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DISPERSAL OF *RAFFLESIA PATMA* BLUME ENDOPHYTE IN GRAFTED HOST PLANT (*TETRASTIGMA LEUCOSTAPHYLUM* (DENNST.) ALSTON)

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Abstract: This study provides a hypothetical discussion about the growth of *Rafflesia patma* Blume (syn. *R. horsfieldii* R. Br. (1821); Rafflesiaceae), an endophytic parasite, within a grafted host, a woody vine (*Tetrastigma leucostaphylum* (Dennst.) Alston). Based on the observation of a 10-year old (2007 to 2017) *R. patma – T. leucostaphylum* graft, we hypothesize that *R. patma* moves away from its host rather than remaining in an anchored position from which it flowers, although flower knobs that emerge later may vary in range from the grafting point, i.e., flower knobs may develop close to or far away from the grafting point. Our provisional macroscopic observations point towards a gradual creeping motion of the endophyte towards new host tissues, flowering opportunistically when sufficient nutrients have been found. Much has yet to be discovered about the growth and flowering behavior of *R. patma* and about the dynamics of the *R. patma – T. leucostaphylum* interaction.

Keywords: endophyte, parasitic plant, Rafflesiaceae, *Tetrastigma*, vine.

Introduction

Rafflesiaceae, which are the most minimalistic plants on the planet, having no leaves or roots, grow as an endophytic parasite inside a host, of the genus *Tetrastigma*. There are 12 recorded *Tetrastigma* species (or synonymous taxa) that are capable of hosting *Rafflesia*: *T. leucostaphylum* (Dennst.) Alston. (syn. *T. lanceolarium* (Roxb.) Planch.), *T. loheri* Gagnep., *T. papillosum* (Blume) Planch., *T. trifoliatum* Merr., *T. scortechinii* (King) Gagnep., *T. scariosum* (Miq.) Planch. (syn. *T. pisicarpum* (Miq.) Planch.), *T. coriaceum* (DC.) Gagnep. (syn. *T. tuberculatum* Latiff), *T. diepenhorstii* Miq., *T. quadrangulum* Gagnep. & Craib, *T. scortechinii* (King) Gagnep., *T. curtisii* (Ridl.) Suesseng., and *T. glabratum* (Blume) Planch. [NAIS, 2001; CHEN & al. 2011; MURSIDAWATI & IRAWATI, 2017]. As *Rafflesia* grow within the host woody vine, they are invisible until they reach the reproductive stage when flower bud or knob emerged through the surface layer of the host [WICAKSONO & al. 2016].

There are currently two hypotheses about how the endophyte is able to grow inside the host vine of *Tetrastigma* (Fig. 1). NIKOLOV & al. (2014) claimed that the endophyte grows radially towards the center of the stem in the xylem area. Personal communication with Nikolov in 2017 revealed that since it is very difficult to observe the transverse cross-sectional morphology of the endophyte by microtomy preparation (i.e., for microscopic observation) compared to radial cross-sections, it is difficult to determine whether *Rafflesia*

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grows within a vine. Nikolov hypothesized that *Rafflesia* grows in one certain place and does not traverse the graft. BARKMAN & al. (2017) revealed that for the *Rafflesia* endophyte, the host body serves as an "island" where it grows and creeps inside the host body rather than remaining anchored in the exact same place.

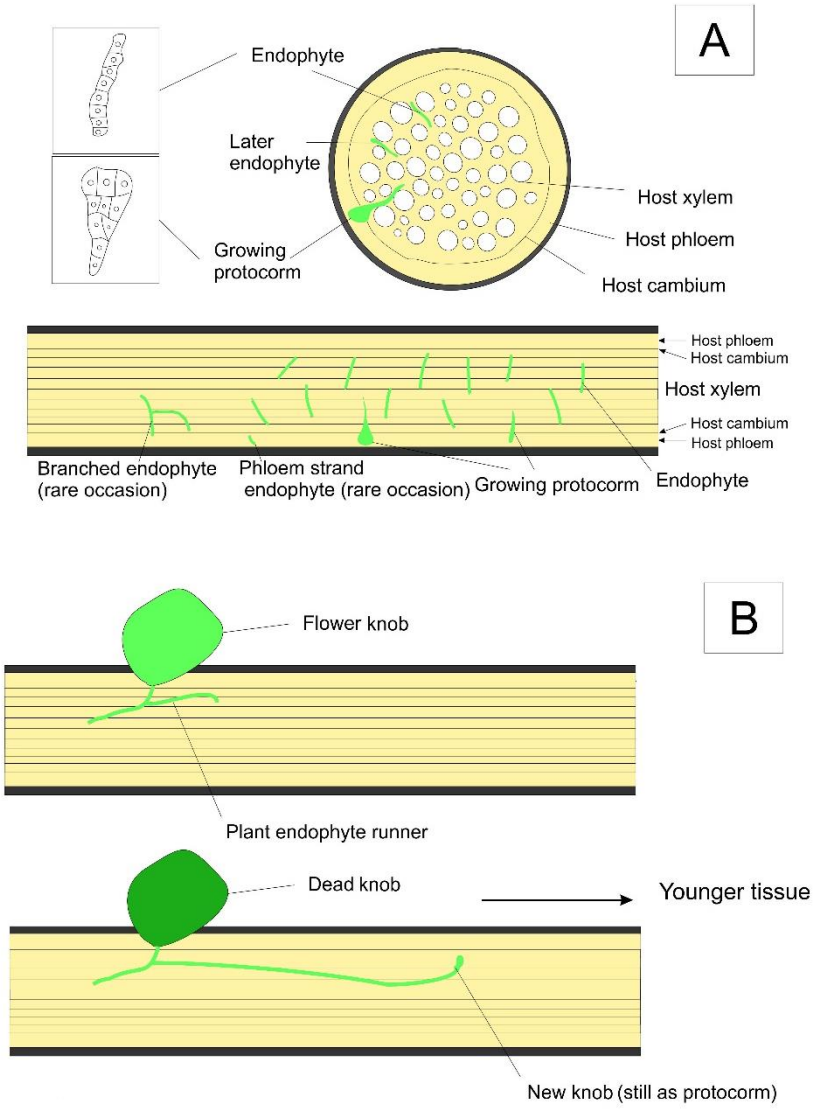

Fig. 1. Two hypotheses that currently describe the growth and distribution of *Rafflesia* endophytes: Anchored endophyte (A) (figure was inspired by NIKOLOV & al. (2014) and depicts a hypothetical growth form following personal discussion with Nikolov); a creeping endophyte as suggested by observations (this study) of a 10-year-old *Rafflesia patma* Blume (syn. *R. horsfieldii* R. Br. (1821)) – *Tetrastigma leucostaphylum* (Dennst.) Alston graft (B).

In this study, *Rafflesia patma* Blume (syn. *R. horsfieldii* R. Br. (1821)) was transplanted by veneer grafting [MURSIDAWATI & al. 2015] in 2007 using a scion from Pangandaran, West Java, Indonesia, and a rootstock from an uninfected host in Bogor Botanical Garden. *R. patma* first bloomed in 2012. The host was identified as *T. leucostaphylum*.

Since 2007, the original grafting spot has disappeared and has become covered by the convoluted stem of *T. leucostaphylum* vines. We made an approximated grafting site and pinned it as the center point of the distance measurement (Fig. 2). The distance of the knobs from the center point, diameter, and age of the knobs were measured (Tab. 1).

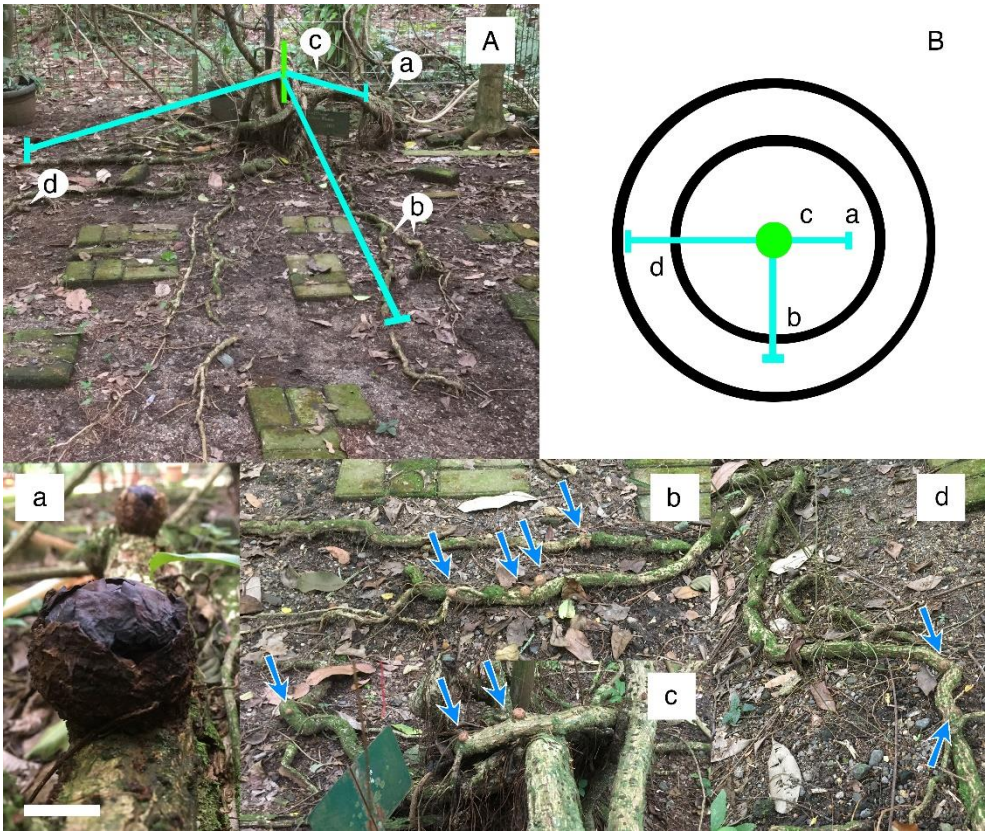

Fig. 2. Measurements of the host vine, *Tetrastigma leucostaphylum*, in Bogor Botanical Garden, Indonesia (A). Top view showing the center point (green) and distance (cyan) covered by the endophytic parasite, *Rafflesia patma* Blume (B). Spots in A have been enlarged in a-d for easier visualization. Flower primordial knobs (enlarged image in a) of *R. patma* are shown by blue arrows. Scale bar (in a): 2 cm.

Tab. 1. *Rafflesia patma* Blume (syn. *R. horsfieldii* R. Br. (1821)) flower knob distribution on/along the host vine (*Tetrastigma leucostaphylum* (Dennst.) Alston).

Year	Distance from grafting area (cm)	Flower/knob diameter (cm)	Knob age (months)	Notes
2017	26.5	2	3	
	27.5	1.8	3	
	62	2	3	
	142	3.5	5	
	163	1.7	3	
	167	4.5	6	
	177	2.5	3	
	182	1.7	2	
	188	3	3	
	191	2	3	
	203	1.2	3	
2015	3	44*	>12	Female flower
2014	58	38*	>12	Female flower
	53	44*	>12	Male flower
	95	34*	>12	Female flower, 6 perigones
2013	31	34*	>12	Female flower
2012	58	37*	>12	Female flower
	34	38*	>12	Female flower

Note: Numbers denoted by an asterisk (*) indicate a fully-grown flower rather than a knob (i.e., flower bud)

We observed that *R. patma* within a grafted host (*T. leucostaphylum*) grew away from its old original point in the transferred (i.e., infected) root into a healthy rootstock and stem of *T. leucostaphylum*, leaving behind the old and decayed host tissue. The growth of *R. patma* was shown to be distributed in several directions within *T. leucostaphylum* vines from its original point (Tab. 1, Fig. 2). This movement, which suggests active growth rather than passive sedentary placement and subsequent flowering, might indicate that *Rafflesia*, as an endophyte, in fact creeps inside its host, possibly to obtain more nutrients. HEIDE-JØRGENSEN (2008) stated, about *R. kerrii* Meijer, that flowers often appear on the younger part of *Tetrastigma*, suggesting that the endophyte grows away from older part before the tissue dies. Variation of the distance of flower knob formation in Table 1 also shows that new flowers do not always sprout far away from the grafting point, but might also sprout close to it. This either shows that the flower might grow from an already existing endophyte or from the reversed direction of endophyte growth (Fig. 3). All of these hypotheses related to the growth of the endophyte, as well as flower knob formation, will require detailed miscroscopic and even genetic studies to prove if the same endophyte is responsible for the formation of knobs close to and distant to the grafting point.

This paper provides distribution data for *R. patma* knobs growing on grafted *T. leucostaphylum* vines, suggests that the endophyte moves away in an active process from the point of origin, unlike current model that suggests sedentary growth, including flowering.

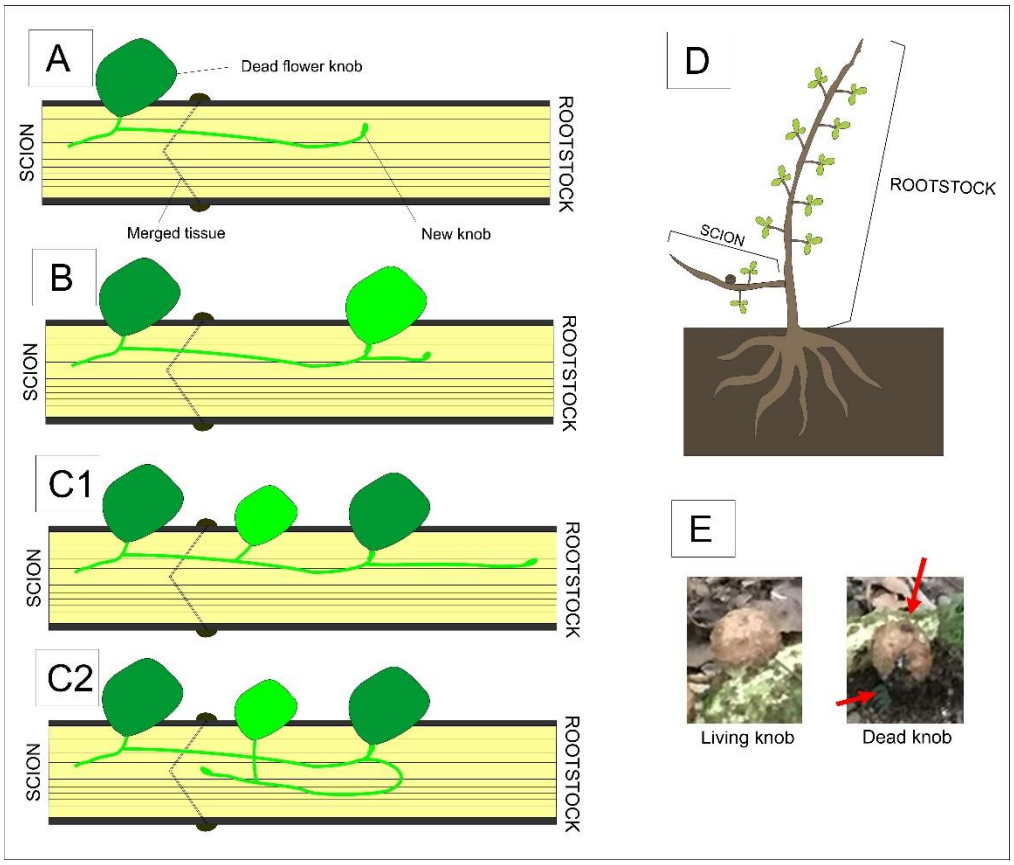

Fig. 3. Hypothetical growth and development of the *Rafflesia patma* Blume flower knob on its grafted host (*Tetrastigma leucostaphylum* (Dennst.) Alston) showing that the endophyte (green) grows towards the rootstock tissue (A) and develops a new flower knob (B). At a later stage, at some moment in time a new flower knob grows closer to the grafting site rather than growing away. Two possible scenarios are depicted: A new flower knob emerges from a previously established endophyte (C1), or the growing point of an endophyte turns back and forms a new flower knob (C2). The picture (D) shows a simplified illustration of a scion and rootstock in the grafted host plant, and (E) shows a living or newly emerged flower knob (left) and an aborted or dead knob (right) with red arrows pointing to the blotched and blackened dead tissues.

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