Borneo Journal of Resource Science and Technology (2016) 6(1): 50-54

SHORT COMMUNICATION

Effects of Inoculum Concentrations of *Colletotrichum gloeosporioides* on Disease Development and Severity on Leaves of Rubber Tree (*Hevea brasiliensis*)

SANGEETHA SIVA SANGU* & SEPIAH MUID

Department of Environmental Science and Ecology, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia

ABSTRACT

Colletotrichum gloeosporioides is among the main causal agent of anthracnose of the rubber tree (*Hevea brasiliensis*). This disease is considered as one of the major foliar diseases that causes declining yields of rubber production in Asia. In order to understand the effect of environmental variables on anthracnose, the effect of inoculum concentrations on the disease development and severity was studied. Spore concentration of 10^6 spores/ml produced the most prominent disease development. Disease was most severe when the leaves were kept in continuous moisture condition for four weeks. Fungal response study showed that the fungus needed 120 hours after inoculation (hai) to penetrate and colonize leaf cells. Knowledge of this factor on disease development can help improve management tactics based on the control of environmental factors.

Keywords: Anthracnose, foliar, fungal response, natural latex

Hevea brasiliensis Müell. Arg., the rubber tree is one of the most important plant species in the world industry. The most essential feature of this tree is the white latex formed when the bark is cut or tapped. It is this white latex which is vulcanized to form rubber (Navie & Adkins, 2008).

Rubber tree is susceptible to several foliar diseases including anthracnose, resulting in lesion forming and affecting the flush of new leaves produced following the 'wintering' effect (Waller, 1992). *Colletotrichum gloeosporioides* was identified as one of the causal agents of anthracnose on rubber tree in 1905 from Ceylon. The disease was also observed in Malaysia, Brazil, India, Indonesia, and Thailand (Jayasinghe *et al*., 1996).

Anthracnose causes declining yields of rubber in Asia (Thambugala & Deshappriya, 2009). Inoculum concentration influences infection and development of disease on crops, such as cucumber (Dean & Kúc, 1986), tomato fruit (Dillard, 1989), round-leaved mallow and velvet leaf's seeds (Makowski, 1993), and Azalea leaves (Bertetti *et al*., 2009). However, in Malaysia, more information is needed on the effects of inoculum concentration on anthracnose of rubber seedlings. Detailed knowledge of these effects on *C. gloeosporioides* may provide helpful information to direct research towards optimizing suitable fungicides to control this species.

Diseased rubber leaves showing symptoms of anthracnose were collected and cut to leaf segments of 1.0 × 2.0 mm and surface sterilized. Then, they were placed on potato dextrose agar (PDA) and left at room temperature for 10 days. Colonies identified as *C. gloeosporioides* based on their morphological and microscopic appearance were transferred to fresh PDA plates, and a series of subcultures was made to obtain pure culture. After two weeks, 10 ml of sterile distilled water were added to the spore production plates, and spores were dislodged

*Corresponding author: *sangeethasivasangu@yahoo.com*

by gentle rubbing with a flamed, glass slide. The spore concentration was counted using a hemocytometer. Leaves were inoculated on 15 points on its surface with 60 µl of inoculum.

Experiments were done on 16 healthy detached and attached leaves respectively of rubber seedlings. For the disease development experiment, equal numbers of leaves were prepared with wounded treatment and with not wounded one. Leaves were wounded by piercing 15 small holes using a sterilised and flamed needle. Then, equal halves of those leaves were sterilized and not sterilized. Sterilization was done to avoid presence of other microbes on the leaves by washing leaves with 50% of ethanol. All the leaves were inoculated with *C. gloeosporioides* of concentrations 10^2, 10^4, and 10^6 spores/ml on separate leaves. Five replicates were done for each concentration. Control leaves were wet and stored. Data observation was done weekly for a month by observing the number of black spots formed on the leaf surface. The inoculum concentration that had caused the most number of spots is the cause of most prominent disease development.

For the disease severity experiment, another set of leaves were prepared and inoculated with inoculum concentration that had caused most prominent disease development in the earlier experiment. Disease severity was observed weekly for four weeks by observing the number of black spots formed on the leaf surface. The duration taken for most number of spots to be formed was observed.

Plastic bags were used to maintain a post-inoculation high humidity environment. The inner side of the bag and the leaves were wet before inoculation by spraying distilled water.

Using the IBM SPSS Statistics ver. 22 program, one-way ANOVA was used to analyse the data.

Five healthy and sterilised rubber leaves were inoculated with the concentration that had caused most number of spots formed on leaf surface in the disease development experiment. Observation was done for five days using the five leaves, each for a day, under the Scanning Electron Microscope to observe the growth and colonization of the *C. gloeosporioides* on the leaves.

Effect of inoculum concentration on disease development study

Spore inoculum concentration of at least 10^2 spores/ml was needed to cause disease development. Concentration of 10^6 spores/ml caused the most prominent disease development on the leaf. The higher the inoculum concentration, the more the number of spots formed on the leaf surface. The results are summarised in Table 1.

It was reported that inoculum concentration of 10^6 spores/ml was the optimum concentration to cause disease development on leaves (Bertetti *et al*., 2009; Dillard, 1989; Forcelini, 2013; Makowski, 1993). However, a concentration higher than 10^6 spores/ml was not applied because higher concentration causes germination auto-inhibitors due to limitation of nutrient and high temperature stress (Leandro *et al*., 2003).

Effect of inoculum concentration on disease severity study

At one week after inoculation, black spots were observed on the leaves. Disease severity was most on the fourth week after inoculation. The number of spots increased with the weeks. Results were summarised in Table 2. Chongo and Bernier (2000) stated that the disease severity was the highest at the fourth week after inoculation. This is due to spore germination which was favoured when leaves were kept in continuous moist condition for longer period (Moral *et al*., 2012).

Table 1. Average disease development (number of spots) leaves of rubber tree when inoculated with different concentrations of inoculum.

Type of leaves	Week	Concentrations of inoculum (spores/ml)			
		Control	10^2	10^4	10^6
Unwounded and sterilized attached leaves	W 2	0.60 ± 0.89 ^{b}	0.80 ± 0.84 ^{b}	1.40 ± 0.55 ^{b}	2.60 ± 1.14 ^{a}
	W 4	0.60 ± 0.89 ^{c}	1.20 ± 1.10 ^{bc}	1.80 ± 0.45 ^{b}	4.00 ± 0.00 ^{a}
Unwounded and unsterilized attached leaves	W 2	0.40 ± 0.55^{b}	1.00 ± 0.00 ^{ab}	2.00 ± 0.71 ^{a}	1.80 ± 1.30 ^{a}
	W 4	0.60 ± 0.55 ^{b}	1.00 ± 0.00 ^{b}	2.40 ± 0.55 ^{a}	2.60 ± 1.34 ^{a}
Wounded and sterilized attached leaves	W 2	0.40 ± 0.55^{c}	1.20 ± 0.45 ^{b}	1.40 ± 0.55 ^{b}	4.40 ± 0.55 ^{a}
	W 4	0.60 ± 0.55 ^{d}	1.40 ± 0.89 ^{c}	2.60 ± 0.55 ^{b}	5.00 ± 0.00 ^{a}
Wounded and unsterilized attached leaves	W 2	0.60 ± 0.55 ^{d}	1.80 ± 0.84 ^{c}	4.00 ± 0.71^{b}	7.00 ± 1.23 ^{a}
	W 4	0.80 ± 0.84^{d}	3.00 ± 0.00 ^{c}	4.80 ± 0.45 ^{b}	9.60 ± 1.52 ^{a}
Unwounded and sterilized detached leaves	W 2	0.00 ± 0.00 ^{c}	1.00 ± 0.71 ^{b}	2.20 ± 0.84^{a}	2.80 ± 0.45 ^{a}
	W 4	0.00 ± 0.00 ^{c}	1.60 ± 0.55 ^{b}	3.00 ± 0.00^{a}	3.00 ± 0.00^{a}
Unwounded and unsterilized detached leaves	W 2	0.40 ± 0.55 ^{c}	0.80 ± 0.45 ^{bc}	2.00 ± 0.71 ^{b}	4.20 ± 1.92^{a}
	W 4	0.40 ± 0.55 ^{b}	1.20 ± 0.45 ^{b}	2.40 ± 0.55^{b}	5.20 ± 2.76^{a}
Wounded and sterilized detached leaves	W 2	0.20 ± 0.45 ^{b}	1.40 ± 1.34 ^{b}	3.00 ± 1.23 ^{a}	4.00 ± 1.23^{a}
	W 4	0.40 ± 0.55 ^{c}	1.80 ± 1.30^{b}	3.20 ± 0.84^{a}	4.40 ± 0.89^{a}
Wounded and unsterilized detached leaves	W 2	0.20 ± 0.45 ^{b}	1.20 ± 0.84 ^{b}	4.80 ± 1.79 ^{a}	5.60 ± 1.34^{a}
	W 4	0.20 ± 0.45 ^{c}	2.60 ± 1.52^{b}	5.80 ± 1.30^{a}	7.40 ± 2.61^{a}

Mean ± standard deviation for each concentration, values in row followed by the same letter indicate no significant difference of disease development at p=0.05 level by Duncan test.
Note: superscripts of a, b, c and d indicate there are not significant difference in terms of disease development if values from the same row has the same superscript.

Table 2. Disease severity based on average disease development (number of spots) on leaves of rubber trees which were inoculated with 10^6 spores/ml of inoculum for four weeks of continuous moisture condition.

Type of leaves	Continuous moisture period			
	Week 1	Week 2	Week 3	Week 4
Unwounded and sterilized attached leaves	0.60 ± 0.90^{b}	1.00 ± 0.71^{ab}	1.40 ± 0.55^{ab}	1.60 ± 0.55^{a}
Unwounded and unsterilized attached leaves	0.60 ± 0.90^{b}	1.00 ± 0.71^{ab}	1.40 ± 0.55^{ab}	1.60 ± 0.55^{a}
Wounded and sterilized attached leaves	2.40 ± 2.10^{b}	4.00 ± 1.41^{ab}	5.40 ± 0.55^{ab}	5.80 ± 1.30^{a}
Wounded and unsterilized attached leaves	4.20 ± 0.45^{c}	5.00 ± 0.71^{c}	7.00 ± 0.71^{b}	9.60 ± 1.14^{a}
Unwounded and sterilized detached leaves	0.40 ± 0.55^{c}	1.20 ± 0.84^{bc}	2.00 ± 0.71^{ab}	2.20 ± 0.45^{a}
Unwounded and unsterilized detached leaves	1.60 ± 0.55^{b}	2.20 ± 0.84^{b}	2.40 ± 0.55^{ab}	3.20 ± 0.45^{a}
Wounded and sterilized detached leaves	0.00 ± 0.00^{c}	0.80 ± 0.45^{b}	1.20 ± 0.45^{b}	2.00 ± 0.71^{a}
Wounded and unsterilized detached leaves	2.60 ± 0.55^{b}	3.00 ± 0.71^{ab}	3.40 ± 0.90^{ab}	3.80 ± 0.45^{a}

Mean ± std. dev. For each concentration, values in row followed by the same letter indicate no significant difference of disease development at p=0.05 level by Duncan test.
Note: superscripts of a, b and c indicate there are not significant difference in terms of disease development if values from the same row has the same superscript.

Fungal response of *Colletotrichum gloeosporioides* on rubber tree leaves

The fungus took 120 hours after inoculation (hai) to completely penetrate and colonize the leaves. At 24 hai, the spores merely adhered to the leaf cells (Figure 1(a)). At 48 hai, the spores had germinated and hypha was formed (Figure 1(b)). A series of hyphae was observed at 72 hai (Figure 1(c)) and septa had formed on the hyphae at 96 hai (Figure 1(d)). At 120 hai, the mycelium had spread on the leaf cells (Figure 1(e)).

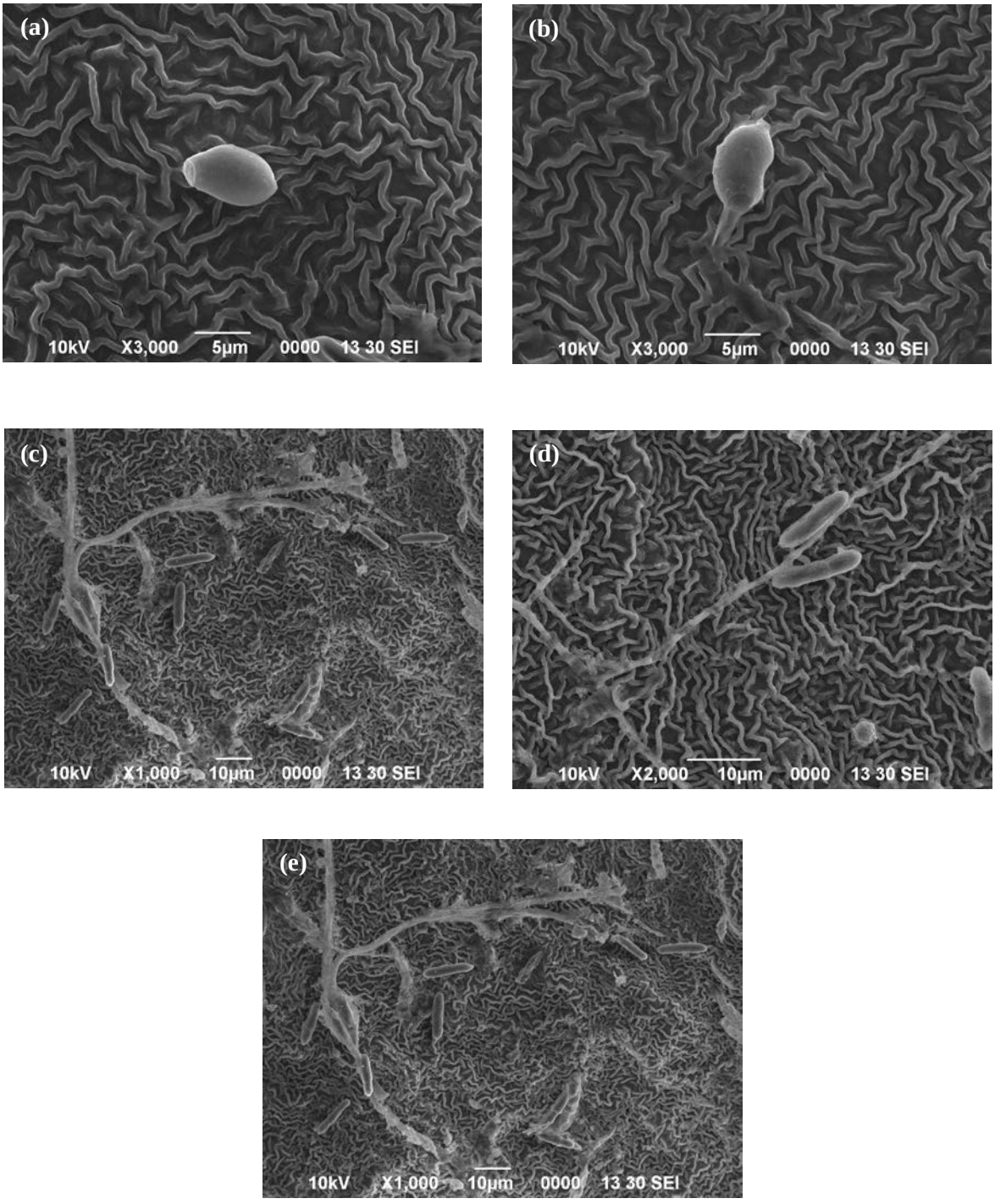

Figure 1. Scanning electron micrograph of the infection process of *C. gloeosporioides* on rubber tree leaves. (a) Ungerminated spore, (b) Germinated spore with hypha on it, (c) Series of hyphae on leaf surface, (d) Septa formed on hyphae and (e) Mycelia formed.

The infection process was observed for five days after inoculation. At this stage, no visible symptom was observed on the leaf surface with naked eyes. According to Bailey *et al*. (1992), *C. gloeosporioides* invades the leaf cell without killing it at this symptomless stage. This stage is when the fungus establishes itself in the leaf tissues. In this stage, this fungus is referred to as intracellular hemi-biotrophic pathogen. Morin *et al*. (1996) showed that an initial sign of penetration on Malvaceae weeds occurred at 36 hai and hypha was formed at 48 hai. At 72 hai, the hyphae were formed and by 112 hai, complete penetration and colonization occurred.

The observation of disease development and severity and hypha development on *H. brasiliensis* matched the previous reports on different hosts. These results can be applied to help improve management tactics based on the control of environmental factors.

REFERENCES

Bailey, J.A., O'Connell, R.J., Pring, R.J. & Nash, C. (1992). Infection strategies of *Colletotrichum* sp. In: J.A. Bailey & M.J. Jeger (Eds.), *Colletotrichum: Biology, Pathology and Control*. UK: Redwood Press Ltd. Pp 88-120.

Bertetti, D., Gullino, M.L. & Garibaldi, A. (2009). Effect of leaf wetness duration, temperature and inoculum concentration on infection of evergreen Azalea by *Colletotrichum* a*cutatum*, the causal agent of anthracnose. *Journal of Plant Pathology*, 91(3): 763-766.

Chongo, G. & Bernier, C.C. (2000). Effects of host, inoculum concentration, wetness duration, growth stage, and temperature on anthracnose of lentil. *Plant Disease,* 84: 544-548.

Dean, R.A. & Kúc, J. (1986). Induced systemic protection in cucumber: Effects of inoculum density on symptom development caused by *Colletotrichum lagenarium* in previously infected and uninfected plants. *Phytopathology*, 76: 186-189.

Dillard, H.R. (1989). Effect of temperature, wetness duration, and inoculum density on infection and lesion development of *Colletotrichum coccodes* on tomato fruit. *Phytopathology*, 79: 1063-1066.

Forcelini, B.B. (2013). *Effect of inoculum concentration, temperature and wetness duration on anthracnose fruit rot development on different strawberry cultivars*. A published Master thesis, University of Florida. http://ufdc.ufl.edu/UFE0046391/00001.

Jayasinghe, C., Fernando, T. & Priyanka, U. (1996). *Colletotrichum acutatum* is the main cause of *Colletotrichum* leaf disease of rubber in Sri Lanka. *Mycopathologia*, 137: 53-56.

Leandro, L.F.S., Gleason, M.L., Nutter, F.W. Jr., Wegulo, S.N. & Dixon, P.M. (2003). Influence of temperature and wetness duration on conidia and appressorial of *Colletotrichum acutatum* on symptomless strawberry leaves. *Epidemiology,* 93(4): 513-520.

Makowski, R.M.D. (1993). Effect of inoculum concentration, temperature, dew period, and plant growth stage on disease of round-leaved mallow and velvetleaf by *Colletotrichum gloeosporioides* f. sp. *malvae*. *Phytopathology*, 83: 1229-1234.

Moral, J., Jurado-Bello, J., Sánchez, M.I., Oliveira, R. & Trapero, A. (2012). Effect of temperature, wetness duration, and planting density on olive anthracnose caused by *Colletotrichum* spp. *Phytopathology,* 102: 974-981.

Morin, L., Derby, J.L. & Kokko, E.G. (1996). Infection process of *Colletotrichum gloeosporioides* f. sp. *malvae* on Malvaceae weeds. *Mycological Research,* 100(2): 165-172.

Navie, S. & Adkins, S. (2008). *Environmental weeds of Australia*. Australia: CRC for Australian Weed Management.

Thambugala, T.A.D.P. & Deshappriya, N. (2009). The role of *Colletotrichum* sp. on the *Colletotrichum* leaf disease of *Hevea brasiliensis* - a preliminary study. *Journal of National Science Foundation of Sri Lanka*, 37(2): 135-138.

Waller, J.M. (1992). *Colletotrichum* diseases of perennial and other cash crops. In Bailey, J.A. & M.J. Jeger, (Eds.), *Colletotrichum: Biology, Pathology and Control*. UK: Redwood Press Ltd. Pp 167-185.