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THE POTENCIAL OF INTERSTOCK USE TO REDUCE DIPLODIA DISEASE (BOTRYODIPLODIA THEOBROMAE PATH.) ON CITRUS PLANT

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ABSTRACT

Botryodiplodia theobromae Path. has caused a damage to stems and death of citrus plants in 22 production centers in Indonesia. The purpose of this research is to determine the potential use of rootstock and interstock in inhibiting the diplodia disease. The research was conducted at the screen house of Indonesian Citrus and Subtropical Fruits Research Institute from July to December 2016. There are 24 treatment combinations consisting of 8 interstocks on 3 commercial citrus varieties. The results showed that the scion of sweet-lime combined with the interstock of Volkameriana, Troyer citrange, and Kanci have the smallest area of disease exposure (0 cm²). Therefore, this combination has the highest potential to inhibit the rot of orange B theobromae stem (there is no symptom = tolerant). The scion of Keprok Batu 55 with the interstock of Carrizo citrange, Swingle citrumello, Poncirus trifoliata, and the scion of sweet lime on Carrizo citrange, Poncirus trifoliata have the least potential to inhibit the rot of the stem compared to other interstock treatments (moderate = slightly resistant). The plants combined with Volkameriana interstock are taller than the other while the plants combined with Citromello and P.trifoliata interstock have the shortest size (stunted).

KEY WORDS

Botryodiplodia theobromae, citrus, diplodia, interstock.

Extreme climate change has changed the disease status of citrus crops in Indonesia. The facts in the field show that, currently, not the Citrus Vein Phloem Degeneration (CVPD) that attacks the citrus plantation but diplodia disease. The cause of this disease is Botryodiplodia theobromae Path. which is synonymous to Lasiodiplodia theobromae (Griff. & Maubl (Juan et al., 2013). Diplodia disease is very substantial because it can kill plants even in the form of seeds or already in the form of mature plants.

The symptoms of the disease are marked with wet spots on the stems, branches, and twigs, followed by broken skin plants secreting a gum like golden-brown glue. Diplodia is not a new disease in citrus crops, its attack has been detected years ago. However, at that time, diplodia is considered not as harmful as CVPD. In 2000, Semangun reported that diplodia disease had attacked citrus crops in Java, Sumatra, Kalimantan, and East Nusa Tenggara. Allegedly, it is due to the climate change in 2009 that tend to be wet until now causing the disease becomes worse.

The geographical distribution of these diseases is very wide. Today, the diseases can be found in all Indonesian citrus centers of more than 22 provinces, districts, and cities. From the report of the Directorate of Plant Protection in 2010 to 2013, there were 577,678,00 citrus trees in Indonesia attacked by diplodia (processed from diplodia attack data source per year by the Directorate of Horticultural Protection 2014). This can be said that the average rate of the attack is 144,419 trees per year. Salamiah (2009) mentioned that the disease tends to attack citrus plants aged >10 years than the plants aged >4 years and 4 to 10 years. In Magetan, East Java, 85% tress from 500 ha of orange crops (Citrus grandis L) have been attacked by diplodia disease. The attack is known to be on the rate of mild to moderate attack (22-37%). It is assumed that the transmission of this disease is through the splash of the water from the ground. Therefore, a high rainfall will accelerate the transmission of this disease. Weed-free citrus plantation will be quickly exposed to this disease as they directly struck by the rain. In contrast, a plantation that is covered with grass is relatively safer from

the attack of the disease. The use of immature manure can also accelerate the transmission of this disease. *B. theobromae* fungus has a variety of host (CABI, 2007) such as citrus, chocolate, rubber, mangosteen, banana, mango, pineapple, avocado, melon, coconut, and eggplant. This fungus is also reported to attack papaya (Nishijima, 2003), jackfruit, mango, mangosteen, beans (Haggag, 2006), banana, lychee, cashew (Alam et al., 2001), coconut (Warwick et al., 1991), apple (Letham, 1989), cocoa (Mbenoun et al., 2008), and guava (Masilamani & Muthumary, 1996).

Currently, more than 95% of citrus crops in Indonesia use the rootstock of *Citrus limonia* Osbeck or often called as *Japansche citroen* (JC) (Balitjestro 2006). JC is more preferred because it has high adaptability and compatibility with all types of oranges in Indonesia as well as resistant to drought and salinity. However, JC is susceptible to diseases such as CVPD, CTV, CEV, root rot, and diplodia (Supriyanto & Setiono, 2006 in Sugiyatno & Palupi 2017). To overcome the growth and resistance to disease, scientists have introduced other rootstocks such as *Poncirus Trifoliata*, Troyer Citrange, Carizzo Citrange, Cleopatra Mandarin, Volkameriana, Citrumello, and Flying Dragon (Hardiyanto et al. 2010 in Sugiyatno & Palupi 2017).

Interstock is a third plant that serves to prevent the incompatibility in plants characterized by death in young plants, inhibition of plant growth, yellowing and falling leaves, as well as asymmetrical growth in rootstock and stem (Ashari, 2006). The use of interstock is expected to become a bridge connecting the rootstock and stem so that the plant will grow normally (Sugiyatno, 2013).

The research in concern to the use of interstock has been documented. The interstock of Citrumello has the potential to encourage the growth of vegetative crops. Meanwhile, Rangpur Lime, Flying Dragon, and Troyer have the potential to control plant growth. The interstock of Flying Dragon is also known to potentially stimulate the efflorescence and fertilization of Pamelon Nambangan (Susanto et al., 2010). On the rootstock of Catania 2 Volkamer Lemon, the Flying Dragon interstock will decrease the size of the plant, while on the rootstock of Davis A Trifoliata, the Flying Dragon interstock will increase the size of the plant (Nunez et al., 2011). Moreover, the use of Flying Dragon interstock on different rootstock of Star Ruby grapefruit, tangerine Mikhal, and Nova, will lead to the differences in vegetative growth (Ashkenazi et al., 1992 in Nunez et al., 2011). Izquierdo et al. (2004) stated that the use of Lemon interstock could increase the age of the plant, production, and fruit quality as well as reduce the thickness of the stem skin on the connected parts. In Malaysia, the use of interstock may inhibit the progression rate of HLB disease in citrus plants (Shokrollah et al, 2011).

The effect of interstock on the resistance of infectious diseases through plant propagation has not been studied although there are some indications that certain rootstocks such as the *Trifoliata* group are resistant to CTV and *Phytophthora*. The use of citrus seeds with interstock has several benefits such as increasing productivity and increasing resistance to certain diseases especially for stem disease (Kresdorn 1978). It is also believed to be beneficial for a high-density spacing planting system (SITARA = **Sistem tanam rapat**). The citrus tolerance to abiotic and biotic factors depends on many factors including the types of the rootstock, N fertilization, soil, and climatic conditions (Garcia-Sanchez & Syvetisen 2009; Gimeno et al. 2009a; Saleh et al. 2008), as well as the interstock grafting between rootstock and stem because it can increase the growth of the plant, productive age, fruits, and quality (Gillzquierdo et al., 2004). In addition to that, it can also increase the tolerance to salinity including citrus disease that is susceptible to salinity. (Gimeno et al., 2009b). The disease that is sensitive to salinity is rotten stem disease (*Phytophthora* spp.) and diplodia (*B. theobromae*). The purpose of this research is to know the influence of seven (7) types of interstock in 3 commercial citrus varieties towards the disease of diplodia.

METHODS AND MATERIALS

The research was conducted at the screen house of Indonesian Citrus and Subtropical Fruits Research (ICISFRI) from July to December 2016. In the observation, the researchers

used plants aged >1 year. The preparation of the interstock took place one year beforehand by connecting the type of interstock based on the treatment requirements (15 cm from the base of the stem with a grafting incision of 1.5 cm followed by connecting it with the scion in accordance to the treatment that is approximately 10 cm above the interstock joint in the same grafting model). Then, the grafted eye is inserted and tied with an elastic plastic strap starting from below rotating upwards. After 2-3 weeks, when the grafted eye is still fresh, the plastic strap is opened.

The test plants were grown on 3-liter polybags with mixed soil and compost (1:1 v/v). In the dry season, watering is carried out twice per week in a field capacity condition. Basic fertilizer is given in the form of NPK (15-15-15) with a dose of 20 g/tree plus ZA 10 g/tree. Then, every 2 months, ZA will be added by 15 g/tree. Pest or disease control is done in accordance with the condition of the plant to prevent the attack of the destructive pest on leaves. Weeding the weeds is performed every once a week and at the time of fertilization. The weeding will be done more intensively in rainy season by retracting it directly.

Twenty four combinations of interstock (*Japanese citroen*, *Rough lemon*, *Carrizo citrange*, *citrumello*, *Poncirus trifoliata*, *volkameriana*, *Troyer citrange* and *Kanci*) on 3 commercial citrus varieties (*C. reticulata* (Blanko) vr *Keprok Batu 55*, *C. nobilis* vr *Siam Pontianak* and *Citrus kumquat* vr *Kanci*) (table 1) were used. Each treatment was repeated for 2 times and each replication consists of 1 test plant. The *B. theobromae* isolate used for the source of inoculum test was derived from the collection of Magetan Phytopathology Laboratory.

The source of inoculum used is in the form of a pathogen-infected stem that is then transmitted to the treatment plant by inserting it into a healthy stem and fastened with plastic. The outside part is sprayed with sterile aquades once in every three days to keep the moisture of the plants. The stems infected are known from the Pamelo types. The manufacture of the inoculum source is infected by the modification of method from Putra *et al.*, (2013) by taking a healthy 1 cm citrus stem and then sterilized by using 70% alcohol for 1 minute, chlorox for 1 minute, and sterile aquades for 1 minute. Each of that treatment is repeated twice. The inoculum source was incubated in PDA (*Potato Dextrose Agar*) which had a full colony of *B. theobromae*. The incubation was performed for a week until the stem showed such infected symptoms that are browned and covered with mycelium pathogen and issued a yellow liquid exudate (Figure 1).

Figure 1 – The stages of *B. theobromae* inoculation process in the test plant:

- Isolate purification Mg 39.2 from pamelo,
- healthy twigs isolation in pure culture of fungal isolates,
- infected twigs insertion on test plant,
- successful inoculation indicator marked by the occurrence of diplodia symptoms

The observation parameters are:

(a) The percentage of successful inoculation and incubation period. The percentage of successful *B.theobromae* inoculation is observed based on the emergence of disease symptoms in the inoculated plant such as wet decay and gum secretion. The period of the pathogen incubation is the time between inoculation of the pathogen to the initial appearance of symptoms in citrus plant stems. The observations were performed on the infected plant stems. The monitoring of the symptoms is based on Putra *et al.*, (2013) that there is a skin peeling on the stem, a discoloration to brown, a moisture on the surface of wounds, and a discharge of thick-brown liquid from the source of infection (gum or *blendok*). The average incubation data shows the time span between the inoculation process until the occurrence of the symptoms. The observations are made once every 2 days until the symptoms appear.

Table 1 – The list of interstock and scion combination

Combination	Interstock	Scion
Combination 1	Rootstock <i>Japansche citroen</i> (JC) – Control	<i>C. reticulata</i> (Blanko) vr Keprok Batu 55
Combination 2	Rootstock <i>Japansche citroen</i> (JC) – Control	<i>C. nobilis</i> vr Siam Pontianak
Combination 3	Rootstock <i>Japansche citroen</i> (JC) – Control	<i>C. aurantifolia</i> vr Sweet Lime
Combination 4	<i>Carrizo citrange</i>	<i>C. reticulata</i> (Blanko) vr Keprok Batu 55
Combination 5	<i>Carrizo citrange</i>	<i>C. nobilis</i> vr Siam Pontianak
Combination 6	<i>Carrizo citrange</i>	<i>C. aurantifolia</i> vr Sweet Lime
Combination 7	<i>Citrumello</i>	<i>C. reticulata</i> (Blanko) vr Keprok Batu 55
Combination 8	<i>Citrumello</i>	<i>C. nobilis</i> vr Siam Pontianak
Combination 9	<i>Citrumello</i>	<i>C. aurantifolia</i> vr Sweet Lime
Combination 10	<i>Poncirus trifoliata</i>	<i>C. reticulata</i> (Blanko) vr Keprok Batu 55
Combination 11	<i>Poncirus trifoliata</i>	<i>C. nobilis</i> vr Siam Pontianak
Combination 12	<i>Poncirus trifoliata</i>	<i>C. aurantifolia</i> vr Sweet Lime
Combination 13	<i>Volkameriana</i>	<i>C. reticulata</i> (Blanko) vr Keprok Batu 55
Combination 14	<i>Volkameriana</i>	<i>C. nobilis</i> vr Siam Pontianak
Combination 15	<i>Volkameriana</i>	<i>C. aurantifolia</i> vr Sweet Lime
Combination 16	<i>Rough lemon</i>	<i>C. reticulata</i> (Blanko) vr Keprok Batu 55
Combination 17	<i>Rough lemon</i>	<i>C. nobilis</i> vr Siam Pontianak
Combination 18	<i>Rough lemon</i>	<i>C. aurantifolia</i> vr Sweet Lime
Combination 19	<i>Troyer citrange</i>	<i>C. reticulata</i> (Blanko) vr Keprok Batu 55
Combination 20	<i>Troyer citrange</i>	<i>C. nobilis</i> vr Siam Pontianak
Combination 21	<i>Troyer citrange</i>	<i>C. aurantifolia</i> vr Sweet Lime
Combination 22	<i>Citrus kumquat</i> vr Kanci	<i>C. reticulata</i> (Blanko) vr Keprok Batu 55
Combination 23	<i>Citrus kumquat</i> vr Kanci	<i>C. nobilis</i> vr Siam Pontianak
Combination 24	<i>Citrus kumquat</i> vr Kanci	<i>C. aurantifolia</i> vr Sweet Lime

(b) The severity of disease symptoms. A wide observation of the symptoms is done on the infected stem. The observation was conducted once every 7 days until the symptoms appear by measuring vertically (v) and horizontally (h) on the infected parts. The area of the infection symptoms (cm²) is known by multiplying (v) and (h). The observation was carried out 49 days after inoculation.

(c) Plant growth:

- Plant height. Plant height is measured from the edge of the joint (attachment/grafting) between the scion and the interstock to the growing point. The observation was done once every two weeks.
- Stem diameter. The measured stems include rootstock and scion. The stem was measured in diameter by using the sliding term. The measured stems were marked with a black waterproof pen. This observation was conducted once a month.

The data of the observation will be analyzed by using the Analysis of Variance (ANOVA) on 5% level. If there is a significant difference, the results will be tested further by using DMRT Test (*Duncan Multiple Range Test*) on 5% level.

RESULTS AND DISCUSSION

The Percentage of Successful Inoculation and Incubation Period. Based on the results of the study, the percentage of infected plant inoculation showed that almost all inoculation was successful (100%) except in the combination of JC/*volkameriana*/Siam Pontianak, JC/*Citrus jambhiri* vr RL/Siam Pontianak, JC/*Citrus jambhiri* vr RL/sweet lime, JC/*Citrus kumquat* vr Kanci/Siam Pontianak, and JC/*Citrus kumquat* vr Kanci/sweet lime. Scion varieties of Keprok Batu 55 with all interstock combinations produce 100% of infected plants. The infection of diplodia characterized by stem peels, discoloration, or gum secretion begins to appear within 12 to 25 days after the varied inoculation in between treatments. The incubation period was not found in 4 treatment combinations because the plants showed no symptoms such as in the treatment 1). JC/*volkameriana*/C. *aurantifolia* vr Lemon Sweet; 2). JC/*Troyer citrange*/C. *reticulata* vr K. Batu 55; 3). JC/*Troyer citrange*/C. *aurantifolia* vr Lemon Sweet; 4). JC/*Citrus kumquat* vr Kanci/C. *aurantifolia* vr Lemon Sweet (table 2). Previous studies which brought similar inoculation methods on different combinations of rootstocks

and different inoculums proved that the incubation period is varied between 9 to 39 days (Dwiastuti, *et al.*, 2017, Putra *et al.* 2013 and Salamiah *et al.* (2008). The incubation of *B.theobromae* in grape and pear plants was reported fairly rapidly in the study (Verma *et al.* 2010) which is 3 to 7 days after inoculation (HIS or *hari setelah inokulasi*). The incubation period and symptoms arising from *B. theobromae* infection depend on the number and pathogenic virulence, plant species, and climatic conditions (Shahbaz *et al.*, 2009 Adandonon 2014). The isolates of *B.theobromae* from the inoculated citrus on rubber have an incubation period of 14 days. After the inoculation, it is known the the isolates become faster than the isolates from mango, papaya, cashew, or avocado (Febiyanti, *et al.*, 2017). The pathogens can develop well at 80% of moisture especially in high rainfall conditions in between the temperatures of 25 to 30°C (Shah *et al.*, 2010; Khanzada *et al.*, 2006).

Table 2 – The percentage of successful inoculation and incubation period on the treatment of rootstock, interstock, and scion combination

Combination	Interstock	Scion	Successful inoculation percentage (%)	The incubation period (days)
Combination 1	Rootstock <i>Japansche citroen</i> (JC) - Control	<i>C. reticulata</i> (Blanko) vr Keprok Batu 55	100	14
Combination 2	Rootstock <i>Japansche citroen</i> (JC) - Control	<i>C. nobilis</i> vr Siam Pontianak	100	14
Combination 3	Rootstock <i>Japansche citroen</i> (JC) - Control	<i>C. aurantifolia</i> vr Sweet lime	100	15
Combination 4	<i>Carrizo citrange</i>	<i>C. reticulata</i> (Blanko) vr Keprok Batu 55	100	16
Combination 5	<i>Carrizo citrange</i>	<i>C. nobilis</i> vr Siam Pontianak	100	14
Combination 6	<i>Carrizo citrange</i>	<i>C. aurantifolia</i> vr Sweet lime	100	14
Combination 7	<i>Swingle citrumello</i>	<i>C. reticulata</i> (Blanko) vr Keprok Batu 55	100	12
Combination 8	<i>Swingle citrumello</i>	<i>C. nobilis</i> vr Siam Pontianak	100	16
Combination 9	<i>Swingle citrumello</i>	<i>C. aurantifolia</i> vr Sweet lime	100	14
Combination 10	<i>Poncirus trifoliata</i>	<i>C. reticulata</i> (Blanko) vr Keprok Batu 55	100	17
Combination 11	<i>Poncirus trifoliata</i>	<i>C. nobilis</i> vr Siam Pontianak	100	14
Combination 12	<i>Poncirus trifoliata</i>	<i>C. aurantifolia</i> vr Sweet lime	100	12
Combination 13	<i>Citrus volkameriana</i>	<i>C. reticulata</i> (Blanko) vr Keprok Batu 55	100	18
Combination 14	<i>Citrus volkameriana</i>	<i>C. nobilis</i> vr Siam Pontianak	50	21
Combination 15	<i>Citrus volkameriana</i>	<i>C. aurantifolia</i> vr Sweet lime	100	20
Combination 16	<i>Citrus jambhiri</i> vr RL	<i>C. reticulata</i> (Blanko) vr Keprok Batu 55	100	19
Combination 17	<i>Citrus jambhiri</i> vr RL	<i>C. nobilis</i> vr Siam Pontianak	50	18
Combination 18	<i>Citrus jambhiri</i> 6 vr RL	<i>C. aurantifolia</i> vr Sweet lime	50	19
Combination 19	<i>Troyer citrange</i>	<i>C. reticulata</i> (Blanko) vr Keprok Batu 55	100	19
Combination 20	<i>Troyer citrange</i>	<i>C. nobilis</i> vr Siam Pontianak	100	20
Combination 21	<i>Troyer citrange</i>	<i>C. aurantifolia</i> vr Sweet lime	100	-
Combination 22	<i>Citrus kumquat</i> vr Kanci	<i>C. reticulata</i> (Blanko) vr Keprok Batu 55	100	16
Combination 23	<i>Citrus kumquat</i> vr Kanci	<i>C. nobilis</i> vr Siam Pontianak	50	25
Combination 24	<i>Citrus kumquat</i> vr Kanci	<i>C. aurantifolia</i> vr Sweet lime	50	-

Severity of Disease Symptoms. The symptoms of the disease were marked by brownish areas and/or yellow exudate (gum) secretion from the point of inoculation so that

the surface becomes wet and sticky. Another symptom of this disease is the appearance of cracks around the wound area in which the wound becomes rotten in white and discolored to brown (Figure 2). From the observation, it is known that most of the rotten stems (most extensive areas of the rot) are found in the treatment 1). *JC/Carrizo citrange/C. reticulata* (Blanko) K Stone 55; 2). *JC/Carrizo citrange/C. aurantifolia* vr Sweet Lime; 3). *JC/Swingle citrumello/K Stone 55*; 4). *JC/Poncirus trifoliata/C. reticulata* (Blanko) K Stone 55; and 5). *JC/Poncirus trifoliata/C. aurantifolia* vr Sweet lime with a width between 5.08 to 6.15 cm². The width is different from other treatments. All of those five treatments were included in the moderate severity group (>5 to 10 cm²). The treatment classified in mild severity group (1 to 5 cm²) was the most common group of 11 treatment combinations with a mean value of 1.53 - 3.25 cm². This is lower than those 5 treatment combinations which have moderate severity but higher and significantly different than the 4 combinations of tolerant group treatments. The combination of interstock treatment *JC/volkameriana/C. aurantifolia* vr Treatment Sweet lime; *JC/Troyer citrange/C. reticulata* (Blanko) vr Tangerine Rock 55; *JC/Troyer citrange/C. aurantifolia* vr Lemon Sweet and *JC/Citrus kumquat* vr Kanci/*C. aurantifolia* vr Sweet lime showed that there are no symptoms until 16 weeks after inoculation so that it can be classified to the tolerant group (table 3).

The rootstock resistance of Troyer citrange in this study is similar to that have been mentioned by Mulaa *et al.*, 2011 from the results of the research (2006 to 2008) concerning the tolerance of pests or diseases called as the combination of 'Troyer Citrange'/'Washington Navel 39'. In addition, (Mulaa *et al.*, 2011) also found a tolerance on other rootstocks such as 'Rough Lemon'/'Washington Navel 102', 'Citrumello 4475'/'Washington Navel 205', 'Citrumello 4475'/'Washington Navel 216', and 'Carrizo Citrange'/'Washington Navel 205'. On the other hand, (Widyaningsih *et al.*, 2017) mentioned that there is a similarity on the results of *volkameriana* that is more tolerant to CVPD or HLB rather than JC. It is also mentioned that the use of a mixture of rootstock and scion will increase the tolerance against HLB.

Figure 2 – The plant's performance of 8 interstocks inoculated with *B. theobromae*, 3 weeks after inoculation: a). Without interstock, b). *Carrizo citrange* interstock, c). *citrumello* interstock, d). *Poncirus trifoliata*, e). *Volkameriana*, f). *Citrus Jambhiri*, g). *Troyer citrange*, h). *Citrus kumquat* vr *Kanci*. = Gum, = Early rotten stem symptoms, pathogenic fungi develop in between the point of inoculation

As we can see, the penetration of *B. theobromae* into the plant tissue is helped by the wounds inducing the production of enzymes and pathogenic toxins. The main enzymes produced by *B.theobromae* are β glucosidase and cellulase which are used to soften the citrus trunk tissue while toxins are used to kill plant cells. The presence of toxins affects the permeability of cell membranes by inactivating and inhibiting plant enzymes or stopping the activity of the related plant enzymes.

Table 3 – The average of disease severity between the citrus combinations until 16 weeks after inoculation

Combination	Interstock	Scion	Average rotten areas (cm ²)	Severity group
1	2	3	4	5
Combination 1	Rootstock <i>Japansche citroen</i> (JC) - Control	<i>C. reticulata</i> (Blanko) vr Keprok Batu 55	6.12 a	Moderate
Combination 2	Rootstock <i>Japansche citroen</i> (JC) - Control	<i>C. nobilis</i> vr Siam Pontianak	5.48 a	moderate
Combination 3	Rootstock <i>Japansche citroen</i> (JC) - Control	<i>C. aurantifolia</i> vr Sweet lime	6.15 a	moderate
Combination 4	<i>Carrizo citrange</i>	<i>C. reticulata</i> (Blanko) vr Keprok Batu 55	5.78 a	moderate
Combination 5	<i>Carrizo citrange</i>	<i>C. nobilis</i> vr Siam Pontianak	2.70 b	mild
Combination 6	<i>Carrizo citrange</i>	<i>C. aurantifolia</i> vr Sweet lime	5.70 a	moderate
Combination 7	<i>citrumello</i>	<i>C. reticulata</i> (Blanko) vr Keprok Batu 55	5.63 a	moderate
Combination 8	<i>citrumello</i>	<i>C. nobilis</i> vr Siam Pontianak	2.43 b	mild
Combination 9	<i>citrumello</i>	<i>C. aurantifolia</i> vr Sweet lime	1.43 b	mild
Combination 10	<i>Poncirus trifoliata</i>	<i>C. reticulata</i> (Blanko) vr Keprok Batu 55	3.25 b	mild
Combination 11	<i>Poncirus trifoliata</i>	<i>C. nobilis</i> vr Siam Pontianak	5.08 a	moderate
Combination 12	<i>Poncirus trifoliata</i>	<i>C. aurantifolia</i> vr Sweet lime	5.35 a	moderate
Combination 13	<i>volkameriana</i>	<i>C. reticulata</i> (Blanko) vr Keprok Batu 55	1.53 b	mild
Combination 14	<i>volkameriana</i>	<i>C. nobilis</i> vr Siam Pontianak	1.78 b	mild
Combination 15	<i>volkameriana</i>	<i>C. aurantifolia</i> vr Sweet lime	0.00 c	tolerant
Combination 16	<i>Rough lemon</i>	<i>C. reticulata</i> (Blanko) vr Keprok Batu 55	3.88 b	mild
Combination 17	<i>Rough lemon</i>	<i>C. nobilis</i> vr Siam Pontianak	3.43 b	mild
Combination 18	<i>Rough lemon</i>	<i>C. aurantifolia</i> vr Sweet lime	2.80 b	mild
Combination 19	<i>Troyer citrange</i>	<i>C. reticulata</i> (Blanko) vr Keprok Batu 55	0.00 c	tolerant
Combination 20	<i>Troyer citrange</i>	<i>C. nobilis</i> vr Siam Pontianak	1.98 b	mild
Combination 21	<i>Troyer citrange</i>	<i>C. aurantifolia</i> vr Sweet lime	0.00 c	tolerant
Combination 22	<i>Citrus kumquat</i> vr Kanci	<i>C. reticulata</i> (Blanko) vr Keprok Batu 55	5.38 a	moderate
Combination 23	<i>Citrus kumquat</i> vr Kanci	<i>C. nobilis</i> vr Siam Pontianak	2.88 b	mild
Combination 24	<i>Citrus kumquat</i> vr Kanci	<i>C. aurantifolia</i> vr Sweet lime	0.00 c	tolerant

Successfully grafted seedlings were arranged in a randomized complete block design with two replications. Severities grouping on seedling: no symptom = tolerant group, 1- 5 cm = mild group, > 5 – 10 cm = moderate group, > 10 cm = severe group.

The enzymes and toxins produced by *B. theobromae* are used to penetrate the point of infection located in the epidermis and parenchyma cells (Al-Saadoon *et al.*, 2012; Parthasarathy *et al.*, 2016, Umeruzike, 1979). It is suspected that 4 tolerant group treatments with the interstock of *Volkameria*, *Troyer*, and *Kanci* which are connected above JC tend to have a mechanical resistance that inhibits the production of enzymes and pathogenic toxins, especially in the sweet lime.

The development of rotten stem symptoms by the treatment of several interstocks on each scion of K.Batu 55, *Siam Pontianak*, and Sweet lime for 5 to 16 weeks after inoculation is presented in Figures 3, 4, and 5. The treatment with the scion of *K. Batu 55* without interstock showed the highest rod development. The trend itself is increased during the time of observation compared to other treatments (Figure 3) and then followed by the treatment with *Citrumello* and *Poncirus trifoliata*. The treatment that produced the narrowest rotten stem area was found on the RL interstock. In RL interstock, the trend was also decreased until end of the observation.

Figure 3 – Figure 3. The development of rotten stem area (cm) with several interstocks on *Keprok Batu 55* scion at 5 to 16 weeks after inoculation

In the treatment with the scion of *Siam Pontianak* and Sweet Lime without interstock, the highest stem rot development is found. Also, the trend is known to be increased during the time of observation compared to other treatment (Figures 4 and 5). The combination of *Troyer citrange* interstock with *Siam Pontianak* produces the narrowest rotten stem are and decreases the trend compared to other treatments. At the end of observation, there is a healing and no symptoms occur. The development of rotten stem in other treatments looks volatile (some might stagnant, increased, or decreased).

Figure 4 – The development of rotten stem area (cm) with several interstocks on *Siam Pontianak* scion at 5 to 16 weeks after inoculation

On the treatment with Sweet Lime scion, there are 3 types of interstocks used such as *Volkameriana*, *Troyer citrange*, and *Kanci*. It is found that there are no symptoms from start to finish. All of the three interstocks can be grouped into the tolerant group (figure 5). The development of the disease with *P.trifoliata* interstock tends to rise very slowly while the treatment with *Carizo citrange* seems to produce a stagnant result. Last but not least, *Citromello* and *RL* tend to decrease the disease attack.

Figure 5 – The development of rotten stem area (cm) with several interstocks on sweet lime scion at 5 to 16 weeks after inoculation

Plant Growth. The results of the analysis of variance indicate that plant height is not significantly influenced by scion interaction and interstock usage. The average height of *Kepron Batu 55* varieties on various interstock treatments has a plant height of 79.06 cm. It tends to be higher than the height of *Siam Pontianak* and Sweet Lime in various treatments. The height of the scion of *Siam Pontianak* and Sweet Lime is 75.94 cm and 66.73 cm. The differences in scion are believed due to the genetic differences of the scion itself. Makmur (1992) stated that genetic variation occurs as a cause that plants have different genetic characteristics.

Figure 6 – Average Plant Height of several interstock at 23 weeks after inoculation, cm

Plants joined to the *Volkameriana* interstock tend to be higher than the other 6 interstocks. The plant height of *Volkameriana*, *Carizo citrange*, *Troyer citrange*, and *rough lemon* is 90.72cm, 86.33cm, 78.17cm, 78.35cm, and 66.35cm respectively. The shortest plant height is found on *Citromello* and *P.trifoliata* (57.6 cm) (Figure 6). However, the results of this study differ from the research from Susanto *et al.* (2004) saying that Citrumelo and Troyer can stimulate the growth of Cikoneng Pamel.

The main benefits of interstock that can control the growth of the scion also become the substantial factor in the development of citrus cultivation in regulating the density of plants in the field. Short plants will ease the cultivation techniques such as spraying, pruning, harvesting, and others. The use of interstock is also able to overcome the incompatibilities between scion and rootstock.

At 23 weeks after inoculation, the *Siam Pontianak* which was grafted with the interstock of *Citromello* and *P. Trifoliata*, as well as the Sweet Lime which was joined with the interstock of Rough lemon, has the shortest plant height which was 58.25cm, 58.55cm, and 60.70cm. The tallest plant height was obtained by *Kepron Batu 55* which was connected with the interstock of *Carrizo Citrange* and *Volkameriana* as well as *Siam Pontianak* which was joined with *Volkameriana* and then *Siam Pontianak* and *Sweet Lime* which were grafted with *Kanci* (88.15cm, 96.44cm, 90.92cm, 93.4cm, and 89.4 cm respectively) (Table 4).

Table 4 – Mean comparison of citrus plant height combination using the Duncan multiple range test

Interstock	Scion	Plant height (cm)
Rootstock <i>Japansche citroen</i> (JC) - Control	<i>C. reticulata</i> (Blanko) vr <i>Kepron Batu 55</i>	78.25 b
Rootstock <i>Japansche citroen</i> (JC) - Control	<i>C. nobilis</i> vr <i>Siam Pontianak</i>	77.41 b
Rootstock <i>Japansche citroen</i> (JC) - Control	<i>C. aurantifolia</i> vr Sweet lime	70.43 c
<i>Carrizo citrange</i>	<i>C. reticulata</i> (Blanko) vr <i>Kepron Batu 55</i>	88.15 a
<i>Carrizo citrange</i>	<i>C. nobilis</i> vr <i>Siam Pontianak</i>	78.05 b
<i>Carrizo citrange</i>	<i>C. aurantifolia</i> vr Sweet lime	68.31 c
<i>citrumello</i>	<i>C. reticulata</i> (Blanko) vr <i>Kepron Batu 55</i>	65.73 c
<i>citrumello</i>	<i>C. nobilis</i> vr <i>Siam Pontianak</i>	58.25 d
<i>citrumello</i>	<i>C. aurantifolia</i> vr Sweet lime	53.12 e
<i>Poncirus trifoliata</i>	<i>C. reticulata</i> (Blanko) vr <i>Kepron Batu 55</i>	65.10 c
<i>Poncirus trifoliata</i>	<i>C. nobilis</i> vr <i>Siam Pontianak</i>	58.55 d
<i>Poncirus trifoliata</i>	<i>C. aurantifolia</i> vr Sweet lime	50.25 e
<i>volkameriana</i>	<i>C. reticulata</i> (Blanko) vr <i>Kepron Batu 55</i>	96.44 a
<i>volkameriana</i>	<i>C. nobilis</i> vr <i>Siam Pontianak</i>	90.92 a
<i>volkameriana</i>	<i>C. aurantifolia</i> vr Sweet lime	84.80 ab
<i>Rough lemon</i>	<i>C. reticulata</i> (Blanko) vr <i>Kepron Batu 55</i>	66.90 c
<i>Rough lemon</i>	<i>C. nobilis</i> vr <i>Siam Pontianak</i>	71.50 bc
<i>Rough lemon</i>	<i>C. aurantifolia</i> vr Sweet lime	60.70 d
<i>Troyer citrange</i>	<i>C. reticulata</i> (Blanko) vr <i>Kepron Batu 55</i>	81.5 b
<i>Troyer citrange</i>	<i>C. nobilis</i> vr <i>Siam Pontianak</i>	83.5 b
<i>Troyer citrange</i>	<i>C. aurantifolia</i> vr Sweet lime	70.05 c
<i>Citrus kumquat</i> vr <i>Kanci</i>	<i>C. reticulata</i> (Blanko) vr <i>Kepron Batu 55</i>	93.4 a
<i>Citrus kumquat</i> vr <i>Kanci</i>	<i>C. nobilis</i> vr <i>Siam Pontianak</i>	89.4 a
<i>Citrus kumquat</i> vr <i>Kanci</i>	<i>C. aurantifolia</i> vr Sweet lime	76.2 c

Different letters are significantly different and means followed by the same letters are not significantly different at $P = 0.05$. A total of 48 successful grafted seedlings were arranged in a randomized complete block design with Two replications.

CONCLUSION

In general, it is found that there are 3 groups resistance to *B theobromae* combination of interstock. The first is a tolerant group: Sweet Lime scion with the interstock of *Volkameriana*, *Troyer citrange*, and *Kanci* have the smallest attack area (0 cm²). The second one is a group that is quite resistant to mild attack: *Siam Pontianak* scion with the interstock of *Carrizo citrange*, *Citrumello*, *Poncirus trifoliata*, *Volkameriana*, *Rough lemon*; sweet lime scion with the interstock of *citrumello*, *Rough lemon* and *Kepron Batu 55* scion with the interstock of *volkameriana* and *Rough lemon* have the incubation period of 14 to 25 days after inoculation. Lastly, the group that has light resistant with moderate severity: The scion of *Kepron Batu 55* with the interstock of *Carrizo citrange*, *Swingle citrumello*, *Poncirus trifoliata* as well as Sweet Lime scion with the interstock of *Carrizo citrange* and *Poncirus trifoliata* has an incubation period of 12 to 16 days after inoculation. The plants grafted with the interstock of *Volkameriana* are taller than the plants which are joined with the interstock of *Citromello* and *P.trifoliata*.

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