SUSCEPTIBILITY OF YELLOW DWARF COCONUT SEEDLINGS TO *Pestalozzia palmarum* Cooke

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ABSTRACT

The average number of leaf spots from natural infection was generally lower than that obtained when Yellow Dwarf coconut seedlings were artificially inoculated with spore suspensions of *Pestalozzia palmarum* Cooke. It was likewise observed that 200 to 300 spores per drop of inoculum produced the highest number of leaf spot among all the treatments at 37 days after inoculation; 50 to 75 spores per drop of fungal suspension was the minimum concentration of *P. palmarum* and 100 to 150 spores the optimum that can cause disease infection on a susceptible host plant. The severity and the average rate of increase of leaf spot in every count was linearly correlated with the inoculum concentration from the first count at 21 days until the ninth count at 37 days after inoculation.

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KEY WORDS: *Pestalozzia palmarum*. Yellow Dwarf coconut. Inoculation method. Gray leaf spot. Inoculum concentration. Symptomatology. Culture. Susceptibility.

INTRODUCTION

Cadang-cadang, bud rot and leaf blight are considered as the most common and most destructive diseases of coconut in the Philippines. One disease, commonly known as coconut gray leaf spot, can develop into a leaf blight. This gray leaf spot is caused by a fungus, *Pestalozzia palmarum* Cke. The disease is often serious in the nursery and in newly-established coconut plantations.

San Juan and Rebigan (1977) observed that gray leaf spot of coconut can be controlled by several kinds of fungicides such as Daconil 2788, Kocide 101, Dithane M-45, Captex 50WP and Delson. However, the use of fungicides is quite expensive and some coconut farmers cannot afford to buy these chemicals. Meanwhile, selection or breeding for resistant varieties against gray leaf spot is an arduous process, but it is more economical and profitable in the long run.

Varietal screening is necessary in order to select promising varieties which are resistant to coconut gray leaf spot. In many cases, natural infection is used in screening varieties, but this is usually an unreliable method since there are different kinds of fungal spores which cause various foliar diseases on coconut. To ensure a uniform and accurate screening test, it is imperative that the number of spores required to initiate an infection by artificial inoculation be determined in order to have a uniform and accurate screening test.

This study determined the minimum concentration of fungal inoculum that can cause infection and compared the effectiveness of natural and artificial inoculation of *P. palmarum* on coconut.

MATERIALS AND METHODS

Germination of Seednuts. — The seednuts of Yellow Dwarf variety were first trimmed near the eye portion to hasten the sprouting of the germinating embryo. Then the seednuts were placed on a raised bed in a horizontal half-buried position at a distance of 14 cm between nuts.

After the seednuts have germinated, when the shoots were about 5 cm high, the seedlings were transferred into polyethylene bags and allowed to grow until they were ready for inoculation, which was at about 6 months in age.

Isolation of Pathogen. — The organism was isolated from infected coconut leaves collected in the field. The diseased leaves were cut into small pieces and disinfected with 0.35% calcium hypochlorite solution to remove some of the surface contaminants before planting them in solidified potato dextrose agar (PDA) in petri plates. The culture was incubated at room temperature to allow growth and development of the pathogen. The pathogen was transferred as pure culture isolates into flat bottles containing PDA and then allowed to sporulate before it was used as inoculum.

Concentration of Fungal Suspension. — The concentration of inoculum was determined by counting under the microscope the number of spores in a drop of fungal suspension. There were 5 different concentrations of the suspension used in inoculating the test seedlings of Yellow Dwarf variety: 50 to 75, 100 to 150, 200 to 300, 400 to 500, and 600 or more per drop of inoculum suspension.

Comparison of Artificial and Natural

Methods of Inoculation. — Six months after the seednuts have germinated, the plants were inoculated with *P. palmarum.* After preparing and determining the recommended concentration of fungal inoculum as described earlier, artificial inoculation was done by placing the inoculum in a hand sprayer and then spraying it into the test coconut seedlings. The plants were covered with plastic bags after artificial inoculation to maintain high humidity on the leaves and to prevent the spores from being washed out from the leaf surface in case heavy rain occurred before the spores had germinated and penetrated the plant tissue.

For naturally-inoculated plants, seedlings were placed near infected coconut seedlings and trees without spraying them with the fungal inoculum. They were left in the field until the seedlings showed symptoms of the disease. Both artificial and natural methods of inoculation were carried out simultaneously.

RESULTS AND DISCUSSION

Cultural Characteristics of Pestalozzia palmarum.

The organism produced whitish or cottony hyphae, procumbent mycelia and circular colonies in PDA. Visible whitish mycelia were observed from 1 to 2 days. Sporulation started from 12 to 14 days after the pathogen was introduced into the culture media. Aggregates of blackish globs could be seen by the naked eye when the organism was 3 to 4 weeks old, indicating sporulation (Fig. 1).

Fig. 1. Pure culture of *Pestalozzia palmarum* after 3 weeks of incubation in a flat bottle containing potato dextrose agar. Note aggregates of blackish globs on the surface of the culture.

At the early stage of development, the mycelial growth was relatively faster. The diameter of the colony after 24 hr was about 6 mm and after 3 days it increased to around 20.5 mm from the time a small portion of mycelia was introduced into the PDA. After 3 days the increase was relatively slower since the colony had a diameter of about 23 mm after 4 days and after 2 weeks it reached to around 60 mm.

The organism had radial growth, i.e., the mycelia were equally dispersed from the point of origin thereby forming a circular colony.

The spores were hyaline in color at younger stage and somewhat dark brown when older. The spores of *P. palmarum* were observed to have 2 slender appendages at the tip portion and usually had 2 to 3 septations of the cell.

Description of Symptoms.

The artificially-inoculated coconut seedlings showed disease symptoms 17 to 20 days after inoculation. The initial symptom of the disease was the appearance of a water-soaked spot on the infected tissue. The water-soaked lesions were not very distinguishable 17 days after inoculation but they became more pronounced after 20 days. The infected tissue remained water-soaked for around 4 to 5 days from the time the lesions appeared, gradually turning yellowish in color after 1 to 2 days. Usually the spot measured about 1 to 2 mm in diameter at the beginning which gradually increased in size as the disease progressed. Most of the leaf spots were circular.

The lesions were more numerous in relatively older than in younger leaves. Furthermore, the lesions were usually more prevalent at the terminal portion compared to the basal portion of the leaves probably because the base contained certain substances at higher levels that could inhibit rapid disease development. Martinez (1979) also observed the same phenomenon wherein cercospora leaf spot of cassava was more prevalent in the older than in younger leaves.

Comparison Between Different Concentrations of Fungal Inoculum.

The counting and observation of leaf spots at two-day intervals were started 21 days after inoculation. The leaf spots that appeared in every counting were marked with ink so that in the next counting, only the unmarked lesions were included.

Variations in the average number and the rate of increase of leaf spots per treatment are given in Table 1. The average number of leaf spot between treatments differed significantly during the first count except in 200 to 300 and 400 to 500 spores wherein the number of leaf spots were more or less equal.

The results show that the number of leaf spot in every count was linearly correlated with the inoculum concentration from the first count at 21 days until the ninth count at 37 days after inoculation (Fig. 2). It was noticed that concentrations of 200 to 300 spores produced the highest number of leaf spot among all the treatments at 37 days after inoculation. Perhaps this concentration of inoculum contains the maximum level of spores that can cause infection to the susceptible host. Beyond this level, further increase in the inoculum concentration resulted in the decrease of the number of lesions or disease severity. These findings are similar to the report of Warren (1974) that disease severity of hypocotyl rot of susceptible lima bean seedlings due to *Rhizoctonia solani* was linearly correlated with inoculum concentration up to a certain level of pathogen population. Further increase in inoculum concentration resulted in relatively small increase in disease severity. Richards (1923) also showed that there was a linear relationship up to a given level of inoculum, but further increase in inoculum concentration caused only small increase in disease severity. These results contradict the report on Verticillium wilt disease by Mosser and Sackston (1972) who showed that there was direct linear relationship between concentration of inoculum and the speed of development of the leaf symptoms and also the degree of stunting of the

Table 1. Average number of coconut gray leaf spot per plant (Variety Yellow Dwarf) from 21 to 59 days after inoculation with *Pestalozzia palmarum*.[1]

Days After Inoculation	Number of Spores Used in Artificial Inoculation					Natural Inoculation
	50-75[2]	100-150	200-300	400-500	600 +	
21	59.25[3]	78.75	104.50	106.00	120.00	98.25
23	74.50	89.75	107.50	108.75	134.75	103.25
25	81.50	148.50	153.25	145.25	164.75	111.50
27	90.50	159.75	162.00	151.75	173.00	112.50
29	90.25	170.00	163.50	157.50	175.75	114.75
31	105.25	175.25	167.00	161.50	179.75	117.25
33	121.25	190.50	180.00	174.50	200.50	119.25
35	133.25	198.50	214.75	187.75	215.50	123.00
37	136.50	213.00	225.75	204.75	217.50	123.23
39	158.00	249.50	253.25	235.50	233.00	127.00
41	200.00	268.75	290.75	252.00	251.00	129.25
43	214.50	286.25	328.00	266.75	270.75	137.50
45	237.25	303.50	358.25	286.25	297.25	152.25
47	259.25	322.75	379.50	306.50	319.25	171.00
49	270.00	334.50	399.00	321.00	329.25	177.25
51	279.00	343.25	399.00	328.00	337.25	183.00
53	302.75	363.00	414.75	345.00	344.50	191.00
55	310.25	370.75	427.00	352.50	348.75	196.50
57	317.75	379.75	438.25	358.25	354.50	202.00
59	326.25	392.00	452.50	363.75	361.00	209.75

[1] Leaf spot symptoms appeared 17 to 20 days after inoculation.

[2] Number of spores based on per drop of fungal suspension; 100 ml was used per inoculation for every treatment.

[3] The average number of gray leaf spot per treatment of both artificially and naturally inoculated plants subtracted from the average number of gray leaf spot from the uninoculated control.

susceptible inbred line CM 162. They also said that leaf symptoms were less severe and developed more slowly at lower inoculum concentration.

As a result of this study, it can be postulated that there is an inhibition or antagonistic effect on the activities of the pathogen if the population density is beyond a certain level. Germination of the spores may have been inhibited due to competition of the organism among themselves or to the rapid accumulation of substances produced as a result of their activities in the host. Warren (1974) observed that decrease in disease severity due

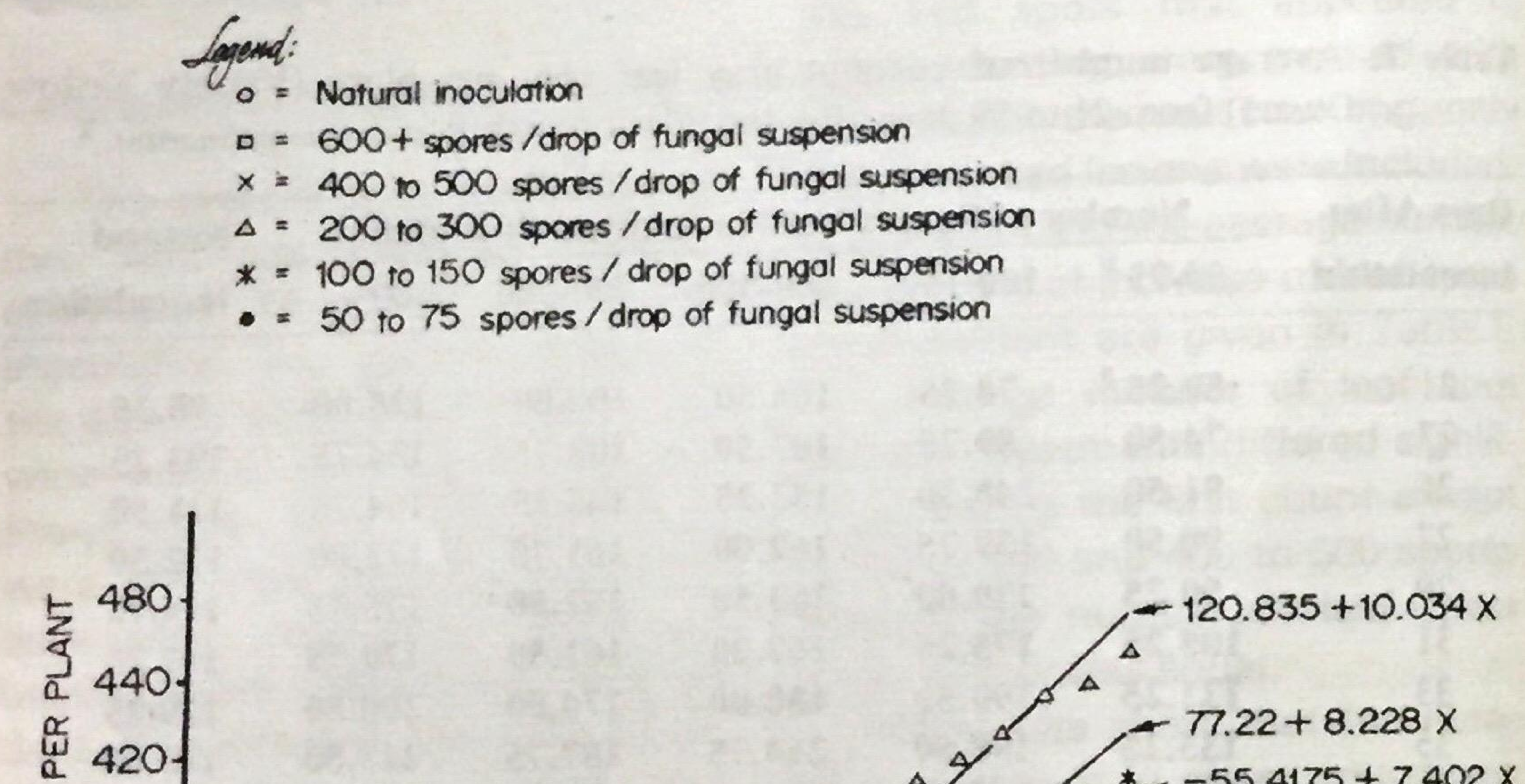

Fig. 2. Relationship between the number of coconut gray leaf spots per plant and the number of days after inoculation with *Pestalozzia palmarum*.

to further increase of inoculum concentration was due to the competition among the organism and not because of inhibitory substances that were produced.

The results of the experiment also showed that 50 to 75 spores per drop of fungal suspension was the minimum concentration for *P. palmarum* to cause or initiate infection on a susceptible host plant. Since the concentration of fungal inoculum that produced the highest number of leaf spot was from 200 to 300 per drop of fungal suspension, this concentration could be considered as the maximum level of inoculum that can cause a high degree of disease occurrence on a susceptible host. As stated earlier, a further increase in inoculum concentration may result in the reduction of disease severity. The number of leaf spots produced from 100 to 150 spores per drop of fungal suspension is less compared to 200 to 300 but relatively higher in comparison to 50 to 75 spores per drop of inoculum. Therefore, 100 to 250 spores per drop of fungal suspension could be considered as the optimum concentration of *P. palmarum* that can cause disease infection on a susceptible host plant.

Comparison of Artificial and Natural Methods of Inoculation.

Table 1 indicates that the average number of leaf spot during the first count for naturally-inoculated seedlings was higher compared to 50 to 75 and 100 to 150 but lower in comparison with 200 to 300, 400 to 500 and 600 or more spores per drop of fungal suspension for the artificially-inoculated plants. This was probably because by the time the healthy test seedlings were placed near the seriously infected seedlings, which served as the source of inoculum for naturally-inoculated plants, strong wind and rain often occurred which caused the efficient dissemination or transmission of the pathogen. However, the increase of leaf spot in naturally infected plants, for all counts, was relatively slower compared to all treatments using artificially-inoculated plants. This result suggests that artificial inoculation of *P. palmarum* is a more accurate and effective method for evaluating the resistance of different promising varieties of coconut against coconut gray leaf spot disease. In natural inoculation method, the test plants did not receive a uniform concentration of spores from the air since it was impossible for the dispersed spores to be equally distributed to all test plants. Furthermore, other fungi such as *Helminthosporium haloides* could also cause leaf spot disease on coconut similar to the leaf spot produced by *P. palmarum*. Therefore, to ensure that the disease produced is caused by a specific pathogen, artificial inoculation must be done in testing the resistance of coconut to a particular disease.

Biweekly Increase in the Number of Gray Leaf Spot.

The data on the biweekly increase in the number of gray leaf spot of both naturally-and artificially-inoculated plants are graphically presented in Fig. 2.

The analysis of variance showed that the differences in the number of leaf spot counted at 3 and 4 weeks after artificial inoculation were highly significant between treatments but not significant between replications. On the other hand, the differences in the number of lesions counted at 5, 6, 9, and 12 weeks after inoculation were not significant both between replications. These results may be explained by the fact that during the early counts, at 3 and 4 weeks after inoculation, treatments inoculated with lower concentration also had correspondingly lower number of leaf spots but after 4 weeks, the increases in the number of leaf spot on these treatments were relatively faster compared to the treatments inoculated with higher concentration. Therefore, the figure on the total number of leaf spots counted in the treatments inoculated with lower concentrations were statistically similar to the total number of leaf spots counted in the treatments inoculated with higher concentrations beginning from the fifth week after inoculation. As pointed out earlier, these observations can be further explained by the inhibitory or antagonistic effect on the activities of the pathogen at higher concentrations of inoculum so that after the population density has reached a certain level there is very little change going on for a period of time.

The two-day interval in counting the number of gray leaf spot per plant was terminated at 59 days after inoculation because the rate of increase in the number of lesions had started to decline at this period. The leaf area which was not yet infected must have been reduced due to the increase in the number and size of the individual lesions. The counting of leaf spots was changed to one-week interval and the gathering of data for this experiment lasted up to 12 weeks after inoculation.

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