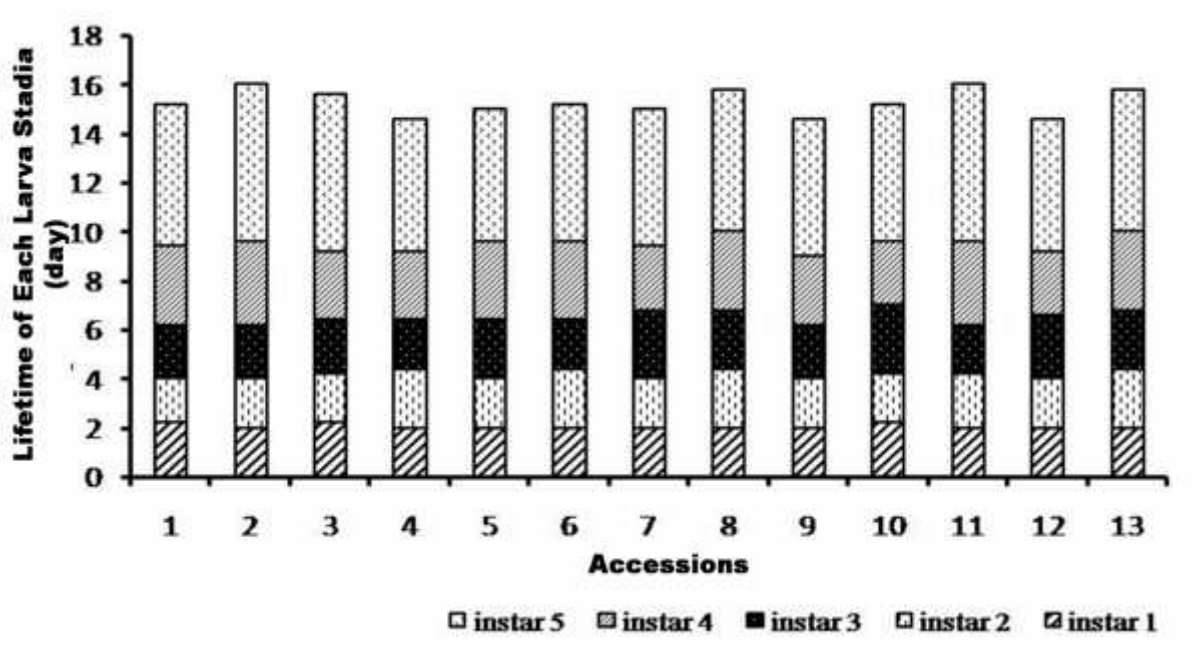

Figure 2. Length of life of each instar stadium on *D.bisaltide* larvae.

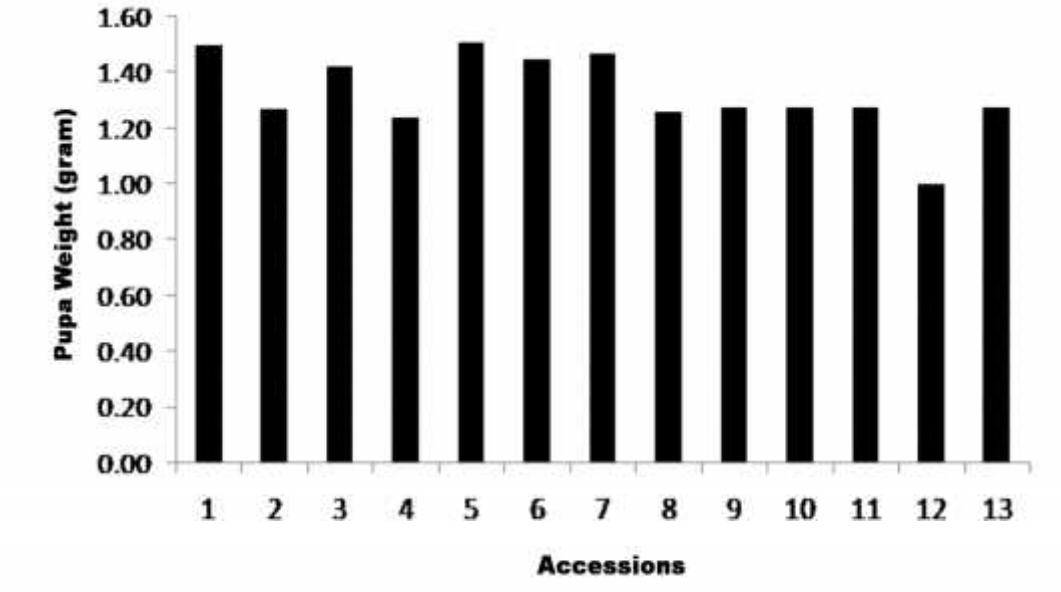

Figure 3. Pupal weights on 13 caricature plant accessions

The correlation analysis showed that leaf area consumption correlated with fiber content (-0.72) and alkaloid content (-0.69) (Table 6). Both of them can be used as direct characters for path analysis. While organic carbon content correlated with alkaloid content (Table 6) which alkaloids was correlated directly to leaf area consumption, so the organic carbon can be used as indirect character in the path analysis. Direct and indirect values are shown in Table 7.

Table 6. Correlation of intermorphological characters and phytochemical substances on caricature plant with feeding preference of *D. bisaltide.*

	Antho	Chl	Caro	C	N	C/N	Ca	Fiber	Alkaloids	Saponins	Flavo	Terpen	Steroid
Chl	-0.99**												
Caro	-0.89**	0.83**											
C	-0.19	0.14	0.33										
N	-0.29	0.30	0.25	0.53									
C/N	0.25	-0.27	-0.13	-0.13	-0.91**								
Ca	0.30	-0.32	-0.21	-0.52	-0.43	0.25							
Fiber	0.06	-0.15	0.31	0.00	0.01	-0.03	0.23						
Alkaloids	-0.05	0.04	0.07	-0.57	-0.15	-0.11	0.41	0.36					
Saponins	0.48	-0.50	-0.33	0.12	-0.29	0.38	-0.33	0.14	-0.53				
Flavo	0.38	-0.38	-0.36	-0.40	0.02	-0.21	0.14	0.28	0.38	0.12			
Terpen	0.20	-0.25	0.02	0.11	-0.47	0.60*	-0.25	0.13	-0.10	0.55	0.11		
Steroid	-0.52	0.54	0.35	0.01	0.27	-0.30	0.19	-0.06	0.00	-0.46	-0.37	-0.84**	
Fed area	-0.36	0.40	0.18	0.44	0.13	0.09	-0.36	-0.72**	-0.69*	-0.09	-0.52	0.03	0.07

Notes: Correlation based on Pearson's method. *=Significant at the 5% level, **=Very significant at the 1% level.
Antho = anthocyanin; chl = chlorophyll; caro = carotenoids; C = carbon organic; N = nitrogen; Ca = calsium; Flavo = flavonoid
Tannin and glycoside cannot be correlated because there was not much data variation.

Table 7. Direct and indirect effects of variables on *D. bisaltide* larval feeding preference based on pathways analysis.

Parameter	Direct effect (c)	Indirect effect through parameters			Total effect
		Fiber	Alkaloids	Organic C	
Fiber	-0.606		-0.115	-0.000	-0.722
Alkaloids	-0.323	-0.218		-0.146	-0.687
Organic C	0.258	0.001	0.183		0.441
Total C	-0.671				
Residue	1.671				

Path analysis showed that the alkaloid and fiber content had negative effects on feeding preferences directly. In contrast, organic carbon content positively influenced the feeding preference of *D. bisaltide* larvae.

Pearson correlation analysis also showed almost no correlation between fiber and organiccarbon contents. In this study, the organic carbon analyzed was the carbon element binding organic materials, such as carbohydrates. Furthermore, the fiber component is cellulose (there is no component of organic matter in it).

Path analysis showed that alkaloids and fiber had direct negative effects on larval feeding preference. Singh and Chaudhary (1979) proposed 3 cross-interpretations of data from the path analysis: 1) If the correlation value between the character and target is almost equal to the direct influence value, then the correlation can describe an actual relationship. Selection will be based on the character effectively. 2) If the correlation is positive but the direct effect is negative or 0, then the indirect effect is the cause of the correlation. Indirect influence should be considered. 3) If the correlation is negative and small but the direct effect is positive and large, then the selected character should be limited to eliminate the indirect effect in order for the value of direct effect to be more useful. Using the Singh and Chaudhary interpretation, fiber and alkaloid as direct characters in the path analysis have negative values (CX2 = -0606; -0.323), almost equal those of the correlation values (RX2 = -0722; -0.690). It indicates that both fiber and alkaloid content can be used as selection criteria for the resistance trait to *D. bisaltide* larvae.

DISCUSSION

The consumed leaf area by *D. bisaltide* larvae in this experiment was similar to that reported by Kristina and Mardiningsih (2008) in *D. polibete.* They reported consumed leaf area to be 470.36 cm^2 on the purple leafy clone of the caricature plant and 392.43 cm^2 on the greenish chimera clone. However, the values they got were greater than what Rojak and Rochimat (2007) obtained in their experiment using a petri dish instead of a whole plant. In their experiment, the larva was placed in a petri dish and was fed one caricature plant leaf a day. It was suggested that having infested larvae on the plant minimizes stress on the larvae and there were more leaves to be eaten daily.

The resistance level of each caricature plant accession to *D. bisaltide* larvae is determined by the use of the box plot method. As a result, 3 accessions (of the 13) were resistant and 3 others were susceptible. However, all resistant accessions affected larval growth lightly, as proven by larval growth parameter. Ellis (1998) reported that the diet of the caterpillars affected the time to pupation. In this experiment, time to pupation did not differ regardless of whether the larvae were fed leaves of resistant or susceptible accessions. All the larvae finished the 5 stages of the instar phase. Pupal weights also did not differ. Sartiami *et al.* (2009) mentioned 5 instars with 19.7 days at the larval stage. It shows that the caricature plant is a suitable host for *D. bisaltide* larvae.

Observations on morphological and phytochemical characters were made to determine the factors influencing caricature plant resistance to larvae of *D. bisaltide*. The data were then used for path analysis. The results showed fiber and alkaloid contents in the leaf having a direct negative effect on larva *D. bisaltide* feeding preference on certain caricature plant accessions. In contrast, organic carbon content in the leaf proved to have indirect and positive effects on larval feeding preference. Both fiber and alkaloid contents were found to be suitable selection criteria. Almost all accessions had high alkaloid concentrations, but among them, accessions 7 and 12 contained the highest fiber concentration. Based on this experiment, fiber content would be a more effective selection criterion than alkaloid content.

Alkaloid has been known as a feeding deterrent for many Lepidopteran species. Aniszewski (2007) reported that these compounds were toxic and inhibit larval growth and development. Plants with high concentrations of alkaloids tend to be more resistant to herbivores than species with low alkaloid content (Villarino and Ravetta 2007). Among the 13 accessions, almost all had

alkaloids in high concentration. It was only in accessions 9, 10, and 11 that this compound was lower. The findings indicate that *D. bisaltide* larvae can sequester or that they do not ingest alkaloids; these compounds therefore do not affect growth.

Furthermore, the organic carbon effect on *D. bisaltide* indicates that the larvae need carbohydrate-based compounds to grow, not protein. Tate and Wimer (2002) explained that carbohydrates are stored as glycogen, trehalose, glucose, and maltose. The Nymphalidae accumulate carbohydrates during the larval stage for reproductive activity (Bauerfeind and Fischer, 2005). That is why these insects tend to like caricature plant with high organic carbon content. When the insects enter the pupal phase, the carbohydrate will be used to produce energy. Based on the results, it is concluded that all accessions do not use the antibiosis mechanism as a defense strategy, but rather that caricature plants use antixenosis for *D. bisaltide*. It is because of the different composition of plant leaf pigments. Some publications confirm that that flavonoids are attractive to insects (Panda and Khush, 1995; Wink, 2006; Kristina and Mardiningsih, 2008). Guo *et al.* (2008) and Cuttriss *et al.* (2006) specifically state that anthocyanin content is involved in antibiosis. But this statement cannot be validated because it used the non choice method in this experiment. According to Villarino and Ravetta (2007), antixenosis is a mechanism that prevents the arrival of herbivores, while antibiosis is the strategy that prevents herbivores from continuously feeding on the plants.

Based on resistance grouping using the box plot method, accessions 7, 8, and 13 are the putative resistant accessions to *D. bisaltide* larvae. Fiber content in the leaves of the plant can be used as selection criterion to screen for resistance trait. Based on fiber content, accessions 7 and 12 can be selected for further development.

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