# PEST INCIDENCE-SEVERITY OF *TENUPALPUS PACIFICUS* TOWARDS LEAF MASS AREA (LMA) ON SEVERAL *Dendrobium* SPP.

(*Tingkat Kejadian-Keparahan hama* Tenupalpus pacificus *terhadap Luas Massa Daun (LMD) pada beberapa* Dendrobium *spp.*)

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**ABSTRAK**

Beberapa anggrek jenis Dendrobium spp. terkenal akan keindahan dan kegunaan obatnya. Upaya pelestariannya masih menjadi prioritas utama di antara tanaman anggrek di Kebun Raya Bogor (BBG). Namun, tungau laba-laba merah (*Tenupaipus pacificus*) menyebabkan kerusakan pada daun beberapa spesies dendrobium di rumah kaca anggrek. Kami melakukan penilaian penyakit dengan tingkat insiden dan keparahan pada spesies yang paling banyak diserang seperti *Dendrobium bigibbum, Dendrobium lineale, Dendrobium sanguinolentum* dan *Dendrobium secundum*. Kami mengukur massa daun (LMA) sebagai salah satu sifat defensif yang mungkin mempengaruhi pola serangan hama tersebut. Penilaian menunjukkan bahwa *D. secundum* memiliki tingkat rata-rata insiden dan keparahan tertinggi (masing-masing 0,84 dan 0,30) di antara spesies lainnya. Pola ini berkorelasi dengan nilai rata-rata terendah dari masa daun (41,86 g $m^{-2}$) dibandingkan dengan *D. lineale* yang memiliki pola yang berlawanan baik pada LMA maupun pada insiden dan keparahan. Korelasi positif dengan tingkat keparahan yang cenderung lebih rendah dari insiden menunjukkan bahwa T. *pasificus* masih berada dalam level pandemi tetapi belum mencapai kerusakan fatal ataupun kematian karena dua spesies lain, *D. bigibbum* dan *D. lineale*, memiliki nilai LMA yang tinggi yang dapat memperlambat perilaku hama. Hasil ini diharapkan menjadi dasar manajemen pengendalian dan sanitasi pada hama tersebut.

**Kata kunci : anggrek, luas massa daun, penaksiran penyakit, tungau laba-laba merah**

***ABSTRACT***

*Many* of Dendrobium *spp. are famous due to its beauty and medicinal uses. Their preserving efforts are still top priority among orchid plants in Bogor Botanical Garden (BBG). However, false spider mite (*Tenupaipus pacificus*) caused damages on leaves of several dendrobium species under greenhouse conditions. We examined diseases assessments of incidence-severity to the most visually attacked species such as* Dendrobium bigibbum, Dendrobium lineale, Dendrobium sanguinolentum and Dendrobium secundum. *We also measured Leaf Mass Area (LMA) as a defensive trait that might influence patterns of the pest's attack. The assessment showed that* D. secundum *had the highest average incidence-severity (0.84 and 0.30 respectively) among other species. This pattern was correlated with its lowest average LMA (41.86 g $m^{-2}$) compared to* D. lineale *which had the opposite patterns both LMA and incidence-severity. Positive correlation on incidence-severity with tendentiously lower severity than incidence suggest that* T. pasificus *was still in outbreak but it had not reached fatal damages or deadly result since two other species,* D. bigibbum *and* D. lineale*, had high LMA that could slow the feeding rate of pest's behavior. This result is expected to be basic sanitary control management to overcome the pests.*

***Keywords: diseases assessments, false spider mites, orchids, leaf mass area***

## I. INTRODUCTION

Dendrobium orchids have appealing and great characteristics and diverse of flower colors, making them has high economic value as ornamental flower (Bulpitt et al. 2007, Huda-shakirah and Mohd, 2021). In China, several

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*Dendrobium* spp. are even grown for herbal drug manufacturers (Bulpitt et al. 2007). Famous as its beauty and medicinal uses, it is necessary to do conservation efforts, one of which is by ex-situ preservation in the botanical garden. However, several dendrobium species preserved in the BBG were shown of feeding injuries on their leaves. The leaf damages were also firstly characterized by silverish or bleached spot appearances and sometimes with tiny bright orange dots. Based on previous studies on several orchid species and laboratory observation, the symptoms were caused by false spider mites, either *Tenupalpus pacificus* (Leonhardt and Sewake 1999, Labanoswski and Soika 2011, Denmark 2018, Wildaniyah et al. 2018). The most part of affected leaves tends to be lower surface with yellow brownish dark color that indicates the leaf tissue oxidizes. This happens because the mites suck leaf sap and chlorophyll. As the damage in the lower surface of leaves become larger and darker, upper leaf will later turn become rusty brown and dark in color (Leonhardt and Sewake 1999, Labanoswski and Soika 2011). Although the greenhouse has a wide variety of orchid (106 genera), the signs and symptoms of pest's attack tended to be visually centralized on dendrobium which were spotted to be the most on *D. bigibbum, D. lineale, D. sanguinolentum* and *D. secundum*. In further report, false spider mites were also reported to attack other orchid genera such as Aerides, Phalaenopsis, Cattleya, Coelogyne, Cypripedium, Grammatophyllum, Oncidium, and Saccolabium (Denmark 2018, Wildaniyah et al. 2018).

Since this pest has a wide variety of orchid host and can damage the aesthetic, medical, and economical value of the orchids, it is necessary to conduct a study to recognize the causal pest species of false spider mites. Secondly, pest assessments of incidence and severity are fundamental starting point for describing an endemic in plant pathology (Carisse et al. 2013, Bock et al. 2015). Third, a simple parameter on leaf economic spectrum such as LMA (Leaf Mass Area) is also useful to identify orchid species' defensive characteristics to the pests because it will later influence pattern's attack to deal with the pests. Fourth, it is also possible to develop predicting incidence-severity relationships at similar or greater level of ranking system to prevent identical cases on different species or even genera of orchid collections in the BBG. Therefore, the aims of this research were to (i) to identify the most vulnerable of Dendrobium species, (ii) to identify species resistance by measuring their LMA, and (iii) to predict statistically the relationships of false spider mites' incidence and severity. Based on previous studies, our hypotheses are: First, *D. lineale* exhibits the lowest pest incidence-severity from false spider mites. This species actually contains high fiber

content that tends to have greater mechanical and structural resistances to protect leaves from herbivores (Onoda et al. 2011, Yonzone et al. 2012). Fiber is usually owned more by species of plants that have priority allocation of construction costs on their growth for survival and defense mechanisms against herbivore called conservative carbon strategies by having low protein content (nitrogen and phosphorus) and low water content, but they have high LMA, tougher leaves and high defensive compounds (such as lignin and phenolic compounds) which are expensive to synthesize (Wright et al. 2004, Kitajima and Myers 2008, Onoda et al. 2017). Second, as anti-herbivore defensive compounds and LMA would have positive correlation, *D. lineale* should have the highest LMA than other three species. Third, the prediction model of incidence-severity relationship may have similar hierarchical level. So, it will be possible to develop statistically correlation from incidence levels that are easier to measure.

## II. MATERIALS AND METHODS

The observations and measurements were conducted from January to March 2021 in the orchid greenhouse of BBG with temperature ranging from 22 to 31°C, humidity between 60-85% while raining could reach 95%, under reduced 60-70% of sunlight cover net.

### A. Materials and Tools

The used tools were microscope, camera (NIKON W300), examples of printed damaged scales from 1 to 10, oven, ruler and ImageJ Software. The used materials were filter papers and white paper.

### B. Research Procedures

#### 1. Pest identification

All collections of *D. bigibbum*, *D. lineale*, *D. sanguinolentum*, and *D. secundum* were used from Dendrobium collection table area in the greenhouse of BBG. As much as 6 individual pots for each species (24 pots in total) were taken randomly from the total of 58 individual pots in the collection for measuring all leaves' severity and incidence of each individual sample. Microscopic scanning (Fig. 1) was performed with one leaf sample for each species to identify the species false spider mite attacking the plants. The mites found in the scanning were photographed, identified by Tissue Culture Laboratory, Bogor Botanical Garden, BRIN (National Research and Innovation Agency) and compared with the morphological characteristics of false spider mites with previous studies from Labanoswski and Soika (2011).

#### 2. Incidence and severity measurements of leaves samples

To determine incidence and severity, whole leaves in each individual were checked and counted its damage. Incidence (I) was obtained by counting number of damaged leaves by the mites and calculated as the

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proportion of damaged leaves from total leaves in one plant. Severity (S) was assessed by measuring all damaged leaves using Horsfall-Barratt (H-B) scale, originally invented for leaf damages, that divides percent severity into interval scale categories from 0 to 11, where 0 = 0, 1 = 0-3%, 2 = 3-6%, 3 = 6-12%, 4 = 12-25%, 5 = 25-50%, 6 = 50-75%, 7 = 75-88%, 8 = 88-94%, 9 = 94-97%, 10 = 97-100%, 11 = 100% (Horsfall and Garrett, 1945). For each plant, severity (S) is calculated based on Disease Severity Index (DSI) (Kone et al. 2017, Wildaniyah et al. 2018), where:

$$DSI = \frac{\sum(class\ frequency\ x\ number\ scale\ per\ category)}{(total\ leaf\ of\ plants\ x\ maximal\ disease\ score)} \ldots (1)$$

To reduce wider subjectivity and error in direct visual estimations of H-B scale as it was suggested and analyzed from previous studies (Bock et al. 2010), we made proportion percent example of damages in each leaf using image analysis with ImageJ Software (Fig. 2) by adjusting the right RGB color model for thresholding of damaged area from 11 leaves with different scale 1 to 11 then compared the examples with leaf samples during severity's observation. We used this comparison method because we were prohibited to take all leaf samples (as disposable sample) for measuring as the orchids in BBG.

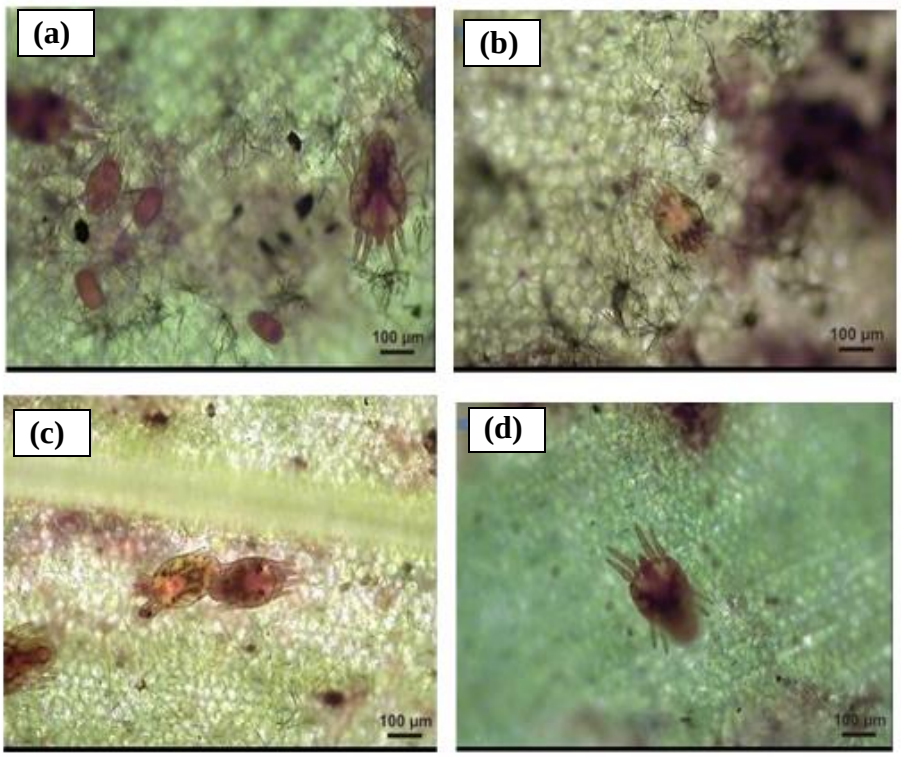


Figure (*Gambar*)1. False spider mites from family Tenuipalpidae, *T. pacifus*, on leaves of (a). *D. bigibbum*, (b). *D. lineale*, (c). *D. sanguiolentum*, and (d). *D. secundum* with 10x magnification on microscop

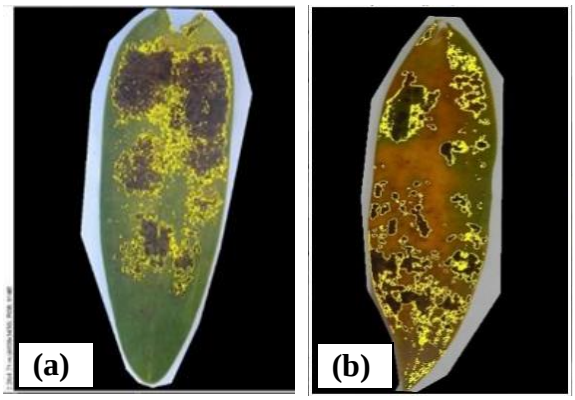


Figure (*Gambar*) 2. Measurement damage examples by false spider mites (yellow lines) with ImageJ software on (a). *D. secundum* and (b). *D. lineale*.

### 3. LMA measurement on leaves samples

A leaf from each individual sample from previous incidence and severity measurements was taken to measure its LMA. The leaves were scanned and later dried in an oven at 70°C until obtaining constant weights (took 7 days as orchids have high water content). Afterward, they were weighted to get dry mass while leaf areas were measured by ImageJ software.

### C. Statistical analysis

R software version 4.0.3 (R Core Team 2020) was used for graphics (ggplot2 package) and statistical analysis (*ggpubr* package) with significant level of $\alpha = 0.05$. The incidence-severity relationship used pair data of $I > 0$ and $S > 0$. As the value result of incidence and severity did not behave the assumptions of normality (after Shapiro-Wilk test), we performed Kruskal-Wallis test to show the comparison among Dendrobium species' incidence and severity of false spider mites. Then, we tested the significant difference among species with *post hoc* multiple comparisons of Tukey's method at 0.05 significance level (*multcomp* packages). To calculate correlation incidence-severity, we used Spearman's correlation coefficients as well as to establish correlation between LMA and incidence-severity. The purpose of this analysis was to measure both strength and direction of incidence-severity relationship and to assess whether LMA, as one of defensive traits, was correlated with the incidence-severity.

## III. RESULT AND DISCUSSION

### A. Result

Physical defects from *T. pacifus* (Fig. 1) on leaves caused different variety of incidence and severity on each species. Kruskal-Wallis test showed significantly difference of incidence among species (Fig.3a). *D. sucundum* had the highest average value incidence with 0.84. Meanwhile, other three species, *D. bigibbum* showed the average incidence of 0.38, followed by *D. sanguinolentum* with 0.21 and *D. lineale* that had the lowest average incidence of 0.17. Futhermore, *post hoc* test showed significantly different between *D. lineale* and *D. bigibbum* despite their overally lower incidence, while *D. sanguinolentum* was not different with both species. On the contrary,

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*D. sucundum* had significantly different with the other three species by having almost completely high incidence ranging from 0.61 to 1. Meanwhile, disease severity showed almost similar patterns to disease incidence (Fig 3b). *D. sucundum*, again, had higher range of severity from 0.22 to 0.44 with the average of 0.30 compared to the others. While *D. bigibbum*, *D. sanguinolentum* and *D. lineale* had lower average severity of 0.10, 0.07, and 0.05 respectively. This result was also supported by post hoc analysis where those three species were on the same group but they were significantly different with *D. sucundum*. Both incidence and severity on *D. sucundum* were higher than other three species.

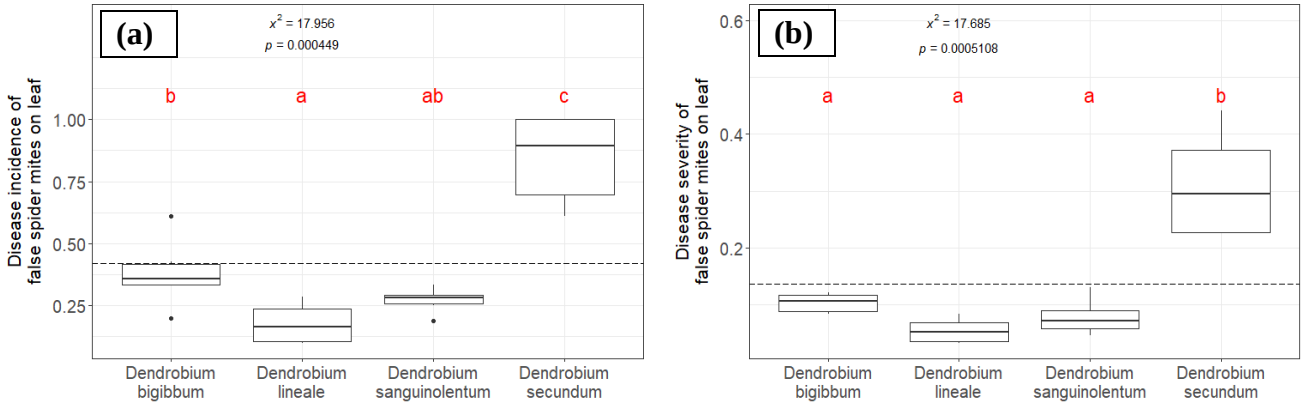


Figure (*Gambar*) 3. Disease a). incidence and b). severity on leaves of several Dendrobium species caused by *T. pacifus*. Black-dashed line is total average of incidence and severity of all species. Different red letters indicate tukey's test significant differences among species.

Incidence-severity relationship was positively and strongly correlated (Fig.4). The correlation was significant ($P < 0.05$) and the coefficients of determination was very high (R = 1). Estimated intercept ($\beta_o$) was -0.02241536 and the slope parameter ($\beta_1$) was 0.37951787. Based on average and covariance analysis, *D. sucundum* were highly exposed both disease incidence and severity while others were relatively low. *D. sucundum* had greater range of standard error compared than others. Overall, this correlation pattern was quite similar to the result of incidence-severity. Although all species showed symptoms and signs with high incidence, they still tended to have low severity (1-1 arbitrary line in Fig. 4).

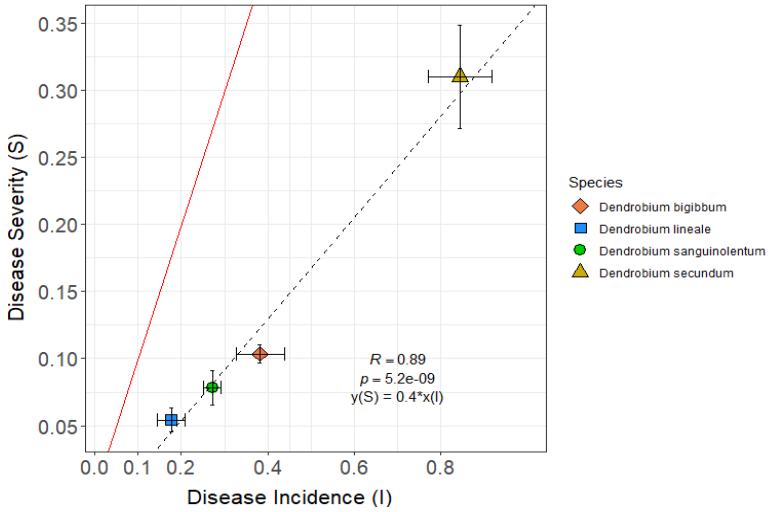


Figure (*Gambar*) 4. Correlation between incidence (I) and severity (S) of all Dendrobium species. Each point indicates the species average value with standard error. Black-dashed line is Spearman's correlation coefficient (*R*) of all species. Red line is 1-1 arbitrary line that depicts tendency in incidence or severity.

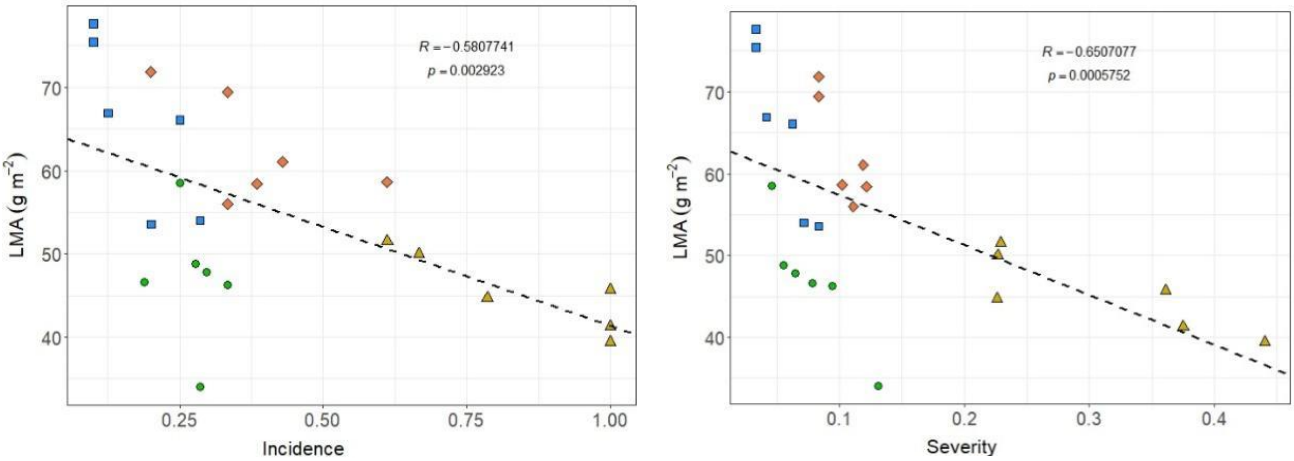


Figure *(Gambar*) 5. Correlations across 24 individuals of 4 Dendrobium species for (a). LMA-incidence (I) and (b). LMA-severity (S). Black-dashed line is Spearman's correlation coefficient (*R*) of all species.

Correlations between LMA and incidence-severity were similar with negative associations of all examined Dendrobium species (Fig 5a and 5b). Both had significant relationship with R = - 0.58 for incidence and R = - 0.65 for severity. LMA association for linier regression analysis model could be calculated as y = -23.72*I + 65.08 on incidence and y = -60.97*S + 63.47 on severity. *D. lineale* showed higher range distribution of LMA trait (53.55 – 77.62 g $m^{-2}$) among four species with low incidence-severity while opposite patterns happened on *D. secundum* with LMA range distribution of 39.45 – 50.03 g $m^{-2}$.

## B. Discussion

This comparative study highlighted the current condition of four Dendrobium species in the BBG to the incidence-severity of false

spider mites from *T. pacifus*. There are many reports for this pest, yet the current update of this pest damaging orchid is still important as this pest is capable of attacking not only Dendrobium species but also other genera and other plants (Leonhardt and Sewake 1999, Denmark, 2018, Wildaniyah et al., 2018). Surrounding environment of the green house also triggered the pest's outbreak significantly as study conducted by Wildaniyah et al. (2018) states that the highest rate attack of *T. pacifus* happens at temperature between 30 and 32ºC. The pest is also capable of completing one life cycle much faster with a humidity of 61.6 – 67.5 %. Susceptible polyculture (mixing of vulnerable genera in one location of BBG with *Phalaenopsis*, *Coelogyne*, and *Grammatophyllum*), low sanitation, and no isolation control in the greenhouse will result in an outburst of the pest population (Wildaniyah et al. 2018).

This study found that *D. sucundum* were highly vurnerable to *T. pacifus*'s attack due to its high incidence and severity (Fig 3). This result was also supported by the average and standard error in the covariace analysis (Fig 4). This species vulnerability might also related to their leaf traits as they are also involved in plant ecological strategies. This pattern was shown on *D. sucundum* with its lower LMA while other species had generally higher LMA. Cheap constructed leaf, mostly with shorter life-span, low LMA and high $N_{mass}$, are leaf traits on acquisitive plant species which prioritize on fast Relative Growth Rate LMA (Kitajima and Myers 2008). This type of plant have difficulties to maintain its survival whenever facing biotic and abiotic threats as trade-offs in allocation patterns in resources that is used more to growth rate than for defense (Wright et al. 2004). In addition, although we did not measure other leaf traits such as leaf thickness, compared to *D. lineale* which tactility has more fleshy leaves, *D. sucundum* is likely to have thin leaves by touching that are susceptible to physical attack such as herbivores and fungal pathogens. Greater leaf thickness will increase mechanical strength of leaf biomechanics which is also positively correlated with material resistance of leaf such as leaf toughness and leaf tissue density (Onoda et al. 2011). Basically, leaf thickness together with tissue density and structural resistance would determine LMA involving about carbon fixation on construction costs (Wright et al. 2004, Onoda et al. 2011). In result, higher LMA and leaf thickness on *D. lineale* will result on more physiologically and structurally resistant than lower LMA and thinner leaves in similar material of leaf. So, higher LMA on *D. lineale* would increase leaf mechanical resistance and resulted in low severity and incidence of false spider mites.

Besides having a high LMA, the low of incidence and severity on *D. lineale* might also

be resulted on greater fiber content which later also contributes to greater resistance from pests and diseases' attacks (Kitajima and Myers 2008, Yonzone et al. 2012). Fiber content consists of many elongated fiber cells, made from cellulose, lignin and pectin, that are connected and unified (Smole et al. 2013). Higher lignin is also correlated to higher leaf thickness, LMA (Leaf Mass Area), leaf lifespan and tissue density that serve as anti-herbivore and anti-pathogens (Lebrija-trejos and Wright 2016, Zhang et al. 2017). Positive correlation between fiber content and LMA finally caused false spider mite to use more energy for digesting and resulted in low incidence and severity on *D. lineale*.

The research revealed that preference and spread of false spider mites to invade was similar in severity and incidence among four Dendrobium species. According to 1-1 arbitrary line, all species depicted greater tendency to have higher disease incidence than severity meaning that the pest spread wider but caused low severity to the plants. This high incident was resulted on current environmental factors such as humidity and temperature in the greenhouse that were apparently favoring the false spider mite to enhance quick life cycle. For this period, the pests seemed still to be under threshold. However, the invaded plants still needs to be isolated for preventing further expansion of the mites. Different placement for susceptible genera of orchid in the greenhouse and sanitary control management might also determine the damage level due to *T. pasificus* can also attack different genera of orchids that later could make outbreak even worse. Based on visual observation, the signs and symptoms of this pest were already seen on orchid collection of *Coelogyne* spp. such as *Coelogyne pandurata*, *Coelogyne rochussenii*, and *Coelogyne asperata*. Different technique actually can be applied during watering by high pressure of spraying on the back side of orchid leaves where the mites were mostly concentrated (Wildaniyah et al. 2018). This is very effective but needs more effort to spray each leaf. Other is appropriate chemical treatments with careful handling such as incompatible pesticide application with too low dose could make the pests become resistant and too high dose could instead kill predatory mites (Puspitarini et al. 2012, Kole et al. 2019). So further research is needed to study of the use of safe chemical treatment both for the orchid plants and also for predatory mites.

## IV. CONCLUSIONS

Pest's incidence was relatively high among Dendrobium species while its severity was seemingly lower. This suggests that *T. pasificus* was already outbreak but it had not caused critical damages or even dead among four Dendrobium species. The lower pests' severity on *D. lineale* positively correlated to the its higher LMA. This leaf defensive trait could be

used as preventive strategies in outbreaking area of false spider mites. However, without proper control health management, the pest's incidence-severity level could be even worse as some vulnerable genera of orchids such as *Coelogyne* spp. in BBG had already showed similar symptoms to this pest's attack.

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