



Insects as Carriers of *Ralstonia solanacearum* Phylotype IV on Kepok Banana Flowers in South Minahasa and Minahasa Districts

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Abstract : The scope of this study is the management of insects that carry the cause of banana blood disease (BBD), *Ralstonia solanacearum* Phylotype IV. The objectives of this study are: (1) to study the diversity and density of visitor insect populations to the Kepok banana flower, and (2) to identify insects in the Kepok banana flower that act as carriers of *R. solanacearum* Phylotype IV, and the population density of these bacteria was carried by each insect. Sampling of banana plantations is done based on purposive sampling method. Insect collection uses a modified insect net, and insect collection uses modified insect nets, and insects were morphologically identified. This bacterial isolation was carried out based on the spread method on NA + TZC media. Inoculation of bacterial isolates was carried out by injection method on the tip of an mature Kepok banana. Density of insects visitors banana flower per tree in South Minahasa and Minahasa regencies are as follows: *Oscinella* sp. (15.50 and 18.08 individuals), *Aphis mellifera* (0.50 and 1.58), *Chelisoche morio* (0.28 and 0.20 individuals, and *Dolichoderus* sp. (1.44 and 6.21 individuals). All insects on the Kepok banana flower in South Minahasa and Minahasa carry *Ralstonia solanacearum* Phylotype IV. *Oscinella* sp., *Aphis mellifera*, *Chelisoche morio*, and *Dolichoderus* sp. in both districts it brought 17,636.39 and 75,533.33 CFU / ml, 15,666.67 and 17,400.00 CFU / ml, 113.33 and 2,667.67 CFU / ml, and 2,400.00 CFU / ml and 21,133.33 CFU / ml.

Keywords : *Ralstonia solanacearum* Phylotype IV, density, *Oscinella* sp., *Aphis mellifera*, *Chelisoche morio*, *Dolichoderus* sp.

1. Introduction

One of the main reducing factors of banana cultivation is the attack that causes banana blood or wilt disease, *Ralstonia solanacearum* (Smith) Yabuuchi et al., Phylotype IV¹. The spread of these pathogenic bacteria can be easily spread by banana flower insects because of the ooze texture of bacteria that comes out of

male flowers and fresh wounds caused by the death of bract such as mucus. In Sumatra, studies have been conducted on the potential of insects on banana flowers as carriers of *Ralstonia solanacearum* Phylotype IV². Their results showed that these pathogenic propagules could be attached to and consumed by *Trigona* sp., *Apis dorsata*, *Apis cerana*, *Erionota thrax*, *Oecophylla*, and *Nezara viridula*.

The dynamics of insect populations visiting Kepok banana flowers such as diversity and density differ due to temperature, humidity and the amount of pollen and nectar, as well as the diversity of plants around the park, and habitat that is conducive to insects to complete their life cycle. The passive distribution of *Ralstonia Solanacearum* Phylotype IV can be carried out by an insect visiting a part of the plant that is the source of the inoculum.

The difference in the number of *R. solanacearum* IV Phylotype cells carried by the visitors of the kepok banana compound is related to foraging behavior, especially the length of the period spent on a flower. Each flower visitor insect takes foraging time and the number of flowers visited per minute is different for a flower^{3,4}.

The objectives of the study were: (1) to study the diversity and density of insect population of visitors to the Kepok banana flowers, and (2) to identify the visitors of the Kepok Banana flowers insects that act as carriers of *R. solanacearum* Phylotype IV, and the population density of these bacteria carried by each insect.

2. Experimental

2.1. Collection, Identification, Insects Population Density

Insects visiting healthy banana flowers and BBD infected are captured using an insect net that has an additional two wire circles (one smaller wire circle is placed about 20 cm below the wire circle as the mouth of the net, and one of the smallest wire circles sewn about 30 cm below the second wire circle), and the bottom of the net is only tied with a rubber band. The captured insects are collected at the end of the net and put into a killing bottle with chloroform vapor for one minute to kill the insect. Insects are then placed on a paper and sorted according to species and counted by each species. Each group was placed in small bottles / pots containing 70% alcohol and taken to the Plant Pest and Entomology Laboratory, Faculty of Agriculture, Sam Ratulangi University to be identified by the Insect Identification Team.

Insect identification was carried out at the Entomology and Plant Pest Laboratory. The references used as a reference for identification^{5,6,7}.

2.2. Insect Species as Carriers of *Ralstonia solanacearum* Filotype IV

To determine the *R. solanacearum* carrier insects and how many inoculums they carry, the insects were collected by net as stated earlier. In each garden, insects were collected on 5-7 Kepok banana flowers to estimate the *Ralstonia solanacearum* Phylotype IV CFU that they brought. Five individuals of each insect species were placed in a vial or pot. In the laboratory, insects were washed with 10 ml of sterilized deionized water (SDW). This washing liquid or solution was diluted two times (10^{-1} and 10^{-2}) with SDW. Each dilution level was pipetted 0.5 ml and on a plate by the spread method at NA + TZC and incubated at room temperature for 2-3 days. Furthermore, the virulent *R. solanacearum* colony forming units (CFU) were calculated with a colony counter. To further ensure that these bacterial colonies are virulent, they were inoculated by a suspension of these bacteria injected into the tip of a mature kepok banana. Preparation of bacterial suspension as follows: (1) bacterial colonies according to insect species were scraped by adding 20 miles of sterile water to the plate then scrapping with a round-tipped ose needle, and (2) the suspension of each bacterial isolate was sucked with a needle sterile injection syringe and injected into mature kepok bananas through the tip.

3. Results and Discussion

3.1. Insect Species and Density in Kepok Banana Flowers

Species and density of insects that visit banana flower per tree in the Minahasa Selatan and Minahasa Districts at 08.00 - 10.00 as follows: *Oscinella* sp. (15.50 and 18.08 individuals), *Aphis mellifera* (0.50 and 1.58), *Chelisoche morio* (0.28 and 0.20 individuals, and *Dolichoderus* sp. (1.44 and 6.21 individuals) (Figure 1). An interesting phenomenon regarding the population density of insects visiting the Kepok banana flower is

the population density of *Oscinella* sp., *A. mellifera*, and *Dolichoderus* sp. In Minahasa Regency it is higher than in South Minahasa Regency. Factors that affect the density of visitors to a flower based on several reports are determined by the number of individual flowering plants, intensive agriculture with an increase in single plants (monoculture), crop rotation, the use of chemicals carelessly and inappropriately to reduce weeds, pests and diseases plants, and destruction of natural habitats, and discoloration of flowers^{8,9,10}. Kepok banana plantations in Minahasa District are relatively far from each other, and among the banana gardens many species of flowering plants grow. According to⁹ that the density of flower visitor insect populations in an area where many flowers are available is higher than that of a few flowering plant species.

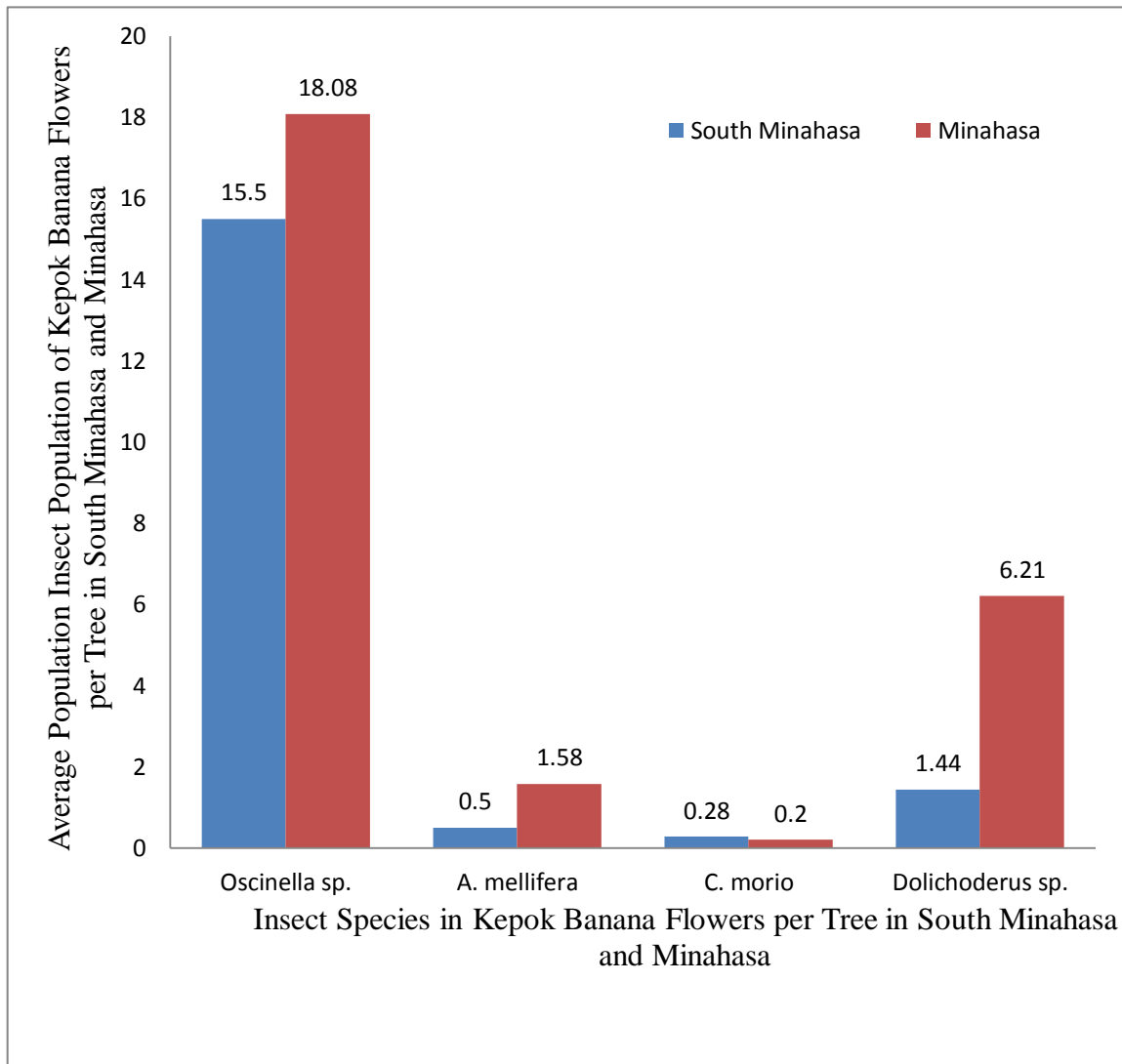


Figure 1. Insect species and density of Kepok banana per tree in South Minahasa and Minahasa Districts

The density of the flower visitor insect population is also influenced by the age of the flower. Kepok banana flowers in Minahasa District are generally younger than in South Minahasa Regency. According to⁸ that flower visitor insects avoid older flowers because they are already inefficient as a food source and so that repeated visits do not occur.

Around the Kepok banana garden in South Minahasa District there are fewer sources of food for flower-visiting insects because there are more chemicals used to control weeds and disease pests than in Minahasa District. The phenomenon is in accordance with that proposed by¹⁰ that the excessive use of chemicals in an agricultural area can reduce the density of flower visitor insect populations.

The black earwig, *C. morio*, is known as a predator of several insect species, for example coconut palm beetles, *Brontispa longissima* Gestro in Asia and the Pacific¹¹. This black earwig can also consume coconut

flowers and organic ingredients¹². So, *C. morio* in Kepok banana flower in South Minahasa Regency and Minahasa can play a role as a forager and/or predator.

The population of *C. morio* in South Minahasa District was somewhat higher compared to that in Minahasa District probably due to differences in habitat and humidity. According to¹³ that black earwig prefers dark and moist habitats.

Dolichoderus sp. many are also found in South Minahasa and Minahasa Districts. The density of the ant population is high because of the many banana flowers found mealybug. *Dolichoderus* sp. and mealybug are ecologically related by mutual symbiosis. The results of research¹⁴ show that these ants are not predators but only obtain food from secretions of white lice that contain lots of sugar.

3.2. Insects in Kepok Banana as Carrier *R. solanacearum* Phylotype IV, and Density of the Bacteria Population Taken by Each

The colonies of *R. solanacearum* Phylotype IV on NA + TZC isolated from *Oscinella* sp, *A. mellifera*, *Dolichoderus* sp, and *C. morio* have morphological features of round, convex and whitish margins and mid-pink margins. These characteristics are in accordance with *R. solanacearum* Phylotype IV virulent which was characterized by² namely elevation of convex, fluidal colonies and white and middle pink margins.

Koch postulates of carrier insects *Ralstonia solanacearum* Phylotype IV can be seen in (Figure 2). Symptoms of this pathogen attack on the middle fruit flesh are reddish brown necrosis and blackish brown. The symptoms of this pathogen attack were consistent with the results of the study put forward by¹⁵, which was due to the attack of *R. solanacearum* Phylotype IV, the internal parts of bananas turn reddish brown.

The source of the *R. solanacearum* Phylotype IV inoculum in the banana flower is the bacterial exudate (ooze) coming out of the tip of the male flower (Figure 3).

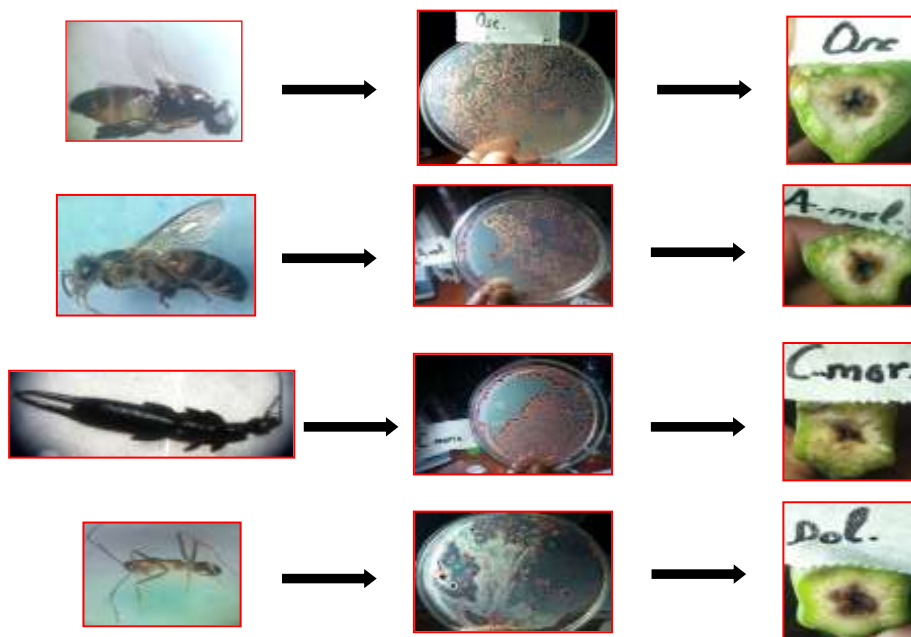


Figure 2. Koch Postulate for Insects Transporting *Ralstonia solanacearum* Phylotype IV.



Figure 3. Ooze of *Ralstonia solanacearum* coming out of the tip of a banana flower¹⁶.

and / or fresh injuries resulting from the death of the bract^{16,17}. Ooze bacteria consist of a mixture of extracellular polysaccharides produced by bacteria and the bacteria themselves. This polysaccharide consists of galactose, glucose, manosa, uronic acid and fructose. Some insects are attracted to sugars (sugars) in bacterial exudates¹⁷. Ooze bacterial texture that is like mucus so it is easy to stick to something that touches it¹⁸. According to², *R. solanacearum* Phylotype IV can be isolated from insects that visit diseased banana flowers.

The position, texture and nutritional value of *R. solanacearum* Phylotype IV on the Kepok banana flower enables and facilitates all visitor insects on this flower to become sticky with exudates containing bacteria and also eaten by insects. When the insect's body and mouth parts are contaminated with these pathogens, they move to healthy bananas and land on new wounds or natural or artificial injuries, and humid environmental conditions, bacteria can multiply, move into plants and start a new infection.

3.3. Population Density of *R. solanacearum* Phylotype IV Carried by Insects

All visitors of the banana flower carry *R. solanacearum* Phylotype IV. *Oscinella* sp., *A. mellifera*, *C. morio*, and *Dolichoderus* sp. in the two districts each brought 45,544.44 and 58,822.22 CFU / ml; 65,877.78 and 44,711.11 CFU / ml; 38,455.56 and 76,377.78 CFU / ml; and 30,544.45 and 78,788.89 CFU / ml (Figure 4).

Insects in healthy kepok banana flowers carry blood-borne bacteria. This fact proves that these insects have visited the sick banana flower, then foraging the healthy banana flower. According to¹⁷ that this pathogenic bacterial ooze is found at the ends of banana flowers and injuries due to the death of brachkteola-brachkteola. Ooze has a bacterial texture like mucus so it is easy to stick to something that touches this ooze. Important information in Figure 4 was *Oscinella* sp. foraging the sick banana flower carrying large quantities of *R. solanacearum* Phylotype IV cells compared to other visitor insects. This fact occurs because these flies are attracted to rotten flowers due to the attack of pathogenic bacteria. This fact was consistent with the results of research² that flies are attracted to tissue that rot due to attack by pathogenic bacteria.

Another fact why the number of individuals *Oscinella* sp. a lot of foraging for banana flowers that this insect has specialized as a flower visitor insect, and because of its small size it easily reaches the end of the nectar outlet¹⁹. The nectar canal is also an outlet for the ooze *R. solanacearum* filotype IV, therefore this bacterium is easily attached to *Oscinella* sp.¹⁶. Besides that, *Oscinella* sp. has a short proboscis that makes it easy to suck nectar²⁰.

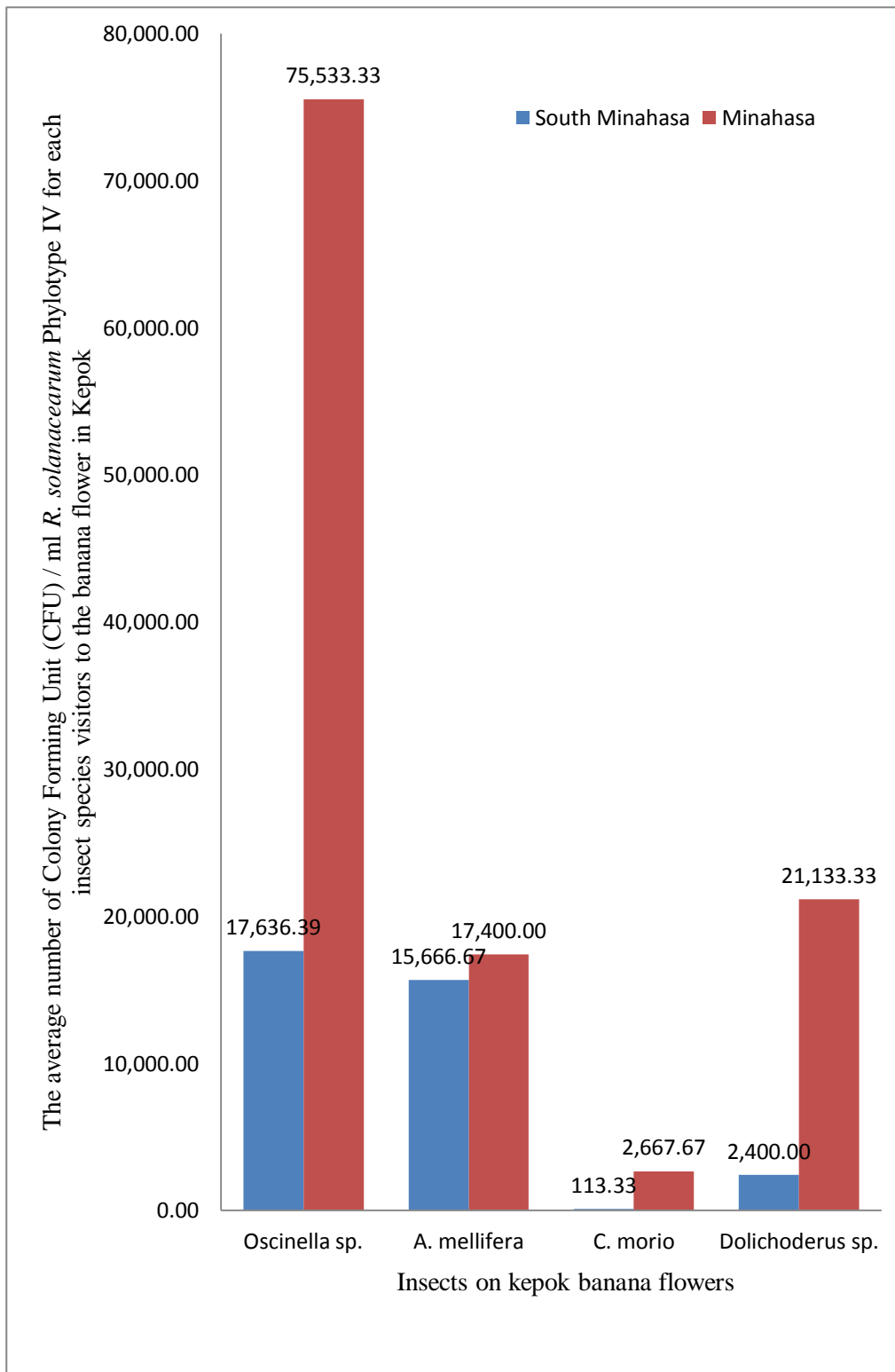


Figure 4. The average number of Colony Forming Units (CFU) / ml *R. solanacearum* filotype IV for each species of visitors of Kepok banana flower in South Minahasa and Minahasa Districts

4. Conclusions

Species and density of insects visitors Kepok banana per tree in South Minahasa and Minahasa Districts were as follows: *Oscinella* sp. (15.50 and 18.08 individuals), *Aphis mellifera* (0.50 and 1.58), *Chelisoche morio* (0.28 and 0.20 individuals), and *Dolichoderus* sp. (1.44 and 6.21 individuals). All visitors to the Kepok banana

flowers in South Minahasa and Minahasa Districts carry *R. solanacearum* Phylotype IV. *Oscinella* sp., *Aphis mellifera*, *Chelisoche morio*, and *Dolichoderus* sp. in both districts it brought 17,636.39 and 75,533.33 CFU / ml, 15,666.67 and 17,400.00 CFU / ml, 113.33 and 2,667.67 CFU / ml, and 2,400.00 CFU / ml and 21,133.33 CFU / ml.

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