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Septobasidium pseudopedicellatum Burt. Caused Velvet Blight Disease on Black Pepper in Indonesia.

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Abstract

Velvet blight disease on black pepper was considered as a new disease, and great possibility to spread easly to the other district in Indonesia. The research aims was to diagonsis the velvet blight disease on black pepper, and to determine the level of the attack and its association to insects. The samples were taken from two regencies (Bengkayang and Sambas) as a central black pepper plantation in West Kalimantan. Measurement of disease occurance has conducted on 50 orchards randomly selected in these district. Postulat Koch has done at one year seedling. According to morphology of velvet blight fruiting bodies of the samples, the pathogen was identified as *Septobasidium pseudopedicellatum* Burt. In The pathogenicity test on black pepper seedlings showed that the symptoms of velvet blight formation occur at wounded stems and at node. the fruiting body were common found scale insect, *Unaspis* sp. The insect function in this case hasn't been known. Occording to field observation found that the average of disease incidance (I) 83% ± 22.7, and disease severity (S) 37.52% ± 17.05). A linier model of I and S relationship based on asinh-asinh transformation was A(I) = -0.11 + 0.86 (A)S and R^2 = 0.815 with pearson correlation 0.905.

Keywords: black pepper; Indonesia; scale insect; *Septobasidium pseudopedicellatum;* disease pathogen.

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1. Introduction

Black pepper has been planted a long time ago in Indonesia. It was knew as a king of spices. Its almost 99% cultivated by the farmers and they produced both black and white pepper. From existing production most (85%) intended for export, both raw or already processed form. Looking at this reality, black pepper will be a great potential as a source of Indonesian foreign exchange.

There were quite a lot of diseases found in black pepper and considered disturbing. One of them is a velvet blight disease that was classified as a minor likes antrachnose [17]. The disease was found in India, Malaysia and Indonesia. In Indonesia it found in West Kalimantan and East Kalimantan. The attacking in West Kalimantan has reached 19.11% of the plantation which has reached 8,415 ha [3]. The level of attacks varies from relatively mild to severe. The attack has occured in seedling, and continues to develop until the old ones.

The farmers in West Kalimantan have considered the disease as a harmfull. The disease has been spread, and still haven't found a method to inhibit its attack. The disease caused wrinkle on fruit spikes and break twigs. When the attacking occurred on main stem the plant will die. The symptoms also found in the leaves. The pathogen grew rapidly in rainy season, the fruiting bodies can reach in 20 cm within a few months. Sometimes the pathogens can cause broken twig or branch, thus increasing yield losses.

The typical symptoms of the genus Septobasidium on annual plants is the formation of resupinate fruiting bodies, ashly to braown color with varying sizes 1 mm - 25 cm. Among the specieses are distinguished in form, the amount of septa and the spores on a basidium; present and shape of pillars [13]. Overall for Septobasidium identification based on morphology commonly still refer to [11]. The research carried out before this was in its early stages to see the association between *Septobasidium* sp. with many plants in much countries. The study did not focus on the Septobasidium status as a plant pathogen that caused velvet blight disease symptoms. The diagnosis of crop diseases is very important, its can be used as the basic of the control strategy.

In some species of Septobasidium associated to insect. In this association does not have an infection to insects as well as in most hypocrealean ascomycet which is as an enthomopathogen. Two species of insects belongs to Diaspididae made assiation to *S. humile* and *S. petchii* at several plants in the Lauraceae, but did not show infection [14]. But between Aspidiotus (Diaspididae) and Septobasidium, it is known that fungi beside provide a shelter but was also infect the insects [10].

The purpose of the study was to diagnosis velvet blight disease on black pepper in Indonesia, and to measure the disease occurance in endemic areas of central black pepper plantation in West Kalimantan.

2. Materials nnd Methods

2.1 Diagnosis of plant pathogen

The stem / twig specimens that show velvet blight symptoms collected from two districts, the acreage of black pepper-cultivating land in West Kalimantan. Sampling was conducted in the period May to October 2012. Macromorphology and microscopic examination of the fruiting bodies done in order to identify the species of Septobasidium. Identification of fungi using monographs [4; 9; 11]. Examination focused on the form of pillars, basidia and basidiospores, sterigmata, probasidia.

Isolation of fungal fruiting bodies (hymenium and pillar) and infected twigs tissue carried out on potato dextrose agar (PDA, Himedia M096). Isolation of fungi from twigs done first by removing the fruiting bodies and surface desinfection with NaOCl 3% in 2 minutes, and than dilute in 70% alcohol in 3 minutes. Isolates used for identification of the pathogen too, and as inoculum in conducting pathogenicity tests or Koch's postulates.

2.2 Pathogenicity test

This assay used 3 fungi isolates, Sdr, Glg and Plh. Pieces mycelia that grown on PDA medium (size ± 1 cm^2) placed on the branch surface and at node of black pepper seedlings. Seedling was treated by drying for 2 months before be used it. Dried treatment caused plant susceptibility increased for pathogen infection [8]. Implementation phases Koch's postulates followed [2].

Attaching the inoculum done on wound and unwound branches, and on the node of branch. At the site of inoculation wrapped with moisted cotton wool. Inoculated seedlings were put into a transparent plastic bag and then sprayed with sterile water. These were incubated for a week. The symptom development was observed weekly. The seedlings that have been transmitted incubated in a shaded nursery. Weeds around the nurseries were allowed to grow in order to keep the humidity is high.

2.3 Insect-fungi association

Insect examination was done by peeling the fruiting bodies of. Samples taken from two locations. The samples were taken to represent all the level of velvet blight symptoms based on fruiting body shape. The level of symptoms include fruiting body unformed pillars, ashy colored; the pillar has been formed with the fruiting body size 10-15 cm; the size of the fruiting bodies more than 20 cm and or have already formed aerial branches. On each type of symptoms observed the number and kind of insects present.

2.4 Assesment of disease occurance

Measurement of the incidence and severity of disease were determined from 25 orchard each in two districts. The orchard was observed at least has 500 plants. Observations were made on 10 plants randomly selected. Disease incidance (I) was calculated by the following equation [7]:

$$I = \frac{\text{No. of infected plant units}}{\text{Total number of plant units assessed}} \text{ X } 100\%$$

Meanwhile, the disease severity (S) was determined according to modify rating scale proposed by (1) in which scale 0= no symptom, scale 1= 0-30% of branch infected, scale 2= 30%-70% branch infected, scale 3= more than 70% branch infected or infection at main trunk . S was measured using equation as follows:

$$S = \frac{\Sigma(a \times b)}{N \times Z} \quad X\ 100\%$$

$\Sigma(a \times b)$ = sum of the symptomatic plant and their corresponding score scale.

N = total of number sampled plant

Z = highest score scale

Disease occurance data was employed to examine posibility a relationship between incidense (I) and severity (S). Prior to analysis of disease occurance, the test normality was employed to determine whether those data should be transformed either to log, ln, arcsine, asinh or square root. Linier regression analysis using Minitab 16.

3. Results

3.1 Disease pathogen diagnose

The symptoms were found in stems, branches and spikes when field observation lead to velvet blight disease. The typical symptoms of the genus Septobasidium is resupinat fruiting body formation, and usually divided into 3 layers, subiculum, pillars and hymenium [9; 11; 12]. The shape and thickness of the fruiting bodies was varies, so made it difficult in species identification [13].

The results of samples examination showed the differencies in fruiting body. The symptoms divided into 3 groups according to its macromorphology, such as: 1. Patches that form fruiting bodies covered with mycelia ashy to pale brown color (Figure 1A). On this stage the fruiting body only found subiculum that developed like velvet. The patch usually 1-8 cm in size. 2. Cinnamon brown color fruiting bodies and have formed the pillars beside subiculum and hymenium. In this stage fruiting bodies showed black pillars and unbranched (fig 1 D). Beside on subiculum, pillars could also form on rhizomorph. 3. Dark brown color fruiting bodies, sometimes hymenium already broken,. This patch might extends for more than 30 cm. It also formed fruiting bodies that occur as a result of branching aerial rizomorfa that lead into the air and was followed by the formation of pillars and hymenium (Figure 1B). Fungi have not been form basidium and spores on PDA. They only formed probasidium and houstoria.

The results of microscopic examination of fruiting bodies obtained that the basidium equipped with tapered sterigmata and spores (Figure 2). Based on micromorphology observations and compared to monographs [4; 9; 11] the velvet blight disease on black pepper in West Kalimantan caused by *Septobasidium pseudopedicellatum*.

Koch's postulates showed a good result, the fungus developed well. The fungus developt at wounded branches and on unwounded node. The development took first at cotton wool, or branch node, and then spread to form

resupinate fungus body. The results acquired from Koch postulates was the early stages of fruiting body formation, which is the formation of subiculum. This event took 7 weeks after inoculation. Only Glg and Sdr isolates showed the early velvet blight symptom on Koch's postulates. Plh isolate, which formed early symptoms did not develop to form fruiting bodies. Mycelia of Plh isolate that formed a thin layer like a cobweb on the surface of branch of black pepper seedlings disappear. So there were no infection.

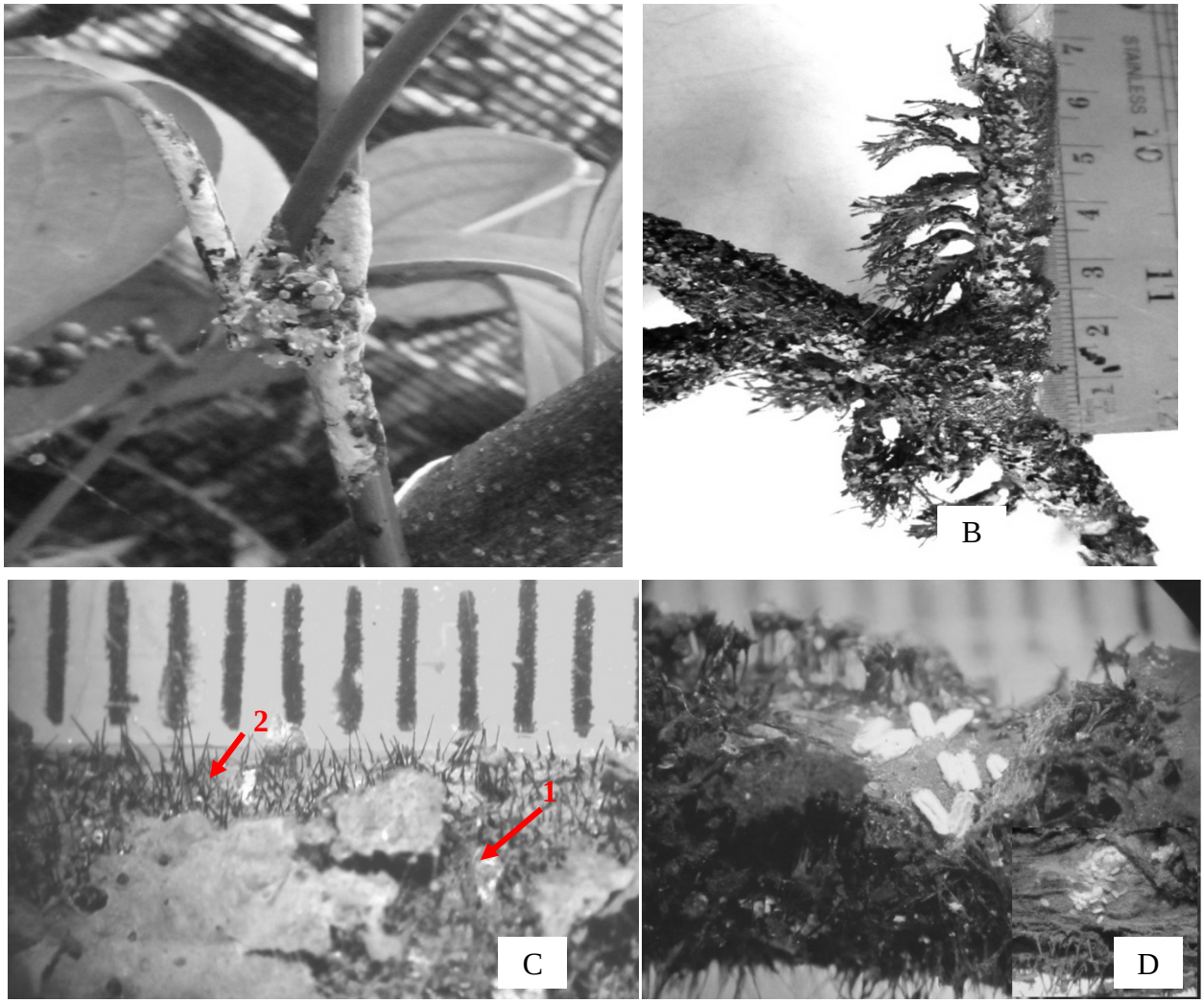

Figure 1. Velvet blight in difference symptoms. A. pillars have not been formed, B. old spesimen, hymenium cracked, formed aerial fruiting bodies, branched, entangled, C. pillars grown erect on rhizomorph (1) and subiculum (2), D. associated to scale insect, *Unaspis* sp. .

The infections of the pathogen successfull after seedling treated by drying. No watering at the growing media until soil was dry. Drying treatment process for 2 weeks, the growing media have been dried, hard and clumping. When inoculation has done the seedlings showed leaf green but a little bit dull, as a sign that the plant started to crash due to drying.

On previous occasions, the transmission is done with the same method by put a piece of mycelia (± 3-4 cm^2) on injured branches always fail. Black pepper seedlings before being used for transmission, be placed in the dark

room for a week. Darkening is done with the expectation that stem tissue becomes softer, and so it will be easier infect by pathogen.

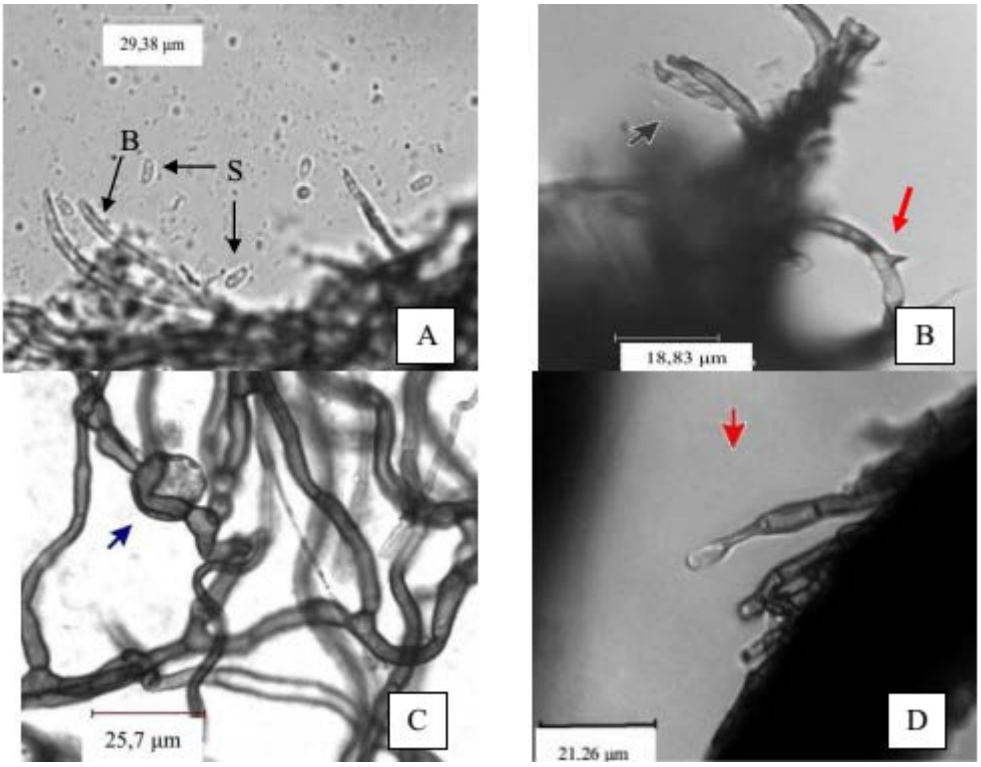

Figure 2. Microscopic of *S. pseudopedicellatum*. A. spores and basidium, B. basidium with sterigmata. C. globous probasidium, D. spore at sterigmata at the end of basidium.

Predispotition to successful transmission is also done by giving high doses of N fertilizer. Each seedling was given 100 g of urea by spreading on the growing media, around the stems of black pepper seedlings. Due to the high N fertilizer use, 400 g / plant causing depressed plant growth [19], becoming more succulent. In plants that grow less normally makes it more susceptible to pathogen infection.

3.2 Insect-fungi association

All of fruiting bodies samples from twig and branch were peel off in order to discover the insects. The most of insect in fruiting bodies were scale insects, *Unaspis* sp. The adult female scales are mussel or oyster-shell shaped, brown or brown-black with a lighter coloured margin, moderately convex and often have a distinct longitudinal dorsal ridge. The exuviae are terminal and brownish-yellow. The scales attained a length of 2.25 mm. The male scales are white, felted, elongate, slender, oval with three longitudinal ridges (Figure 1D). The exuviae are terminal and brownish-yellow. The insects were found on twig/ branch bark or subiculum of fruiting bodies. Detailed morphology description, illustration and keys are provided by [21].

At fruiting bodies with no pillars only 60% (from 15 samples) found scale insects. In older fruiting bodies (size 10-15 cm) which the pillars have been formed there only 58.3% (from 36 samples) have scale insects. In the

older one, fruiting body has already formed aerial branches 100% were found the scale insect. In this stage insect populations were low, most of them have died.

3.3 Assessment of disease occurance

The disease insidance in two region was 0 - 100% with mean 83% ± 22,7 and disease severity 0 – 90% with mean 37,52% ± 17,05. There was a linear relationship between disease severity and disease incidense (Figure 3).The equation for the ralationship was A(I) = -0.11 + 0.86 A(S) with R2 value = 81.5%, and the correlation around 0.905. Incidense and severity were transformed to arshin prior analysis. The disease assessment were made in the tropical ecology, and at perennial plant. This condition make the pathogen development is relatively stable.

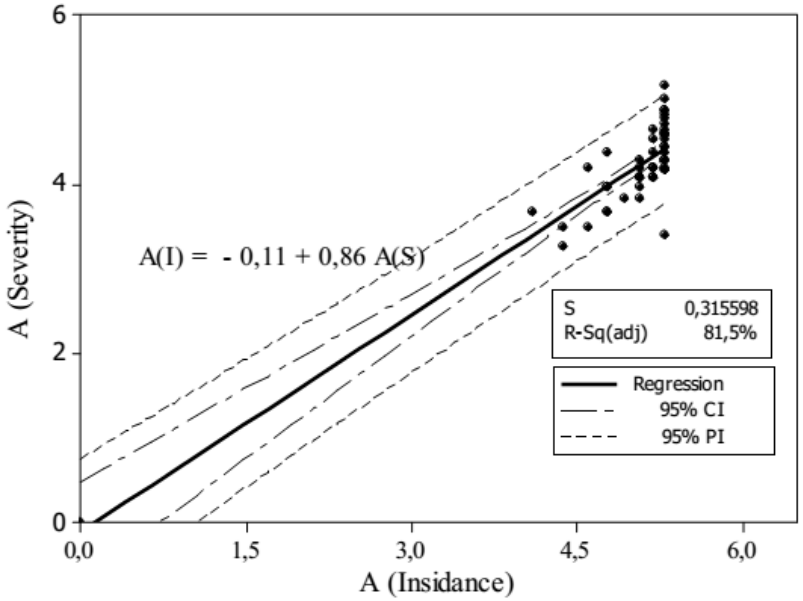

Figure 3. The relationship between incidense and severity of velvet blight disease

4. Discussion and Conclusions

The result of microscopic observation showed straight-bent cylindrical basidium (4,94 ± 1,14 x 27,75 ± 2,56) µm with 3-4 segment at hymenium; subglobose or pyriform probasidium, diameter 10.77 µm ± 1.3. Sterigmata 3 – 5 µm long, lateral or sometimes terminal on the end cell. Spores hyaline, bent-elliptic 2.71 ± 0.64 x 7.78 ± 0,68 µm, usually becoming three septate. Pillars simple, dark color, erect 0.70 mm ± 0.12 tall, formed at subiculum or rhizomorph. The pillars branch out above to form hymenium. On the old specimens top layer of fruiting bodies formed several hymenium layers, compose loosly packed, and branched. Based on the microscopic and macromorphological characteristics, the cause velvet blight on black pepper in West Kalimantan is *S. pseudopedicellatum* Burt. Identification based on monograph of the genus of Septobasidium [11; 12].

The results of measurements of the spores, the basidium, sterigmata, pillar is smaller than that described by [4; 9; 11]. The smaller size indicates that the differences occure are influenced by the type of host plant and the environment. Description of the *S. pseudopedicellatum* performed on several trees and herbs found in North America. Substrate used as a food source determines the fungus growth, and this can be seen from the size of this part of the body pieces.

On direct observation of fruiting bodies are hard to find probasidium. Probasidium more easily found in pure culture isolation. But in the culture isolation the fungi didn't form a basidium. Septobasidium rarely formed basidium and spores on agar media [9; 13].

Different to *S. bogoriense* fruiting bodies, this species has wider and smaller pillars than *S. pseudopedicellatum*. Basidium of *S. bogoriense* bent and has no or smaller strigmata. It was very difficult to see bent-elliptic spore [11]. At hymenium of *S. pseudopedicellatum* the straight to bent spores be found easily. Phylogeny of Pucciniomycotina has made by [22] shows close kindship between *S. pseudopedicellatum* with *S. cokeri*. *S. cokeri* have fruiting bodies are relatively small (up to 15 cm long). Pillars which arised from subiculum arising singly or sometimes in tufts. Pillars often arise in concentric rows on the margin. In the older parts this concentric arrangement of the pillars is not evident. Pillar also formed unbranched [9].

In Koch's postulat showed succesfull infection in wound twig and branch or on node of black pepper. On that site contained water film longer than other part of the plant, gave more possibilities for the fungus to infect the tissue. The infection occured after drought treatmen. Root development is affected by the condition of balance between water, air and soil rigidity. The all factors affects the soil structure. In good composition plant roots will grow elongated and spread, so that it will make the growth of plants look good. In water shortage conditions will cause soil structure harden, thereby inhibiting root development and function of roots to absorb nutrients and water decrease. This is certainly has an impact on plant growth stunted, increased root exudation, so causing the plant to be weak [20]. In these conditions the infection by pathogens may occur will be greater. Due to drought and heat stress can be a barrier to the growth of plants. The effects of drought caused a lessions, and this are usually due to membranes produced super oxide radicals, hydrogen peroxide, hydroxyl radicals. Other consequences that might happen is a reduction in protein synthesis and protein denaturation and thus the integrity of the membrane reduced [15].

For fungal infection there were not prior scale insect. On the symptons are not always found insects. In the symptom with insects, most the mature insect looked weak but the young crawled easily. Fungi gave a shelter for insect life. In the fruiting bodies was found both the infected insect or uninfected insects. This indicates that infection by pathogens can occur in branches that are not funded by the insects previously, but can also be initiated by the scale insect infestation [4]. Scales insects, *Unaspis* sp. attack the branch due to wound, and this made the pathogens infect easily. Generally the older fruiting bodies tend to inhabited by the scale insect.

In the fruiting bodies of *Septobasidium* sp. not only found *Unaspis* sp., but also can be found other Diaspididae such as *Parlatoria* sp. which was found with *S. petchii*, *Aulacaspis tubercularis* found within *S. humile* [14]. In the fruiting bodies of S. *meredithiae* found *Diaspidiotus liquidambaris* [13]. In the case of velvet blight on black

pepper in West Kalimantan, the insects found in the fruiting bodies of *S. pseudopedicellatum* only *Unaspis* sp. In normal conditions *Unaspis* sp. can also be found on the leaves and fruit of black pepper.

Refers to the observations, the disease was diffuse and then followed by an increase in disease severity. When pathogens disperse and form new infections will always be followed by an increase in disease severity. There was a linier correlation between disease incidense and disease severity.

A relationship between the incidence and severity of velvet blight disease will facilitate the assessment of the disease severity. Its only determined disease incidense to measure disease severity of velvet blight disease on black pepper. To assessed disease severity more difficult and subjectivelly. Similar results were also seen in the soft rot disease on dragon fruit [16] and powdery mildew on strawberry [5]. There was a relationship between disease incidance and disease severity. The relationship between the incidence and severity of velvet blight disease on black pepper which was analyzed on the basis of disease occurance data, will be more stable and can be used for a long time in many places in West Kalimantan and other tropical region.

It is based on several reasons such as: a. The weather was relatively not varied. Ecological conditions such as West Kalimantan has tropical weather conditions are relatively stable. Some weather factors such as temperature, rainfall, humidity, light intensity has not changed much in long time. Relatively stable conditions will lead to the development of plant disease is not impaired. If climatic factors play an important role in altering incidence-severity relationship, these should be more consistent for tropical diseases than that for the temperate diseases [6]. The velvet blight on black pepper is one kind of a tropical disease. b. The presence of pathogens in the field relatively undisturbed. Most of black pepper farmers didn't do disease control. It makes *S. pseudopedicellatum* continues to grow. Disease control activities at least will reduce pathogen propagul populations in the field. Most of farmers in West Kalimantan do not control the pathogen so the development of the disease will continue to progress. The farmers were not spraying fungicide to suppress the development of velvet blight pathogen. Fungicide application may affect the incidence-severity relationship [18] c. The plant always present in the field in long time.

Black pepper one of a perennial crops will always be found easly in the field. The presence of host plants ensure availability of food for pathogens. Production system of the most farmers in west Kalimantan are relatively uniform, and these causing the black pepper health also same. One of the factors that determined the relationship between disease incidence and severity of powdery mildew on strawberry is a production system [5]. These are certainly a factors that causes the pathogen fitness can be maintained. The spread will have a great chance to be followed by new infections, either on the same plant or other black pepper.

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References

[1] K.L. Bowen. "Plant diseases epidemiology", in *Plant Pathology, Consept and Laboratory Exercise*, 2nd edition,; R.N. Trigiano, M.T. Windham, A.S. Windham, Ed. New York: CRC Press, 2004, pp: 281-294.

[2] C.W.D. Brathwaite. 1981. *An Introduction to the Diagnosis of Plant Disease*. San Jose (CR): Inter-Am Inst Corp Agric.

[3] BPTP Bun. 2014. *Laporan monitoring organisme pengganggu tumbuhan Prov. Kalimantan Barat periode triwulan 1 (Januari-Maret 2014)*. Pontianak: BPTP Pontianak.

[4] E.A. Burt. The Thelephoraceae of North America. VII Septobasidium. *Ann. Mo. Bot. Gard*, vol 3, pp. 319-343. 1916.

[5] O. Carisse, A. Lefebvre, H. van der Heyden, L. Roberge, L. Brodeur. "Analysis of incidence-severity relationships for strowberry powdery mildew as influence by cultivar, cultivar type, and production systems." *Plant Disease*, vol 97 no 3, pp. 354-362. 2013

[6] T.Y. Chuang, M.J. Jeger. M.J. "Relationship between incidence and severity of banana leaf spot in Taiwan", *Phytopathology*, vol 77, pp.1537-1541. 1987.

[7] B.M. Cooke. 2006. "Disease assessment and yield loss, 2nd edition," in *The Epidemiology of Plant Diseases.* B.M. Cooke, D.G. Jones, B. Kaye. Netherland: Springer, 2006, pp: 43-80.

[8] T. Corcobado, E. Cubera, E. Juarez, G. Moreno, A. Solla. "Drought events determine performance of *Quercus ilex* seedlings and increase their susceptibility to *Phytophthora cinnamomi*", *Agric. Forest Meteorology*, vol 192-193, pp 1-8. 2014

[9] J.N. Couch. "Septobasidium in the United State", J. *Elisha Mitchell Sci. Soc*., vol.51, pp. 1-77 and 44 plates. 1935

[10] J.N. Couch. "The Biological Relationship between *Septobasidium retiforme* (B.&C.) Pat. and *Aspidiotus osborni* New.and Ckll. with plates 15-19 and 60 text-figures," Paper presented at the International Botanical Congress, Cambridge, England, August 19,1930.

[11] J.N. Couch. *The genus Septobasidium*. North Caroline ,Univ. North Carolina Press, USA, 1938.

[12] J.N. Couch. "A monograph of Septobasidium. Part 1 Jamaican species." *J. Elisha Mitchell Sci. Soc*., vol 44, pp. 242-260. 1929.

[13] D.A. Henk. "New species of *Septobasidium* from south Costa Rica and the southastern United State." *Mycologia*, vol. 94, pp. 908-913, 2005.

[14] R. Kirschner, C. Jen-Chen. "New report of two hypophyllous S*eptobasidium* species from Taiwan." *Fung. Sci*, vol. 22, pp. 39-46, 2007.

[15] J. Lipiec, C. Doussan, A. Nosalewicz, K. Kondracka. "Effect of drought and heat stress on plant growth and yield: a review.", Int Agrophys, vol. 27, pp. 463-477, 2013.

[16] M. Masyahit, K. Sijam, Y. Awang, M.G. Mohd Satar. "First report on bacterial soft rot disease on dragon fruit (*Hylocereus* spp.) caused by *Enterobacter cloacae* in Peninsular Malaysia." *Int. J. Agric & Biol.*, vol.11, pp. 659-666, 2009.

[17] Y.R. Sarma, D. Manohara, T. Premkumar, S.J. Eapen. *Disease and insects pests of black pepper (Piper nigrum L.)*. Jakarta: Int. Pepper Com., 2011. pp. 3-54.

[18] R.C. Seem, J.D. Gilpatrick, R.C. Pearson. "Fungicide influence on the relationship between incidence and severity of powdery mildew on apple." *Phytopathology*, vol.71, pp. 947-950, 1981.

[19] P. Wahid. "Pengaruh naungan dan pemupukan terhadap pertumbuhan dan produksi tanaman lada (*Piper nigrum* Linn.)". Dissertation, Fak Pascasarjana IPB, Bogor, 1980.

[20] A.P. Whitmore, W.R. Whalley. "Physical effects of soil drying on roots and crop growth." *J Exper Bot*, vol. 60, pp. 2845–2857, 2009.

[21] D.J. Williams, G.W. Watson. *The scale insects of the tropical South Pacific region. Part 1. The armoured scales (Diaspididae)*. Wallingford, UK: CAB International, 1988, pp. 185-187.

[22] D.A. Henk, R. Vigalys. "Molecular phylogeny suggest a single origin of insect symbiosis in the Pucciniomycetes with support for some relationship within the genus *Septobasidium*." Amer J Bot., vol. 94 pp. 1515-1526, 2007.