BIODIVERSITAS
Volume 26, Number 8, August 2025
Pages: 3791-3802

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d260812

Insect vectors of banana blood disease in Flores Island, East Nusa Tenggara, Indonesia

MAYAVIRA V. HAHULY[1,♥], I WAYAN MUDITA[1], AGNES V. SIMAMORA[1], PETRONELLA S. NENOTEK[1], SRI WIDINUGRAHENI[1], VIKTORIANUS JEHAUT[1], FADLAN PRAMATANA[2]
[1]Department of Agrotechnology, Faculty of Agriculture, Universitas Nusa Cendana. Jl. Adisucipto, Penfui, Kupang 85001, East Nusa Tenggara, Indonesia. Tel./fax.: +62-380-881085, ♥email: mayavira.hahuly@staf.undana.ac.id
[2]Department of Forestry, Faculty of Agriculture, Universitas Nusa Cendana. Jl. Adisucipto, Penfui, Kupang 85001, East Nusa Tenggara, Indonesia

Manuscript received: 15 November 2024. Revision accepted: 8 August 2025.

Abstract. *Hahuly MV, Mudita IW, Simamora AV, Nenotek PS, Widinugraheni S, Jehaut V, Pramatana F. 2025. Insect vectors of banana blood disease in Flores Island, East Nusa Tenggara, Indonesia. Biodiversitas 26: 3791-3802.* Banana Blood Disease (BBD) poses a significant threat to banana cultivation in Indonesia. Following severe outbreaks on Sumba Island, the disease spread to the neighboring Flores Island in mid-2022, with a particularly pronounced impact on the predominant cultivar, *Musa* sp. ABB 'Kepok plantain.' Despite prior outbreaks, awareness and understanding of the disease and its insect vectors remain limited among agricultural officers, village leaders, and farmers on Flores Island. The pathogen, *Ralstonia syzygii* subsp. *celebesensis* (Rsc), is known to be transmitted by insect vectors; however, no prior data existed regarding vectors on Flores Island. This knowledge gap has impeded effective disease management and allowed the disease to spread unnoticed. This study aimed to assess the spread and incidence of BBD and investigate potential insect vectors involved in its transmission. A survey conducted across five districts in western Flores confirmed the presence of BBD in all surveyed areas, with varying incidence rates among banana cultivars. The highest incidence (65.88%) was recorded in *Musa* sp. ABB 'Kepok plantain,' followed by *Musa* sp. AAB 'Raja Sereh banana,' *Musa* sp. AAA 'Barangan banana,' *Musa* sp. AAA 'Cavendish banana,' and *Musa* sp. BB 'Klutuk plantain.' Insect surveys identified eight species visiting male banana inflorescences. Among them, *Oscinella* sp. (Diptera: Chloropidae) and *Bactrocera* sp. (Diptera: Tephritidae) were the most widespread and abundant. These species have previously been reported as BBD vectors in other regions. They were likely key contributors to the spread of Rsc on Flores Island.

Keywords: Banana blood disease, Island of Flores, *Ralstonia syyzygii* subsp. *celebesensis*, survey method, transmission by insect

INTRODUCTION

Indonesia ranks among the top-10 global banana producers, yielding an average of 9.2 million metric tons annually between 2021-2023, primarily cultivated in Java, Sumatra, and Sulawesi (FAO 2024). However, Banana Blood Disease (BBD), caused by the bacterial pathogen has emerged as a critical threat. Initially devastating banana crops on Sumba Island (Mudita et al. 2018; Ray et al. 2021; Nampa et al. 2022), BBD recently spread to Flores Island, likely transmitted via contaminated harvesting tools used by inter-island traders (Mudita and Benu 2018; Ray et al. 2021, 2022a). Once introduced, BBD rapidly disseminated across Flores' districts, alarming farmers and governments due to bananas' role as a staple food during scarcity (Ray et al. 2021). Compounding this crisis, Flores has become an important supplier of bananas in inter-island trade because many other regions are experiencing production declines due to BBD and fusarium wilt (*Fusarium oxysporum* f. sp. *cubense*) (Drenth and Kema 2021). While Timor Island remains free of BBD (Henuk et al. 2020), the implementation of strict quarantine protocols is critical to prevent the introduction of the pathogen. Despite Indonesia's significant production, exports remain limited, with most bananas consumed domestically, underscoring their socioeconomic importance for food security and smallholder livelihoods (FAO 2024).

In Indonesia, BBD was found infecting different cultivars, but the one most damaged was 'Kepok plantain' (Mudita and Benu 2018; Nampa et al. 2022). 'Kepok plantain' was the host of the disease when it was first reported from Kayuadi and Tanahjampea, before spreading to the nearby small islands and the mainland of South Sulawesi Province (Buddenhagen 2007). There were speculations regarding why this cultivar was susceptible to blood disease, including its inherent genetics, flower morphology, nectar production, and the means of transmission of the bacteria causing the disease. Genetically, banana cultivars are relatively homogenous since they are vegetatively derived from the same wild ancestors, *Musa acuminata* (A) and *Musa balbisiana* (B). However, due to a long selection process in their cultivation, cultivars with B genome have distinct flower morphology compared to cultivars with A genome and produce more abundant nectar, attracting insects as well as nectar-eating birds and bats to visit their flowers (Buddenhagen 2007). The bracts and underneath flowers of the male bud shed-off quickly, leaving fresh scars open on the peduncle and allowing insects, birds, and bats visiting the flowers of a diseased individual to carry bacterial ooze discharging from the scars to healthy individuals they visit later (Buddenhagen 2007; Ray et al. 2021, 2022b).

The bacterium causing BBD was first identified in 1921 as *Pseudomonas* sp. but was later linked to *Ralstonia solanacearum* species complex, which causes moko and bugtok diseases. Initially classified by host range and metabolic properties, it was later divided into four geographic phylotypes and reclassified into three species: *R. solanacearum* (phylotype II from America), *Ralstonia pseudosolanacearum* (phylotypes I from Asia and III from Africa), and *Ralstonia syzygii* (phylotype IV from Indonesia) (Safni et al. 2014; Prior et al. 2016). *Ralstonia syzygii* was further divided into three subspecies based on phenotypic and genotypic traits: *R. syzygii* subsp. *syzygii*, causing sumatra disease of clove; *R. syzygii* subsp. *indonesiensis*, causing bacterial wilt in a wide range of solanaceous plants; and *R. syyzygii* subsp. *celebesensis* (Rsc), causing BBD (Safni et al. 2014, et al. 2018).

The transmission of BBD was previously hypothesized to be related to insects. Research conducted in the early 2000s revealed that Rsc had been isolated from insects such as *Trigona minangkabau*, *Erionota thrax* and other species. More rigorous studies later confirmed that several insect species were responsible for transmitting the bacterium causing BBD (Ray et al. 2021, 2022a). Since no such information was available for Flores Island, this study aimed to determine the diversity of insects associated with banana male flowers that have the capacity to transmit the bacterium to different banana accessions or cultivars, and the spread of BBD in the western part of Flores Island. By understanding the role of insects in transmitting the bacterium to each banana cultivar, more effective control measures can be implemented.

MATERIALS AND METHODS

Study area

This study was conducted as part of a collaborative research initiative addressing the biosecurity threats posed by BBD on Flores Island, East Nusa Tenggara, Indonesia. Focusing on the island's western region, where BBD outbreaks were widely reported by local and national media, the research spanned five districts: Nagekeo, Ngada, East Manggarai, Manggarai, and West Manggarai, arranged geographically from east to west (Figure 1). District selection was guided by a preliminary survey conducted in 2023, which identified a high prevalence of the disease in these areas while confirming the absence of BBD in the eastern districts of Ende and Sikka. Fieldwork, including GPS-mapped geospatial data collection and structured interviews, was conducted from July to October 2023.

Sampling design

The study employed a multi-stage sampling method to conduct a survey aimed at identifying banana accessions or cultivars with blooming male inflorescences, from which insects were collected. Initially, interviews were conducted at the district agricultural services offices to gather information on the distribution of BBD. Based on this information, three sub-districts with the most severe disease incidence were selected. Within each sub-district, two to three villages were chosen for further investigation. In each selected village, interviews were conducted with the village head and/or local leaders to obtain detailed information about the banana cultivars, their locations, disease symptoms, pathogen signs of BBD, and insects visiting banana inflorescences.

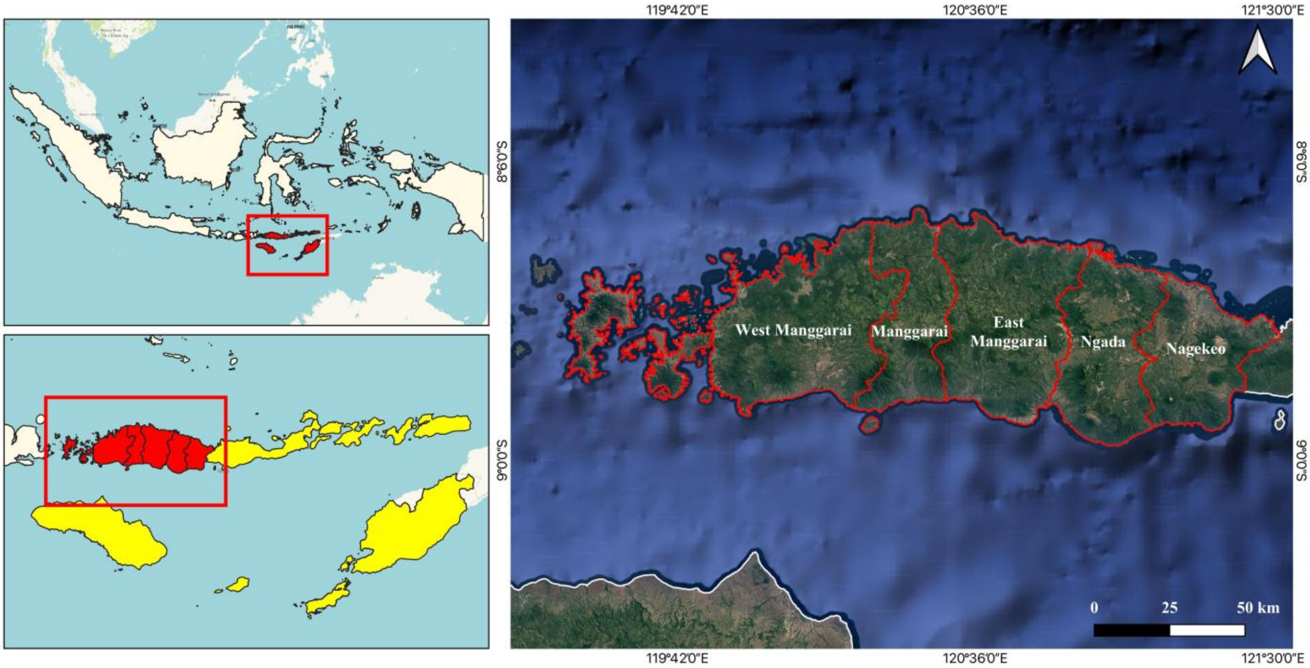

Figure 1. Districts selected as the site of the study in the western part of Flores Island, East Nusa Tenggara, Indonesia

Subsequent to the interviews, field visits were conducted to the specified locations, which included three to five farmer's fields per village. During these visits, the banana cultivars were identified, and the incidence of BBD was assessed. The disease incidence was determined by examining at least 30% of the banana population in each field sample. Additionally, insects visiting the male inflorescences were collected for further analysis. Banana cultivars identification was carried out based on morphological characteristics following the guidelines provided in "Morphological identification of banana plants: a basis for scoring, genome group determination, and research unit and sample unit identification" (Mudita 2023).

Insect collection and identification

Insects were collected from blooming male inflorescences of five banana cultivars, i.e., *Musa* sp. ABB 'Kepok plantain', AAA 'Barangan banana', BB 'Klutuk plantain', AAB 'Raja Sereh banana, and AAA 'Cavendish banana'. Large insects were captured using sweep nets, with pole extensions employed for tall pseudo-stems, the false stem formed by tightly packed leaf sheaths, (>2m height). Male peduncles were excised to facilitate insect capture within enclosed inflorescences. Small insects were collected using yellow sticky traps placed 20-30 cm from inflorescences and bottle traps attached to leaf petioles, both deployed during daylight hours (07:00-17:00).

Collected specimens were preserved in sterile containers, transported in cool-box, and delivered to Entomology Laboratory in Universitas Nusa Cendana for identification. Taxonomic classification was performed using stereo microscopy and validated against GBIF Backbone Taxonomy and contemporary taxonomic literature (Barthélémy et al. 2014; Barthélémy 2021).

Equipment specifications

The Table 1 lists the specific equipment used during the field collection, specimen processing, and laboratory identification stages of the study.

Detailed field collection and trapping protocols

Active collection procedure: The collection process was adapted based on the height of the pseudo-stem of the banana. ***For tall pseudo-stems:*** The handle of the sweep net was attached to the distal end of a retractable aluminum pole. The pole was then carefully extended to position the net over the male inflorescence, enveloping it. A second pole, with a knife affixed to its end, was used to cut the male peduncle. The entire net, now containing the male bud and trapped insects, was lowered for specimen collection. ***For short pseudo-stems:*** The use of extension poles was unnecessary. The male inflorescence was directly enclosed with the sweep net, and the peduncle was cut manually to collect the insect inside.

Passive trapping procedure: To supplement active collection, passive traps were deployed around the inflorescences of the target banana cultivars: *Musa* sp. ABB 'Kepok plantain', *Musa* sp. AAA 'Barangan banana', *Musa* sp. BB 'Klutuk plantain', *Musa* sp. AAB 'Raja Sereh banana', and *Musa* sp. AAA 'Cavendish banana'. (i) One yellow sticky trap and one bottle trap were positioned near each male inflorescence. (ii) Traps were hung from leaf petioles, using aluminium poles where necessary to achieve the correct height. They were positioned to be level with the male bud at approximately 20-30 cm. (iii) The sticky traps were placed in the morning (approximately 07:00) and retrieved in the late afternoon (approximately 17:00) to capture diurnally active insect. Upon retrieval, the traps were examined, and all captured insects were carefully collected.

Following collection, each distinct insect species was documented. ***Photography:*** A 100-mm macro lens kit attached to a cell-phone via a multi-function clip was used to photograph each species for a detailed visual record. ***Preservation and transport:*** Two specimens of each insect species were preserved, either in a sterilized bottle or a test tube. For transport from the field site, these containers were placed in a cool box to maintain specimen integrity. The box was then flied to the Entomology Laboratory at the Faculty oof Agriculture, Universitas Nusa Cendana, Kupang. ***Identification:*** In the laboratory, detailed morphological examinations were conducted using an Olympus SZ51 stereo microscope. Identification was performed by cross-referencing observations with established taxonomic resources, including: (i) The Global Biodiversity Information Facility (GBIF) Backbone Taxonomy Checklist Dataset. (ii) Provisional distributional checklist of Hong Kong social wasps (Hymenoptera: Vespidae: Vespinae, Polistinae, Stenogastrinae) (Barthélémy et al. 2014). (iii) Identification Guide to Social Wasps of Hong Kong (Hymenoptera: Vespidae) (Barthélémy 2021).

Interactive map link

Data from the interactive link map was used for creating Geographic distribution map of blood disease in 12 banana cultivars/accessions found on the western part of Flores Island presented in Figure 7. The link is https://www.google.com/maps/d/u/0/edit?mid=1enf43hZnRfQNStEfiyuSOJtIbWV4H2w&usp=sharing.

Identification of banana Blood Disease Bacteria (BDB)

Identification of BDB was conducted on specimens from diseased banana cultivars. Specimen collection and isolation followed the procedures described by Ray et al. (2021, 2022b). Diseased plant tissues, specifically symptomatic banana fruits, were collected and transported in a cool box from Flores Island to Kupang by plane.

Specimen processing and taxonomic identification

The symptomatic fruit was then surface sterilized by first washing it to remove dirt and then drying it with tissue paper. The fruit was then sprayed with 70% ethanol until run-off and dried again using sterile tissue paper. After sterilization, the fruit was cut into small pieces measuring approximately 5-10 mm. Two pieces of the tissue were placed into a test tube containing 2-3 mL of sterile water and gently shaken to allow the bacteria to diffuse into the water. Using a sterile loop, the resulting suspension was streaked onto Nutrient Agar (NA) media and incubated for 24 hours at room temperature. One of the bacteria colonies that appeared was then isolated and grown as a pure culture.

Table 1. Equipment for field collection, processing, and laboratory identification of banana-inflorescence insects

Equipment category	Item descriptions	Purpose
Active collection	Retractable aluminum pole	To extend the reach of the sweep net knife for tall pseudo-stems.
	Sweep net	Primary tool for capturing large insects from inflorescences.
	Knife attached to a retractable aluminum pole	To cut the male peduncle of tall plants, facilitating insect collection.
Passive trapping	Yellow sticky plastic sheet traps	To capture small flying insects attracted to the yellow color.
	Bottle traps	To passively collect insects around the inflorescence
Specimen processing	Multi-function lens clip with a 100-mm macro lens kit for a cell phone	To take high-resolution, close-up photographs of each insect species for morphological documentation.
	Sterilized bottles and test tubes	For the secure and sterile containment of collected insect specimens.
	Cool box	For temperature-controlled local transport of specimens to prevent degradation.
Laboratory analysis	Olympus SZ51 stereo microscope on an SZ2-ILST Slim LED Base	For detailed morphological observation and taxonomic identification of specimens.

The bacterial isolate was tested for pathogenicity using a Hypersensitive Response (HR) assay on tobacco leaves. A bacterial suspension from a 24- to 48-hour culture was carefully infiltrated into the leaves. Necrosis within 48 hours indicated pathogenicity, as pathogenic bacteria trigger rapid cell death in tobacco leaves.

Molecular identification of the BBD bacterium was performed using the primers 121F (5'-AAG TTC ATT GGT GCC GAA TCA-3') and 121R (5'-CGT ATT GGA TGC CGT ATT GGA-3') following Ray et al. (2021). Genomic DNA was extracted using the Quick-DNA Magbead Plus Kit (Zymo Research, D4082), ensuring high-quality DNA for downstream applications. PCR amplification was carried out with the (2x) MyTaq HS Red Mix (Bioline, BIO-25048) to selectively amplify the target DNA region. Finally, bi-directional sequencing was performed to obtain accurate nucleotide sequence data for molecular identification.

Data analysis

All resulting data were tabulated using Excel spreadsheets. Interview data were analyzed quantitatively to describe knowledge about BBD and the role of insects in the transmission of the pathogen. Quantitative data on insects visiting banana inflorescences was analyzed for each cultivar using descriptive quantitative analysis. All the necessary quantitative analyses were performed using the open-source statistical computing environment R (version 4.3.2; R Core Team 2013), run via the RStudio integrated development environment (RStudio Team 2021). Pearson correlation analysis was used to evaluate the relationship between insect species richness and BBD incidence.

RESULTS AND DISCUSSION

The findings of this research elucidated the role of insects in the transmission of BDB as well as the current distribution and spread of BBD on Flores Island.

Knowledge about banana blood disease and the role of insects in its transmission

Discussions with district agricultural officers in each sample village revealed that all officers became aware of BBD only after it was detected in bananas within their district. While they knew about the banana cultivars that were infected, they were uncertain about the role of insects in transmitting the disease. Similarly, village leaders and farmers knew about the disease and banana accession or cultivar infection after receiving information from the district agricultural services and observing diseased bananas. However, not all of them could accurately identify the symptoms and signs of the pathogens related to the disease. Table 2 synthesizes responses from district agricultural officers, village leaders and farmers interviewed during fieldwork, highlighting variations in knowledge about BBD symptoms, insect vectors, and cultivar susceptibility.

Table 2 shows that knowledge about BBD and the role of insects in the transmission of the disease varies among interviewees and between different aspects of the disease and the role of insects. This variation indicates that their encounter with the disease is new, and not much effort is made to obtain information about the disease, even though the disease destroyed bananas on Sumba Island earlier. Such a lack of detailed knowledge about the disease and the role of insects in its transmission allows the disease to spread unnoticeably.

The limited awareness of BBD symptoms and insect transmission pathways (Table 2) aligns with studies showing that delayed recognition of emerging plant diseases exacerbates epidemics (Jones 2021). For instance, in Fusarium wilt outbreaks, poor farmer recognition of early symptoms led to uncontrolled spread (Drenth and Kema 2021). Similarly, the lack of consensus on insect vectors in this study mirrors challenges in managing Xanthomonas wilt of banana, where pollinating insects were overlooked as vectors until later stages (Tinzaara et al. 2016).

The respondents’ focus on ‘Kepok plantain’ as the most susceptible cultivar suggests a need for cultivar-specific management strategies, as seen in BBD-prone regions (Ray et al. 2022a). Furthermore, the uncertainty about insect vectors emphasizes the urgency of vector ecology studies to inform containment protocols, as demonstrated in managing bacterial wilt in *Capsicum* spp. (Thakur et al. 2021).

Insects found on male inflorescences

Eight species of insects belonging to three orders, namely Diptera, Dermaptera, and Hymenoptera, were found visiting banana inflorescences. Those insects were *Oscinella* sp. (Diptera: Chloropidae), *Musca domestica* (Diptera: Muscidae), *Bactrocera* sp. (Diptera: Tephritidae: Dacinae), *Chelisoches morio* (Dermaptera: Chelisochidae), *Vespa affinis* (Hymenoptera: Vespidae), *Vespa velutina* (Hymenoptera: Vespidae), *Polistes rothneyi* (Hymenoptera: Eumenidae), and *Dolichoderus thoracicus* (Hymenoptera: Formicidae) (Figure 2). From those eight insect species, *V. affinis*, *V. velutina*, and *P. rothneyi* are pollinator insects.

The banana cultivar visited by the most diverse insects was *Musa* sp. ABB ‘Kepok plantain’ followed by *Musa* sp. AAB ‘Raja Sereh banana’, *Musa* sp. AAA ‘Barangan banana’ as well as *Musa* sp. AAA ‘Cavendish banana’ and *Musa* sp. BB ‘Klutuk plantain’ (Figure 3). *Oscinella* sp. and *Bactrocera* sp. were consistently trapped in *Musa* sp. ABB ‘Kepok plantain’ in all 15 surveyed villages. These were followed by *C. morio*, detected in 11 villages, and *V. affinis* and *V. velutina*, both observed in ten villages. The insect species visiting the most diverse banana cultivars were *Oscinella* sp. and *Bactrocera* sp., each found on all five major banana cultivars, followed by *V. velutina*, which was observed on three cultivars. In contrast, *P. rothneyi* was the most host-restricted, found only on Musa ABB 'Kepok plantain' in three villages. The four other insect species were found visiting two different cultivars.

Table 2. Knowledge of agricultural officers, village leaders, and farmers about Banana Blood Disease (BBD) and the role of insects visiting banana inflorescences in transmitting the disease in the districts of Nagekeo, Ngada, East Manggarai, Manggarai, and West Manggarai, the Province of East Nusa Tenggara, Indonesia

Knowledge aspect	District agricultural officers	Village leaders and farmers
Disease symptoms and signs	All knew symptoms, some knew signs	Some knew symptoms, no signs
Diseased banana accessions/cultivar	Mostly ‘Pisang Kepok’, some others	Mostly ‘Pisang Kepok’, some unsure
Insect species visiting inflorescences	Common names only, no scientific names	Local names only, no scientific names
Insects visited only male or both female and male inflorescences	None was sure	Mixed responses, some unsure
Purposes of insects visiting inflorescences	Mostly nectar, some unsure	Mostly unsure, few mentioned nectar
The ability of insects to transmit blood disease	Some suspected, others unaware	None aware
Reasons for transmission suspicion	Disease starts at bunch, not pseudo-stems	-

Source: Interview data collected in this research (2023)

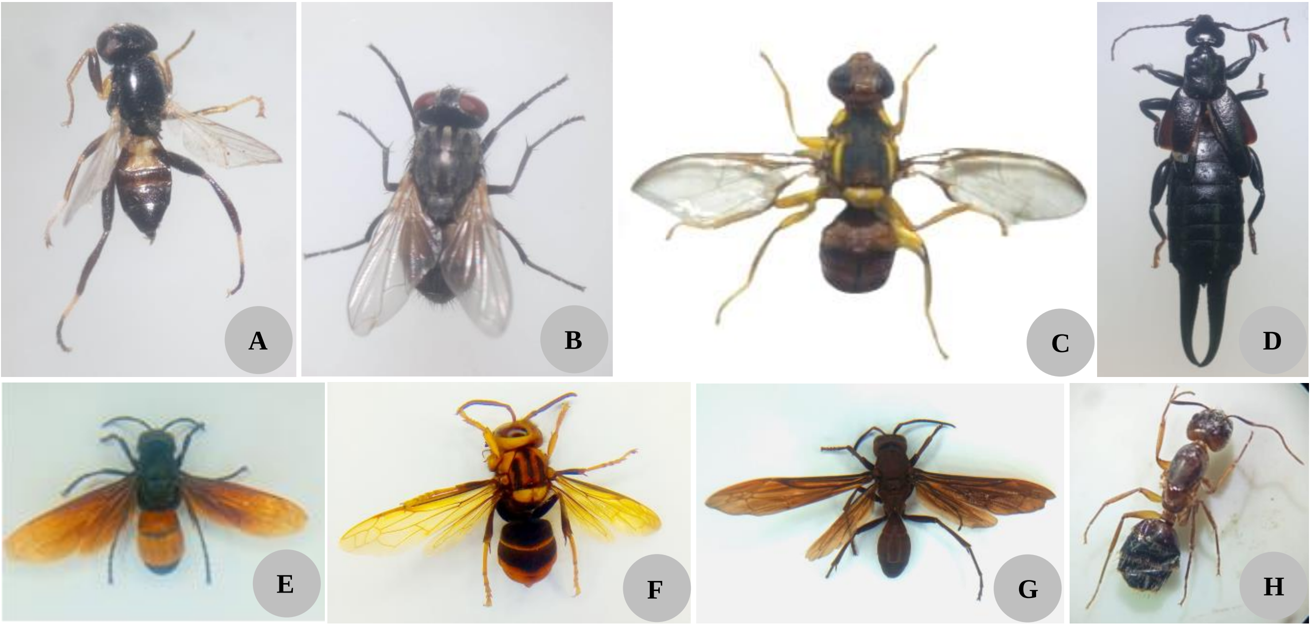

Figure 2. Eight species of insect found visiting banana male flowers in Nagekeo, Ngada, East Manggarai, Manggarai, and West Manggarai Districts in Western part of Flores Island, Indonesia. A. *Oscinella* sp. (Diptera: Chloropidae), B. *Musca domestica* (Diptera: Muscidae), C. *Bactrocera* sp. (Diptera: Tephritidae: Dacinae), D. *Chelisoches morio* (Dermaptera: Chelisochidae), E. *Vespa affinis* (Hymenoptera: Vespidae), F. *Vespa velutina* (Hymenoptera: Vespidae), G. *Polistes rothneyi* (Hymenoptera: Eumenidae), H. *Dolichoderus thoracicus* (Hymenoptera: Formicidae)

Insects play a significant role in spreading bacterial pathogens that cause plant diseases. Research on insect species capable of carrying and transmitting the BDB has been conducted since 2003. Leiwakabessy (2003) reported that certain Diptera families (Chloropidae, Platypezidae, and Drosophilidae) carried the BDB both internally and externally, while other Diptera families (Tephritidae, Dolichopodidae, Culicidae, Calliphoridae, Anthomyiidae, and Muscidae), along with insects from the orders Lepidoptera (Coleophoridae), Hymenoptera (Apidae), and Blattaria (Blattidae), carried the BDB externally. Mairawita et al. (2012) identified *T. minangkabau* (Hymenoptera: Apidae) and *Drosophila* spp. (Diptera: Drosophilidae) as the dominant visitors of banana flowers in West Sumatra; both species were capable of harboring BDB internally and externally. Later, Sahetapy et al. (2020a) confirmed that Diptera families (Drosophilidae, Tephritidae, and Muscidae) carried the BDB both internally and externally. More recently, Montong and Salaky (2019) demonstrated that *Oscinella* sp. (Diptera: Chloropidae), *Apis mellifera* (Hemiptera: Aphididae), *C. morio* (Dermaptera: Chelisochidae), and *Dolichoderus* sp. (Hymenoptera: Formicidae) could transmit the BDB. This collective research underscores the diverse range of insect species involved in the transmission of BBD.

Referring to Leiwakabessy (2003), Sahetapy et al. (2020a, b) and Montong and Salaky (2019), five insect families identified in our study have been reported as capable of transmitting Rsc. These families include Diptera: Chloropidae (*Oscinella* sp.), Diptera: Muscidae (*M. domestica*), Diptera: Tephritidae (*Bactrocera* sp.), Dermaptera: Chelisochidae (*C. morio*), and Hymenoptera: Formicidae (*Dolichoderus* sp.). Among these insects, *Oscinella* sp. and *Bactrocera* sp. was not only found on the inflorescences of *Musa* sp. ABB 'Kepok plantain' across all 15 surveyed villages but was also observed visiting other banana cultivars. These include *Musa* sp. AAB 'Raja Sereh banana' and *Musa* sp. AAA 'Cavendish banana' in two villages, *Musa* sp. BB 'Klutuk plantain' in one village, and *Musa* sp. AAA 'Baranga banana' in four villages.

While our findings highlight insects with high vector potential, future studies should isolate Rsc from collected specimens and validate transmission via controlled inoculation experiments following Koch's postulates. Sahetapy et al. (2020b) confirmed that *Trigona* spp. and *E. thrax* mechanically transmit Rsc under experimental conditions. Additionally, Montong and Salaki (2020) demonstrate that *Oscinella* sp., *A. mellifera*, *C. morio*, and *Dolichoderus* sp. can carry and transmit *R. solanacearum* Phylotype IV in banana, supporting our hypothesis that similar insects observed in our study may act as vectors in Flores agroecosystems. Although the pathogen was not directly detected, the insects identified are considered priority targets for biosecurity measures. These measures include restricting the movement of contaminated tools and insects to prevent the spread of BBD, as recommended for other Ralstonia pathosystems (Prior et al. 2016).

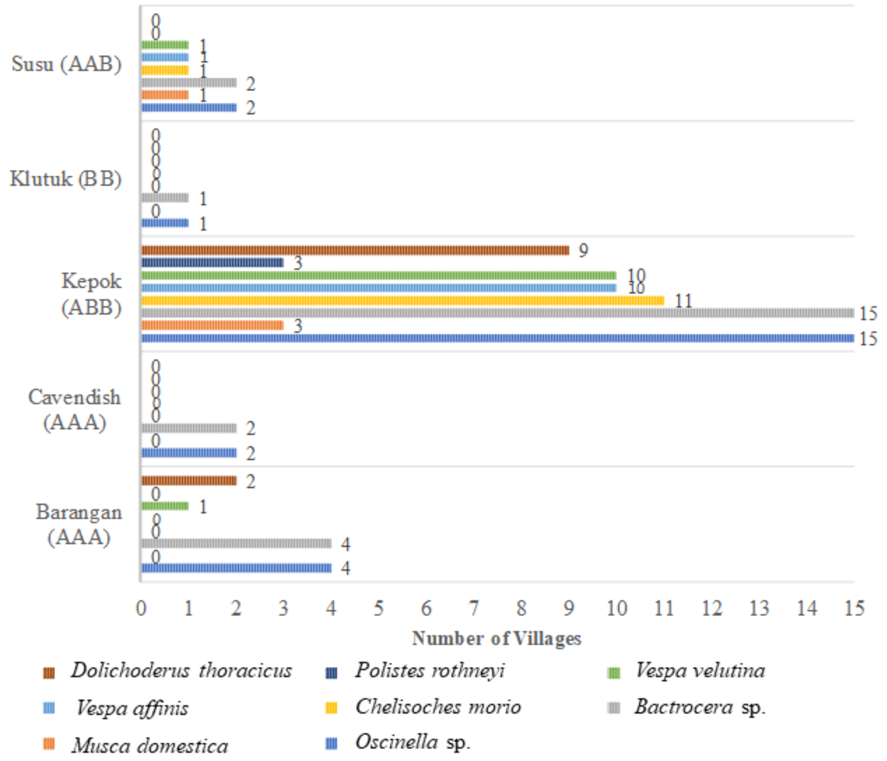

Figure 3. Diversity of insect visiting 5 banana accessions in 15 villages of observation sites in Nagekeo, Ngada, East Manggarai, Manggarai, and West Manggarai Districts, East Nusa Tenggara, Indonesia

Characteristics of infected plants, male inflorescences, and fruits

Symptoms of blood disease in bananas initially appeared on the leaves, which started to turn yellow from the edges. Symptoms spread to all parts of the leaf, and then the leaf hung and dried (Figure 4.A). Infected male inflorescences remained to look normal from the outside, but when cut open, they showed brownish-yellow spots and were soft and slimy (Figure 4.B). At an advanced stage, the male inflorescences dried out, and even included all the young fruit bunches (Figure 4.C). These symptoms were found in various cultivars of bananas with blood disease. Necrosis symptoms were also found in the inner part of the bunch stalk (Figure 4.D). The fruit bunches looked normal, but when cut open the inside part of the fruit was slimy and blackish-brown in color. Signs of blood disease were found in the form of suspensions or colonies of bacteria mixed with white sap, and some are reddish brown in color (Figure 4.D).

These symptoms and signs were most apparent in the *Musa* sp. ABB 'Kepok plantain', whereas symptoms and signs in other infected banana cultivars were found to be slightly varied with respect to wilting of leaves, drying of male inflorescences and young fruit bunches, and color of the affected parts. The findings align with earlier studies by Mudita et al. (2018), and Ray et al. (2021, 2022b), which documented similar symptoms and signs of BBD. Bakar et al. (2018) reported that the disease in Malaysia progresses through four distinct stages, beginning with external symptoms such as leaf wilting and yellowing and advancing to internal damage and plant death. The study also highlighted the host specificity of BDB which infecting banana exclusively. That specificity differentiating Rsc from *R. solanacearum*, which infects a broader host range.

Pathogenic bacteria were isolated from symptomatic banana fruit. The isolates showed a positive reaction in the hypersensitivity test on tobacco leaves. Colonies on Nutrient Agar medium were whitish to creamy in color, raised small circular in shape (Figure 5.A), and Gram-negative. This was similar to the BBD colony that grew in a Casamino Peptone Glucose (CPG) agar medium reported by Ho et al. (2023). The molecular detection of pathogenic bacteria from banana samples exhibiting typical symptoms of blood disease, using specific primers (Ray et al. 2021; Prakoso et al. 2022), yielded positive results indicated by the presence of a 317 bp amplicon (Figure 5.B). This finding is further supported by the results of Lada (2024), who reported that 20 out of 22 sampling points across four of the five districts included in this study (Nagekeo, Ngada, Manggarai Timur, and Manggarai) tested positive when amplified using the same primer pair specific to the blood disease bacterium. Although a positive control was not shown in the gel electrophoresis image presented here, the high specificity of the primers and the consistent amplification across multiple independent samples provide strong evidence for accurate detection of the pathogen. Moreover, the broad molecular confirmation across diverse geographic locations within the study area reinforces the representativeness and validity of using a single sequenced isolate as a molecular reference in this study. BLAST results against NCBI Database showed that the isolate's sequences were in grouped with four isolates of *R. syzygii* and blood disease bacterium (Figure 5.C). The sequence of the isolate Bbd-F-1 has been submitted to the NCBI and assigned the accession number PRJNA1281186.

The current distribution and spread of bbd in Flores Island

Exploration activities conducted in the western part of Flores Island identified 15 banana cultivars and one close relative, *Ensete glaucum*. The cultivars included Ambon Hijau, Ambon Putih, Baranga Merah, Baranga Kuning, Meja, Kepok, Klutuk, Lampung, Mas, Raja, Raja Sereh, Cavendish, Tanduk, Tembaga Hijau, and Tembaga Merah. Among these, BBD symptoms were detected in all banana cultivars except Tanduk, Mas, and Lampung, as well as in *E. glaucum*, suggesting varying levels of susceptibility. The incidence of blood disease varied across banana cultivars, with the highest observed in *Musa* sp. ABB 'Kepok plantain', which recorded an average infection rate of 65.88% (Figure 6). A survey conducted across five districts administrative areas revealed that BBD was present in each district, although the incidence varied among cultivars in different districts (Figure 7).

Figure 4. Symptoms of blood disease in bananas: A. Early symptom appears as yellowing of young leaves starting from the edges toward the midrib, followed by leaf desiccation, B. Male inflorescences initially asymptomatic, later develop central black spots accompanied by internal fluid accumulation and basal yellowing, C. Advanced stage: Dried male inflorescences on banana bunches, D. Bunches may appear healthy externally, but fruits show browning with white and brownish bacterial ooze, and necrosis at the inner stalk edge

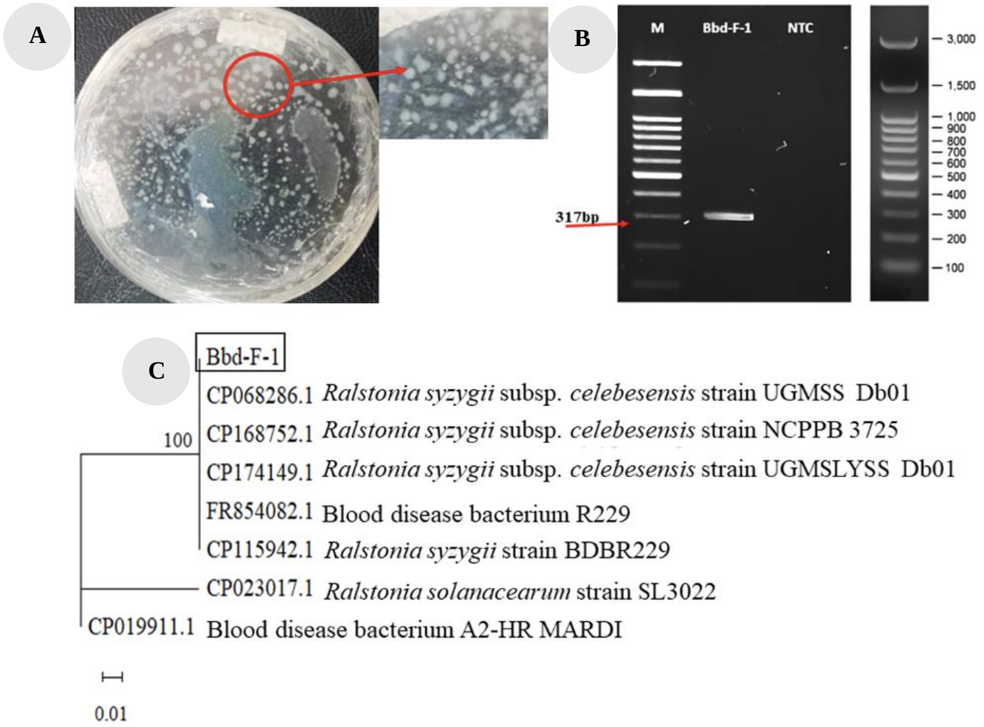

Figure 5. Characterization of bacterial isolates from bananas with symptoms of blood disease. A. Isolate aged 48 hours on Nutrient Agar media, B. PCR amplification using specific primer 121F/121R of Bbd-F-1 samples at 317bp, C. Phylogenetic relationship of isolate Bbd-F-1 with *Ralstonia syzygii* and blood disease bacterium strains based on BLAST results in the NCBI Database, constructed in MEGA12 using the Neighbor-Joining Method with 1000 bootstrap replicates

The highest average incidence across all cultivars was recorded in East Manggarai District. Additionally, a relatively high incidence was observed in the eastern border of the easternmost surveyed Nagekeo District, suggesting that the disease is spreading eastward to other districts, including Ende, Sikka, and East Flores. This spread poses a significant threat to banana production in the region, as the disease is known to reduce yields substantially. Studies reviewed by Jacobsen et al. (2019) indicate that BBD can cause severe yield losses, with average crop losses exceeding 35%. In some cases, losses have reached up to 80% in South Sulawesi and 36% in West Java. This underscores the potential impact of the disease on banana production in Flores Island and highlights the urgent need for effective management strategies. To investigate the possible relationship between insect diversity and BBD spread, a Pearson correlation analysis was conducted using data from 15 sample villages across five districts. The analysis yielded a moderate positive correlation between insect species richness and BBD incidence (r: 0.34), but was not statistically significant (t: 1.304, df: 13, p: 0.216), indicating that the observed trend could be due to random variation.

General discussion

This study showed that BBD was spreading across various banana cultivars in the western region of Flores Island. The disease was particularly damaging to the *Musa* sp. ABB ‘Kepok plantain’ cultivar, which has previously been identified as highly susceptible to BBD in Indonesia (Ray et al. 2022b). Eight insect species were found associated with banana male inflorescences; among them, *Oscinella* sp. (Diptera: Chloropidae) and *Bactrocera* sp. (Diptera: Tephritidae) being the most widespread and abundant. Their consistent presence across all 15 surveyed villages, coupled with their association with diverse banana cultivars, strongly suggests their potential role as vectors in BBD transmission.

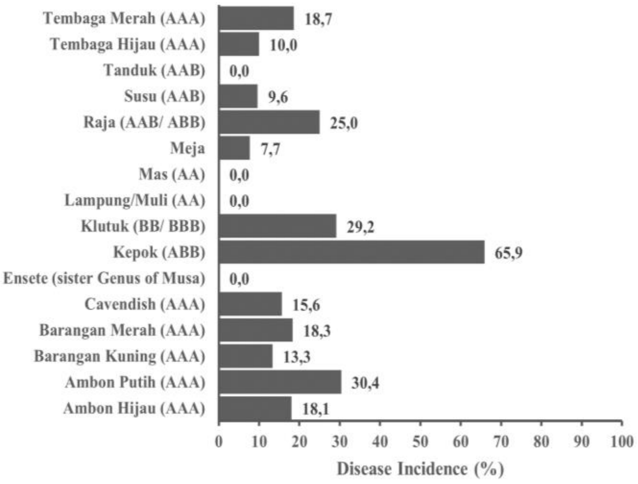

Figure 6. Average of blood disease incidence (%) in 15 banana cultivars and one banana-relative found from the survey conducted in the western region of Flores Island, East Nusa Tenggara, Indonesia

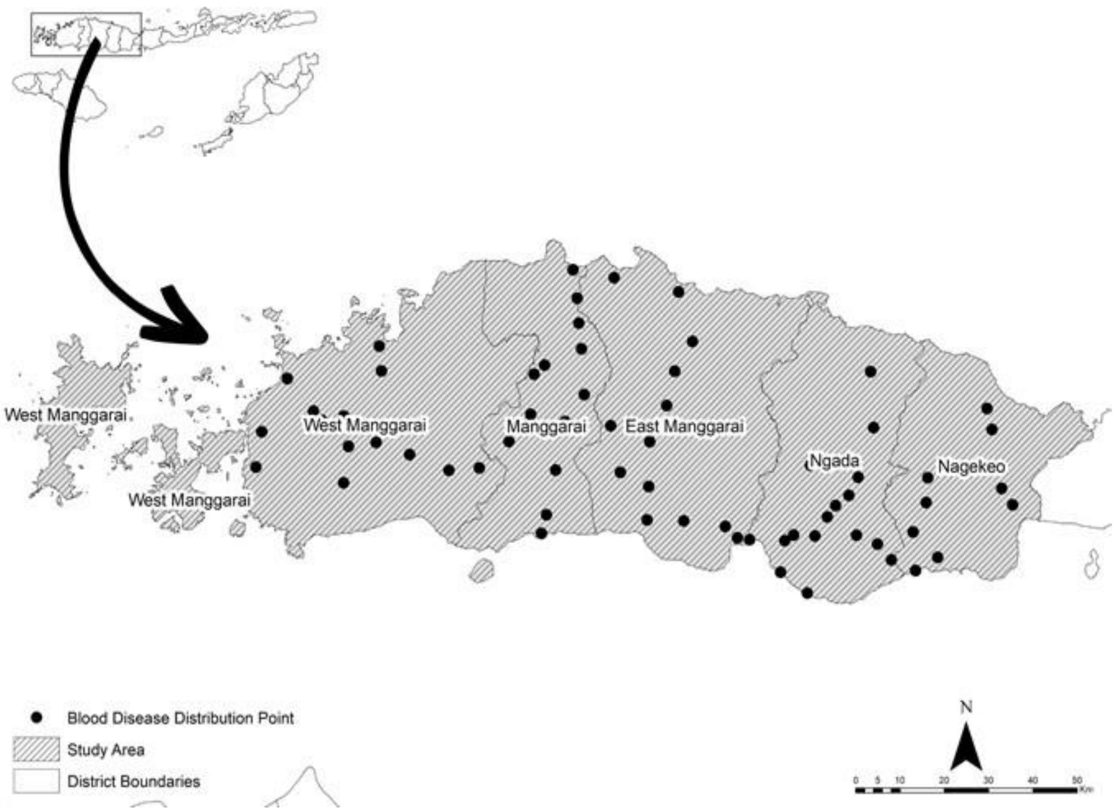

Figure 7. Geographic distribution map of blood disease in 12 banana cultivars/accessions found on the western part of Flores Island, East Nusa Tenggara, Indonesia

Previous research by Leiwakabessy (2003), Montong et al. (2015), Montong and Salaky (2019), and Sahetapy et al. (2020a, b) confirmed the presence of Rsc isolated from *Oscinella* sp., *C. morio*, *Dolichoderus* sp., *Polistes* sp., *Vespa* spp., and *Drosophila* sp. Considering the characteristics of insect species associated with banana male inflorescences, these insects remain the most likely vectors of BBD. Notably, the Vespa species identified in this study are widely distributed throughout Indonesia, including Flores Island (Barthélémy et al. 2014; GBIF Secretariat 2023a, b). These species rely on floral resources for carbohydrates and, importantly, possess the capacity for long-distance flight (Sauvard et al. 2018; Barthélémy 2021). However, we acknowledge that this study did not experimentally demonstrate insect-mediated transmission of Rsc. The proposed vector roles remain based on correlative observations and prior reports (Leiwakabessy 2003; Montong and Salaky 2019; Sahetapy et al. 2020a, b). To strengthen this hypothesis, future research should prioritize controlled inoculation and transmission experiments that satisfy Koch's postulates to confirm the vector competence of these insect species in the spread of BBD.

The likelihood of insect attraction may also be influenced by the Volatile Organic Compounds (VOCs) emitted by banana cultivars. Berhal et al. (2017) highlighted that different banana cultivars emit distinct compositions and levels of VOCs. The Cavendish cultivar (AAA) was found to produce 11 VOCs at relatively low emission levels, with (E,E)-α-farnesene and methyl salicylate being the most prominent. In contrast, the Pacific Plantain cultivar (ABB) emitted 14 VOCs at significantly higher levels, with (Z,E)-α-farnesene, (E,E)-α-farnesene, and (E)-β-ocimene dominating. These VOCs are known to attract a variety of insect species, including pollinators and pests, as they mimic floral or fruity aromas that appeal to insects seeking food or breeding sites. The higher diversity and emission levels of VOCs in the Pacific Plantain, including the ABB genome 'Kepok plantain', likely explain its ability to attract a broader range or greater abundance of insect visitors compared with the Cavendish cultivar or the AAA genome. Similarly, Masriany et al. (2020) found that α-pinene was the most dominant metabolite in both symptomatic and non-symptomatic banana flowers infected with Rsc, with higher production in symptomatic flowers. Alpha-pinene is believed to attract insect vectors of Banana Blood Disease (BBD), as evidenced by the significantly higher preference of *Drosophila* sp., *Rhodesiella bhutanensis*, and *Musca* sp. for α-pinene compared with ethanol as the control.

The moderate but statistically non-significant positive correlation (r: 0.34, p: 0.216) between insect species richness and BBD incidence indicates a possible ecological relationship between insect visitation and disease spread. While this correlation did not reach statistical significance, likely due to the limited sample size or unaccounted environmental factors, the trend suggests that greater insect diversity could be associated with increased opportunities for disease dissemination. Previous findings have established that diverse insect taxa, particularly from Diptera and Hymenoptera, can act as mechanical or biological vectors of Rsc (Leiwakabessy 2003; Mairawita et al. 2012; Montong and Salaky 2019; Sahetapy et al. 2020a). Our findings are consistent with this, especially given the widespread occurrence of *Oscinella* sp. and *Bactrocera* sp. in infected sites. The consistent detection of these insects across all villages and cultivars reinforces the hypothesis that vector presence is integral to disease dissemination. While a causal relationship could not be firmly established, it is plausible that multiple insect species perform similar functions in BBD epidemiology, either through direct bacterial carriage or by facilitating pathogen entry via floral injury.

Given the potential for insects to contribute to the spread of BBD, especially during the anthesis period when floral parts are most attractive, their role warrants more detailed study. Future research should employ larger datasets, controlled transmission assays, and geospatial modeling to clarify the dynamics between insect vectors and BBD spread. Disease management strategies should consider vector monitoring and control as part of integrated banana health management programs in Flores Island and beyond.

The devastation of banana crops by BBD, particularly in the ‘Kepok plantain’ cultivar, and the role of insects in pathogen transmission raised several critical research questions. Similar to outbreaks reported in Sumba Island (Mudita and Benu 2018; Nampa et al. 2022), the loss of ‘Kepok plantain’ threatened local banana biodiversity. Because this cultivar dominated fruit production for inter-island trade, its decline would have directly reduced income for local communities.

Disease management conducted by local Government in each surveyed district have included the eradication of infected banana plants and soil treatment with Trichoderma-based bio-fungicides. However, the eradication strategy appears overly ambitious, given the high density of banana populations and the labor-intensive process of felling or injecting each infected plant with herbicides. Field observations revealed that infected banana pseudo-stems and fruits remained fresh and exuded bacterial ooze for over two weeks after falling, indicating that disease reservoirs persisted in the field. Moreover, treating soil with *Trichoderma* is unlikely to halt disease transmission, as insects, birds, and bats play a significant role in disseminating Rsc. Considering these findings, a shift in management strategy is recommended, moving away from eradication, and focusing instead on preventing pathogen spread. Effective measures include covering banana bunches and removing male inflorescences, which serve as key entry points for insect-mediated infection. The use of the ‘Kepok Tanjung’ cultivar, a Musa ABB ‘Kepok plantain’ variant that naturally lacks male inflorescences, is particularly promising, as it has been reported to be less susceptible to insect-borne Rsc transmission (Hermanto et al. 2013).

Complementary disease mitigation methods involved the use of insect repellents derived from essential oils (citronella, ginger, basil) shown to deter *Drosophila melanogaster* (Anggraeni et al. 2018), and liquid smoke from coconut shells or pinecones that enhanced resistance to Rsc by boosting ethylene, auxin, lignin synthesis, and defense enzyme activity (Aisyah et al. 2018). Recent findings by Sam et al. (2024) suggested that *Lacticaseibacillus paracasei* and *Lactiplantibacillus plantarum*, lactic acid bacteria isolated from fermented milk and kimchi, could also be employed as potential biocontrol agents against Rsc.

However, successful intervention depends heavily on early detection of infection. Since BBD is typically confirmed only after symptom emergence, there is a critical need for rapid diagnostic tools that detect Rsc during early or asymptomatic stages. Talib et al. (2021) developed a Lateral Flow Immunoassay (LFIA) strip capable of detecting Rsc at concentrations as low as 10^4CFU/mL with 100% concordance with PCR-based diagnostics, representing a promising field-based diagnostic tool. In broader spatial assessments, the application of remote sensing technologies using UAV-based multispectral imaging has proven useful in mapping banana diseases. Essential indices such as NDVI, MCARI, NDWI, and soil pH have shown promise in delineating infected areas (Wikantika et al. 2023), and their application could enhance surveillance over large banana-growing landscapes.

In conclusion, BBD was a newly encountered issue for most agricultural officers, village leaders, and farmers on Flores Island, resulting in limited understanding of the disease and its insect vectors. Surveys confirmed that *Musa* ABB ‘Kepok plantain’ was the most severely affected cultivar. The consistent presence of eight insect species on male inflorescences of symptomatic plants across all surveyed locations pointed to a strong likelihood of insect involvement in disease transmission. Current control strategies, including eradication and Trichoderma soil treatments, have proven inadequate, particularly given the role of insect and possibly bird or bat vectors in pathogen spread. Effective disease management requires comprehensive strategies, including preventative measures, advanced diagnostic tools, and adoption of resistant cultivars.

ACKNOWLEDGEMENTS

This study was part of a joint research project funded by Universitas Nusa Cendana in response to the spread of Banana Blood Disease (BBD) and its biosecurity implications on Flores Island, East Nusa Tenggara, Indonesia. We thank the Rector of the University, the Dean of the Faculty, and the Head of the Department for providing the funding and other necessary support.

REFERENCES

Aisyah I, Sinaga MS, Nawangsih AA, Giyanto, Pari G. 2018. Utilization of liquid smoke to suppress blood diseases on bananas and its effects on the plant growth. Agrivita 40 (3): 453-460. DOI: 10.17503/agrivita.v40i3.1390.

Anggraeni T, Nisrine N, Barlian A, Sumarsono SH. 2018. Repellency of some essential oils against *Drosophila melanogaster*, vector for bacterium blood disease in banana plantation. J Entomol 15 (3): 125-134. DOI: 10.3923/je.2018.125.134.

Bakar RAH, Badrun R, Ahmad K, Bakar NA. 2018. Symptomatology and range of the blood disease bacterium A2 HR MARDI strain (*Ralstonia syzygii* subsp. *celebensis*) on Selected Hosts. IOSR J Agric Vet Sci 11 (9): 71-77. DOI: 10.9790/2380-1109027177.

Barthélémy C, Lee JX, Kojima J. 2014. Provisional distributional checklist of Hong Kong social wasps (Hymenoptera: Vespidae: Vespinae, Polistinae, Stenogastrinae). Hong Kong Entomol Bull 6 (1): 3-14.

Barthélémy C. 2021. Identification Guide to Social Wasps of Hong Kong (Hymenoptera: Vespidae). Christophe Barthelem, Hongkong.

Berhal C, De Clerck C, Fauconnier ML, Levicek C, Boullis A, Kaddes A, Haïssam M, Jijakli HM, Verheggen F, Massart S. 2017. First characterisation of volatile organic compounds emitted by banana plants. Sci Rep 7: 46400. DOI: 10.1038/srep46400.

Buddenhagen I. 2007. Blood bacterial wilt of banana: History, field biology and solution. III International Symposium on Banana: ISHS-ProMusa Symposium on Recent Advances in Banana Crop Protection for Sustainable. Greenway Woods, White River, South Africa, 10-14 September 2007.

Drenth A, Kema G. 2021. The vulnerability of bananas to globally emerging disease threats. Phytopathology 111 (12): 2146-2161. DOI: 10.1094/PHYTO-07-20-0311-RVW.

FAO. 2024. FAOSTAT Crops and Livestock Products. Banana Production of Indonesia on 2021-2023. FAO, Rome. https://www.fao.org/faostat/en/#data/QCL.

GBIF Secretariat. 2023a. *Vespa affinis* (Linnaeus, 1764). GBIF Backbone Taxonomy Checklist Dataset. https://www.gbif.org/species/1311357.

GBIF Secretariat. 2023b. *Vespa velutina* Lepeletier, 1836. GBIF Backbone Taxonomy Checklist Dataset. https://www.gbif.org/species/1311477.

Henuk JBD, Kadja DH, Mau YS. 2020. Inventory and identification of banana cultivars and diseases caused by bacterial and fungal pathogens in West Timor, East Nusa Tenggara Province, Indonesia. Intl J Trop Dryl 4 (1): 10-16. DOI: 10.13057/tropdrylands/t040103.

Hermanto C, Eliza, Emilda D. 2013. Bunch management of banana to control blood disease. Aust Plant Path 42: 653-658. DOI: 10.1007/s13313-013-0248-5.

Ho JS, Krishnen G, Jaffar NS, Yeap SK, Ho WY. 2023. Pathogenesis study of blood disease bacterium in local bananas of Malaysia. Physiol Mol Plant Pathol 127: 102087. DOI: 10.1016/j.pmpp.2023.102087.

ITC. 2023. Trade Map. Trade Statistics for International Business Development. List of Importing Markets for a Product Exported by Indonesia. Product: 0803 Bananas, Incl. Plantains, Fresh or Dried. International Trade Centre. https://www.trademap.org/Country_SelProductCountry_TS.aspx?nvpm=1%7c360%7c%7c%7c%7c0803%7c%7c%7c4%7c1%7c1%7c2%7c2%7c1%7c2%7c1%7c1%7c1. Retrieved on 2 April 2025.

Jacobsen K, Omondi BA, Almekinders C, Alvarez E, Blomme G, Dita M, Iskra-Caruana ML, Ocimati W, Tinzaara W, Kumar PL, Staver C. 2019. Seed degeneration of banana planting materials: Strategies for improved farmer access to healthy seed. Plant Pathol 68: 207-228. DOI: 10.1111/ppa.12958.

Jones RAC. 2021. Global plant virus disease pandemics and epidemics. Plants 10: 233. DOI: 10.3390/plants10 020233.

Lada KYP. 2024. Eksplorasi, Identifikasi Morfologi dan Deteksi Molekuler *Blood Disease Bacteria* (BDB) Penyebab Penyakit Darah Pisang di Flores Bagian Barat. [Skripsi]. Unit Pelaksana Teknis Perpustakaan Universitas Nusa Cendana, Kupang. [Indonesian]

Leiwakabessy C. 2003. Potensi beberapa jenis serangga dalam penyebaran penyakit darah pisang (*Ralstonia solanacearum* Yabuuchi et al.). Jurnal Pertanian Kepulauan 2 (2): 137-145. [Indonesian]

Mairawita M, Suswati S, Habazar T, Hasyim A, Nasir N. 2012. *Trigona minangkabau* potential as bacterial spreader agent of *Ralstonia solanacearum* Phylotype IV cause blood disease on banana plants. 2012 International Conference on Biological and Life Sciences IPCBEE. Singapore, 23-24 July 2012.

Masriany M, Esyanti RR, Dwivany FM, Anggraeni TJ. 2020. Banana flower-insect interaction: Alpha-pinene as potential attractant for the insect vector of banana blood disease. Hayati 27 (1): 8-15. DOI: 10.4308/hjb.27.1.8.

Montong V, Salaki C. 2020. Insects as carriers of *Ralstonia solanacearum* phylotype IV on Kepok Banana flowers in South Minahasa and Minahasa District. Intl J Chem Tech Res 13 (1): 199-205. DOI: 10.20902/IJCTR.2019.130124.

Montong VB, Maramis, RTD, Pelealu J, Salaki CL. 2015. Involvement of insects in the transmission of banana blood disease. Intl J Res Eng Sci 3 (1): 41-44.

Montong VB, Salaki CL. 2019. Insects that visit kepok plantain flowers in South Minahasa Regency as carriers of *Ralstonia solanacearum* phylotype IV (causing banana blood disease). Jurnal Enfit: Entomologi Fitopatologi 1 (1): 17-28. DOI: 10.35791/jef.v1i1.27165. [Indonesian]

Mudita IW, Benu FL. 2018. Go bananas no more: Socio-economic and biosecurity implications of blood disease of banana in Sumba Island, the province of East Nusa Tenggara, Indonesia. Hortic Intl J 2 (6): 347-350. DOI: 10.15406/hij.2018.02.00075.

Mudita IW, Drenth A, Markus JER, Ray J, Natonis RL. 2018. The Spread of Blood Disease of Banana in The Island of Sumba, East Nusa Tenggara, Indonesia: What Does It Mean to agrobiosecurity and Why Does It Matter? International Conference and Exhibition on Science and Technology (ICEST). Faculty of Science Engineering in Labuhan Bajo, West Manggarai, East Nusa Tenggara, 26-27 October 2018.

Mudita IW. 2023. Panduan Pengenalan Morfologi Tanaman Pisang: Sebagai Dasar Pemberian Skor Penentuan Kelompok Genom serta Penentuan Satuan Penelitian dan Satuan Sampel. Program Studi Agroteknologi, Fakultas Pertanian, Universitas Nusa Cendana, Kupang. https://www.academia.edu/116669382/Panduan_Pengenalan_Morfologi_Tanaman_Pisang_Sebagai_Dasar_Pemberian_Skor_Penentuan_Kelompok_Genom_Serta_Penentuan_Satuan_Penelitian_dan_Satuan_Sampel. [Indonesian]

Nampa IW, Mudita IW, Widinugraheni S, Natonis RL, Surayasa MT. 2022. Impacts of banana blood disease outbreak to the farmers' households food security in Sumba Island, East Nusa Tenggara Province, Indonesia. IOP Conf Ser Earth Environ Sci 1107 (1): 012089. DOI: 10.1088/1755-1315/1107/1/012089.

Prakoso AB, Joko T, Soffan A, Sari JP, Ray JD, Drenth A, Subandiyah S. 2022. Draft genome sequence of *Ralstonia syzygii* subsp. *celebesensis* from Indonesia, the causal agent of blood disease of banana. Phytopathology 112: 1584-1586. DOI: 10.1094/PHYTO-10-21-0443-A.

Prior P, Ailloud F, Dalsing BL, Remenant B, Sanchez B, Allen C. 2016. Genomic and proteomic evidence supporting the division of the plant pathogen *Ralstonia solanacearum* into three species. BMC Genom 17: 1-11. DOI: 10.1186/s12864-016-2413-z.

R Core Team. 2013. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Ray JD, Subandiyah S, Prakoso AB, Rincón-Flórez VA, Carvalhais LC, Drenth A. 2022a. Transmission of blood disease in banana. Plant Dis 106 (8): 2155-2164. DOI: 10.1094/PDIS-10-21-2373-RE.

Ray JD, Subandiyah S, Rincón-Flórez VA, Prakoso AB, Carvalhais LC, Drenth A. 2022b. Susceptibility of the banana inflorescence to blood disease. Phytopathology 112 (4): 803-810. DOI: 10.1094/PHYTO-07-21-0311-R.

Ray JD, Subandiyah S, Rincon-Florez VA, Prakoso AB, Mudita IW, Carvalhais LC, Markus JER, O'Dwyer CA, Drenth A. 2021. Geographic expansion of banana blood disease in Southeast Asia. Plant Dis 105 (10): 2792-2800. DOI: 10.1094/PDIS-01-21-0149-RE.

RStudio Team. 2021. RStudio: Integrated Development Environment for R. https://www.r-project.org/conferences/useR-2011/abstracts/180111-allairejj.pdf.

Safni I, Cleenwerck I, De Vos P, Fegan M, Sly L, Kappler U. 2014. Polyphasic taxonomic revision of the *Ralstonia solanacearum* species complex: Proposal to emend the descriptions of *Ralstonia solanacearum* and *Ralstonia syzygii* and reclassify current *R. syzygii* strains as *R. syzygii* subsp. *syzygii* subsp. nov., *R. solanacearum* phylotype IV strains as *R. syzygii* subsp. *indonesiensis* subsp. nov., banana blood disease bacterium strains as *R. syzygii* subsp. *celebesensis* subsp. nov. and *R. solanacearum* phylotype I and III strains as *R. pseudosolanacearum* sp. nov. Intl J Syst Evol Microb 64 (9): 3087-3103. DOI: 10.1099/ijs.0.066712-0.

Safni I, Subandiyah S, Fegan M. 2018. Ecology, epidemiology, and disease management of *Ralstonia syzygii* in Indonesia. Front Microbiol 9: 419. DOI: 10.3389/fmicb.2018.00419.

Sahetapy B, Maryana N, Manuwoto S, Latumahina F, Mutaqin KH. 2020a. Identification and detection of blood disease bacterium in the isolates derived from insects' body parts. J Crit Rev 7 (8): 2827-2841.

Sahetapy B, Maryana N, Manuwoto S, Mutaqin KH, Latumahina F. 2020b. Test of Blood Disease Bacterium (BDB) transmission by potential insect vectors. J Trop Plant Pests Dis 20 (1): 71-77. DOI: 10.23960/j.hptt.12071-77.

Sam MKV, Jawan R, Chong KP. 2024. Characterization and identification of potential lactic acid bacteria as biological control agent against *Ralstonia syzygii* subsp. *celebesensis* of the banana blood disease. J Pure Appl Microbiol 18 (3): 38. DOI: 10.22207/jpam.18.3.38.

Sauvard D, Imbault V, Darrouzet É. 2018. Flight capacities of yellow-legged hornet (*Vespa velutina nigrithorax*, Hymenoptera: Vespidae) workers from an invasive population in Europe. Plos One 13 (6): e0198597. DOI: 10.1371/journal.pone.0198597.

Talib MAA, Azeme NFN, Shafie KA, Jaafar NS, Bunawan SN, Haron FF, Mazlan Z, Kadir AAA. 2021. Lateral flow immunoassay detection of *Ralstonia syzygii* subsp. *celebensis* causing banana blood disease in symptomatic and asymptomatic banana plants. J Trop Plant Physiol 13 (1): 51-62. DOI: 10.56999/jtpp.2021.13.1.14.

Thakur H, Sharma A, Sharma P, Rana RS. 2021. An insight into the problem of bacterial wilt in *Capsicum* spp. with special reference to India. Crop Prot 140: 105420. DOI: 10.1016/j.cropro.2020.105420.

Tinzaara W, Karamura EB, Kubiriba J, Ochola D, Ocimati W, Blomme G, Ssekiwoko F. 2016. The banana *Xanthomonas* Wilt epidemic in East and Central Africa: Current research and development efforts. Acta Hortic 1114: 1-10. DOI: 10.17660/ActaHortic.2016.1114.36.

Wikantika K, Ghazali MF, Dwivany FM, Susantoro TM, Yayusman LF, Sunarwati D, Sutanto A. 2023. A study on the distribution pattern of Banana Blood Disease (BBD) and *Fusarium* Wilt using multispectral aerial photos and a handheld spectrometer in Subang, Indonesia. Diversity 15: 1046. DOI: 10.3390/d15101046.