BIODIVERSITAS
Volume 24, Number 7, July 2023
Pages: 4001-4007

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d240737

The relationship between vector insect populations, natural enemies, and disease incidence of tungro virus during wet and dry seasons

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Manuscript received: 27 December 2022. Revision accepted: 21 July 2023.

Abstract. *Hutasoit RT, Jihad M, Listihani L, Selangga DGW. 2023. The relationship between vector insect populations, natural enemies, and disease incidence of tungro virus during wet and dry seasons. Biodiversitas 24: 4001-4007.* Tungro virus is one of the most prevalent viruses affecting rice plants. The tungro virus is frequently found in rice plantations because its green planthopper vector is always present. This study aimed to determine the relationship between the population density of green planthoppers and its natural enemies with the incidence of tungro disease during the rainy and dry seasons in Lanrang, Sidenreng Rappang, South Sulawesi. The research method employed was field monitoring of the population density of green planthoppers, natural enemies, and the incidence of tungro disease. The presence of the tungro virus was confirmed by the molecular method using RTSV and RTBV-specific primers. The results showed three types of tungro vector insects: *Nephotettix virescens*, *Nephotettix nigropictus*, and *Recilia dorsalis*. *Nephotettix virescens* was the dominant vector insect, with the highest population in March and August of 101 and 51 individuals, respectively. During the dry season, the high population of the three vector insects in August was followed by a high incidence of tungro disease in September, reaching 29.38%. Symptoms of yellow leaves have been confirmed by molecular methods, which indicated that the infection was caused by RTSV and RTBV, as evidenced by the amplification of DNA bands measuring 787 bp and 1400 bp. Data on the population of vector insects and the incidence of tungro disease indicated the importance of determining the ideal time to plant to avoid the plant's susceptible phase during the peak vector population between March and August. The dominant natural enemies found during the observations included *Araneus inustus*, *Tetragnatha maxillosa*, *Agriocnemis pygmaea*, and *Menochilus sexmaculatus*. Increasing the population of natural enemies could suppress the population of vector insects.

Keywords: *Nephotettix nigropictus, Nephotettix virescens, Recilia dorsalis,* RTBV, RTSV

INTRODUCTION

Pest infestation and pathogen infection are a hindrance to the cultivation of rice. In Indonesia, tungro disease, a viral infection, ranks fifth of the most important pests and diseases in rice after the brown planthopper, stem borer, rat, and blast. If the virus infection occurs during the early stages of rice growth or the vegetative phase in the nursery, rice productivity is reduced, and crop failure may occur. Potential yield loss due to viral infection varies depending on the age of the plant at the time of infection, location and point of infection, growing season, and rice variety. The younger the plant is infected, the greater the percentage of yield loss.

If the tungro virus infects rice, its yield potential will not be achieved; even plants attacked during the early vegetative phase will not yield rice (Banerjee et al. 2012). Tungro disease affects all phases of plant development, but symptoms are most pronounced during the vegetative phase (Anand et al. 2022). Plants affected by tungro experienced leaf discoloration, stunted, reduced tillers, and delayed flowering accompanied by the presence of vectors of both imago, nymph, and eggs (Kim et al. 2019).

Initially, the distribution of tungro in Indonesia was restricted to a few regions in South Sulawesi, South Kalimantan, West Nusa Tenggara, and North Sulawesi. Still later, it expanded to East Java, Central Java, and Yogyakarta (Satomi 1972). In 1972/1973, there was a transmission of tungro in South Sulawesi, and in 1998/1999, there were heavy outbreaks in Central Lombok and East Lombok, covering an area of 10,000-15,000 ha (Widiarta et al. 1999). Transmission of tungro in Central Sulawesi occurred in Donggala, Tolitoli, and Parigi Moutong, and in MT 2002, the most expansive transmission occurred in Parigi Moutong (Rosida et al. 2020). In Southeast Sulawesi, the transmission of tungro occurred in Konawe, particularly in Wawotobi and Pondidaha (Rosida et al. 2020). The transmission of tungro still occurs in South Sulawesi, West Nusa Tenggara, Bali, West Java, and Central Java.

Tungro disease is caused by the infection of two viruses: *Rice tungro spherical virus* (RTSV) and *Rice tungro bacilliform virus* (RTBV). The virus interacts with the helper component (HC) mechanism to cause plant disease (Sailaja et al. 2013; Ng and Zhou 2015; Huang et

al. 2018). Both types of viruses can only be transmitted by green planthoppers semi-persistently. The population of green planthoppers that act as active transmitters dramatically influences the tungro disease development rate (Sutrawati et al. 2021). The presence of inoculum sources around the plantations and the population density of the first-generation vectors subsequently determine the development of tungro attacks (Chen et al. 2015). The high and low intensity of tungro disease is determined by several factors, including the availability of inoculum sources, vectors, susceptible plants, and environmental conditions (Sutrawati et al. 2021). However, a virus-carrying vector (*viruliferous* vector) is the most critical factor (Hattori et al. 2015).

Nephotettix virescens, *N. nigropictus*, *N. malayanus*, *N. cincticeps*, *N. parvus*, and *Recilia dorsalis* are types of planthoppers that transmit the tungro virus (Hibino et al. 1979; Hattori et al. 2015). *Nephotettix virescens* is the vector of tungro disease with the highest transmission rate in Indonesia (Sutrawati et al. 2021). This efficiency can reach 81% in endemic areas and about 52% in non-endemic areas (Suzuki et al. 1992; Kim et al. 2019), more significantly when insects acquire the virus from young plants (Roshan and Raju 2017). This vector insect also forms colonies earlier, and its population grows faster (Listihani et al. 2022a). In addition, the vector has high adaptability to the environment. It is evident from a variety's susceptibility to the tungro virus and green planthopper (Singh et al. 2015; Jabeen et al. 2017; Zarreena et al. 2018).

Fluctuations of vector populations affect tungro disease if the source of the virus inoculum is already in the field (Mangrauthia et al. 2017). The presence of 30-40% inoculum sources in rice plants, accompanied by an increase in the vector population, causes a high tungro incidence (Kim et al. 2019). The development of subsequent infestations is determined by the plant's source of the inoculum and the first-generation vector's population density (Sutrawati et al. 2021). Therefore, this study aimed to determine the pattern of population fluctuations of vector insects and their natural enemies and the incidence of tungro disease on rainfed land in Lanrang, Sidenreng Rappang Regency, South Sulawesi.

MATERIALS AND METHODS

Determination of research location

The research was carried out in the experimental field of the Tungro Research Disease Station, Lanrang, South Sulawesi, Indonesia. The research was carried out between November 2019 and October 2020. The research method used was weekly monitoring for one year.

Trial plot preparation and maintenance

The experimental field was made of 12 plots measuring 8 m x 8 m per plot. The plots were arranged in such a way with a causeway as a barrier between the plots. Causeway serves as a shelter or alternative habitat for green planthopper vector insects. One observation plot was planted every month. The variety used was Taichung Native 1 (TN1). This variety is susceptible to both green planthopper and tungro virus, so there is no limiting factor for green planthopper and the mechanism of transmission of the tungro virus. Fertilization was carried out two weeks after planting with urea and NPK compound fertilizer at a dose of 250 kg ha^{-1} and 250 kg ha^{-1}, respectively. Pesticides and herbicides were not applied in the observation plots.

Observation on tungro vector population, natural enemies, and incidence of tungro disease

Observations were made every week for one year. The parameters observed were the number and type of individual tungro vector insects, the number of individual arthropods that act as natural enemies of the vector, and the incidence of tungro disease. Observations of tungro vector insects and their natural enemies were performed using a swing net of insects for ten double swings diagonally. All captured arthropods were placed in plastic bags and transported to a lab for identification and counting. Natural enemies were identified using the identification guidance book "Natural Enemies of Rice Pests" (Shepard et al. 1987). The incidence of tungro disease was determined by observing the plants in the observation plot. Plants showing tungro symptoms were sampled and taken to the laboratory for PCR/RT-PCR testing. The tungro virus was confirmed by molecular methods to determine whether the yellow symptoms on the tips of the rice leaves were due to the tungro virus. RTBV was detected by the PCR (polymerase chain reaction) method, and the RT-PCR carried out RTSV (reverse transcription-polymerase chain reaction) method.

Detection of RTBV by PCR method and RTSV by RT-PCR method

Total DNA/RNA extraction was performed using the CTAB (Cetyl Trimethyl Ammonium Bromide) method. In the initial stage, 0.1 g of leaf sample was added with liquid nitrogen and crushed using a mortar and a gun until it became powder. The powder was then put into a 2 mL micro-tube, added 500 µL of CTAB buffer 1% -ME, and incubated at 65°C for 60 minutes for DNA viruses, while RNA viruses were incubated at 65°C for 30 minutes. After that, 500 µL of chloroform: isoamyl alcohol mixture was added with a ratio (24:1) and centrifuged at 12,000 rpm for 15 minutes. Next, 1/10 of the sodium acetate and isopropanol were added to as much as 2/3 of the volume of the supernatant. The sample was incubated for one night at -20°C and followed by 10 minutes of centrifugation at 12,000 rpm. The supernatant was discarded, and the pellet was washed using 500 µL of 70% ethanol and centrifuged at 8,000 rpm for 5 minutes. After that, 500 µL of nuclease-free water was added and stored in the freezer (-20°C) until used for the next step.

RTBV DNA amplification used specific primers for RTBV, namely DAF (5'-GGATTCCGGCCCTCAAAAA CCTAGAAG-3') and DAR RTBV primer (5'-GGGGTACCCCCCTCCGATTTCCCATGTATG-3') with a target of 1400 bp amplification product. RTSV amplification used a pair of primers, RTSV-F2 (GAAGAAGCCTATCATGYTCGCGT) and RTSV-R2 (CCTCCACGATATTGTACGAGG), with a product target of 787 bp.

Visualization of PCR/RT-PCR results

The PCR amplification products were taken as much as 5 µL and electrophoresed on 1% agarose gel in TAE buffer at 50 volts for 50 minutes. Then, the gel was immersed in 0.2% ethidium bromide (EtBr) for 15 minutes. After that, it was washed with ddH_2O for 5 minutes, and DNA was visualized on the gel documentation (GelDoc Axygen).

RESULTS AND DISCUSSION

Determination of tungro-virus infection in rice plants

Three species of tungro vector insects were observed, i.e., *N. virescens*, *N. nigropictus*, and *R. dorsalis* (Figure 1). According to Hibino et al. (1979), there are five types of tungro virus vectors in Indonesia, namely *N. virescens*, *N. nigropictus*, *N. malayanus*, *N. parvus*, and *Recilia dorsalis*. This study found no *N. malayanus* and *N. parvus* that feed on tungro-diseased plants; this pest may not have spread in Lanrang, South Sulawesi. Early in November 2019, the three insect vectors were discovered; *N. virescens* was present in a higher population than other pests (Figure 2).

In August, the population of vector insects increased; this increase in vector insect population was probably affected by the non-simultaneous planting pattern. The rice plants around the observation site were vegetative in August. According to Anand (2022), the population density of *N. virescens* is generally low and only increases when the plant is in its vegetative state. The population of vector insects increases from the immigrant generation to the first generation, then the population density decreases (Otuka et al. 2008). The decline in population density from the first generation to the second generation during the rainy and dry seasons was attributable to imago dispersal activity, as indicated by the short duration of the imago stage in the field (Vu et al. 2014). The three vectors transmit the tungro virus to rice crops, particularly during the vegetative phase, as evidenced by the symptoms of yellowing leaves, twisting, and a lack of tillers (Figure 2).

In rice, RTSV causes mild stunted symptoms and increases the severity of symptoms caused by RTBV, namely yellowing and stunted leaves (Hibino et al. 1979). *Nephotettix virescens* feeding on diseased rice plants could spread RTBV and RTSV simultaneously or independently (Hibino et al. 1979). Different green planthoppers species transmit the tungro virus with varying efficiencies, with *N. virescens* being the most significant vector due to its high transmission efficiency. The two virus particles do not circulate in the vector body. In addition, the virus cannot be transmitted from imago to eggs or between changes in developmental stages. Insects that have acquired the virus immediately transmit it until the acquired virus is depleted, thus losing the ability to transmit the virus. The vector can transmit the virus in the most prolonged six days. The time the insects take to acquire the virus is between 5-30 minutes, while the time needed to transmit the virus is also short, between 7-30 minutes; the virus incubation period in plants is between 6-15 days. Molecular tests verified that rice was infected with RTSV and RTBV, with amplified DNA bands of 787 and 1400 bp, respectively (Figure 3).

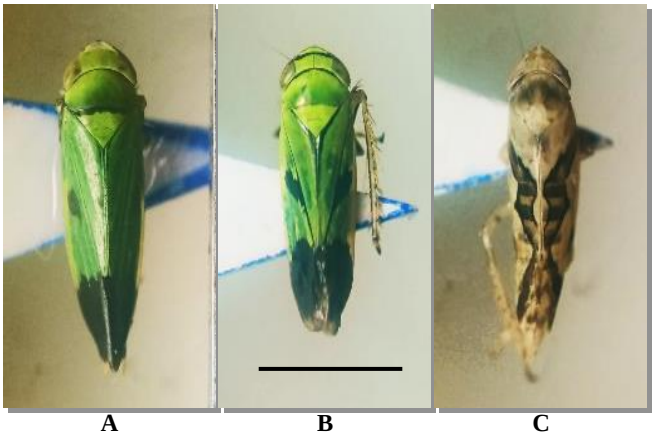

Figure 1. Morphological characteristics of three species of planthoppers: A. *N. virescens*, B. *N. nigropictus*, and C. *R. dorsalis*. Bar = 2 mm)

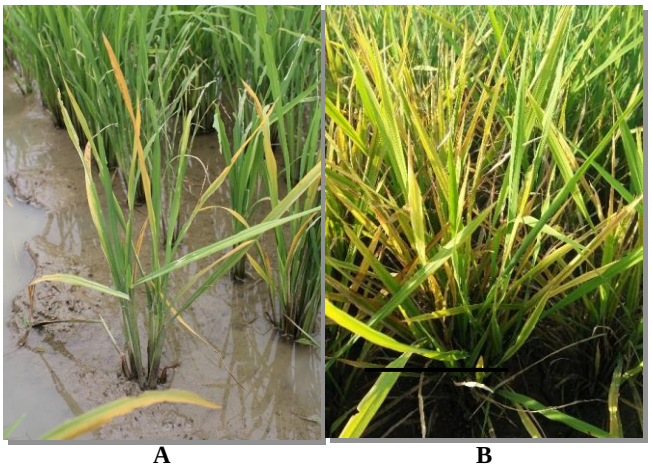

Figure 2. Rice plants infected with the tungro virus show yellow symptoms at the tips of the leaves: tungro virus infects plants in the vegetative phase (A) and enters the generative phase (B)

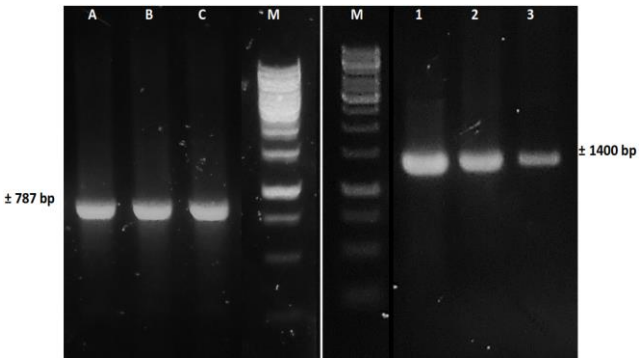

Figure 3. Amplification result of partial CP gene of RTSV by RT-PCR (A, B, C) and RTBV by PCR (1, 2, 3); M. Marker 1 kb DNA ladder

Population dynamics of pest insects and natural enemies

During the wet season, the highest population of *N. virescens* occurred in March, with 51 individuals, while the highest population of *N. virescens* in the dry season occurred in August 2020, with 101 individuals (Tables 1 and 2). Heavy precipitation causes high mortality among the three vector insects from December to February, resulting in a low population. *Nephotettix virescens* dominated the composition of insect vector species during the observation (Table 2). *Nephotettix virescens* is more dominant due to its early colony formation and rapid population growth (Srilatha et al. 2019).

Climate change influences the geographical distribution and population dynamics of insect pests, thereby influencing the pest status of the crop (Lehmann et al. 2020; Triwidodo and Listihani 2020; Skendži'c et al. 2021; Temaja et al. 2022). Regarding increasing population, insects benefit from higher temperatures due to shorter reproductive maturation and increased food quality due to abiotic stress in cultivated plants (Lashari et al. 2012; Blas et al. 2016). This research result is consistent with previous studies; when temperatures are higher during the dry season than in the wet season, the tungro vector population is higher. Some invasive species are sensitive to changes in time, precipitation, and ambient temperature or humidity, whereas others are more sensitive to changes in host pressure (Skendži'c et al. 2021).

During the rainy season, the high population of vector insects in March was followed by an increase in the incidence of tungro disease in April, reaching 1.13% (Figure 4). During the dry season, a high population of the three vector insects occurred in August, followed by a high incidence of tungro disease in September, reaching 29.38% (Figure 5). The relationship between the population of vector insects and the incidence of tungro disease in the field indicates that during the rainy and dry seasons, a high population of vector insects was followed by an increase in the incidence of tungro disease, which is believed to be affected by infected plants (inoculum) and the type and population of infective vector insects. *Nephotettix virescens* is the most important vector in Indonesia because it is the most efficient transmitter of the tungro virus, and its population is predominant compared to other vectors (Sutrawati et al. 2021).

Four weeks after the initial insect vector was discovered at the study site, the first cases of tungro emerged (Figure 5). Immigrant-generation insect vectors are possibly not infectious (*viruliferous*), as no viral transmission occurred, and there were no indications of tungro infection at the study site during the initial observation. It is suspected that insect vectors obtained the virus from tungro-infected plants near the study location. The virus incubation period in plants was affected by the timing of symptoms (Listihani et al. 2018; Listihani et al. 2020; Damayanti et al. 2022; Listihani et al. 2022b; Pandawani et al. 2022; Selangga and Listihani 2022; Selangga et al. 2022; Selangga et al. 2023). According to Listihani et al. (2019), Selangga et al. (2019), and Malathi et al. (2019), the virus incubation period in plants ranges from 6-15 days. The study results of Hutasoit and Ismayanti (2020) showed that the incubation period for the emergence of tungro disease symptoms in the TN1 variety was about 8 days after the plants were inoculated with the tungro virus. The peak of tungro disease incidence is strongly influenced by the vector population if there is a source of inoculum. Initial infection with the tungro virus is determined by the population density of infective vectors migrating to the plantation.

In contrast, the following attacks are determined by the inoculum source in the plantation and the population density of the first-generation vector (Otuka et al. 2008). Even with a low population density, green planthoppers can effectively transmit the tungro virus (Kim et al. 2019). The plants that exhibit symptoms of tungro disease at the beginning of the growing season contribute to the disease's development; crop failure does occur if infection occurs at the vegetative stage. The insect vector population substantially influences the transmission of tungro disease.

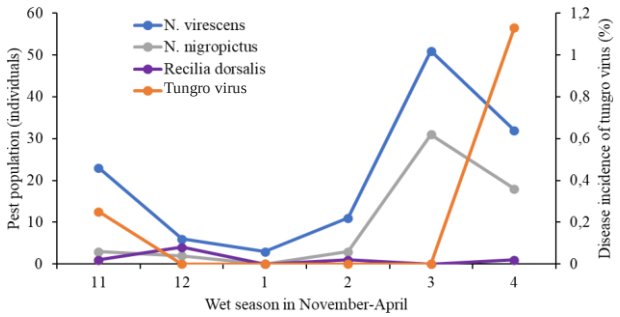

Figure 4. Relationship between populations of *N. virescens, N. nigropictus,* and *Recilia dorsalis* and the disease incidence of tungro virus in the wet season from November 2019 to April 2020

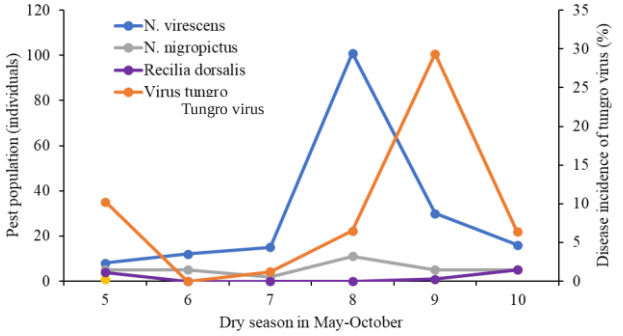

Figure 5. Relationship between populations of *N. virescens, N. nigropictus, Recilia dorsalis* and the disease incidence of tungro virus in the dry season from May to Oct. 2020

Table 1. Population density of pest insects and natural enemies during the wet season from November 2019 to April 2020

Insect species	The wet season in the month of						Average
	11	12	1	2	3	4	
Natural enemies							
Agriocnemis pygmaea (Odonata: Coenagrionidae)	9	13	7	23	56	23	21.83
Menochilus sexmaculatus (Coleoptera: Coccinellidae)	7	66	0	1	19	0	15.5
Ophionea nigrofasciata (Coleoptera: Carabidae)	2	4	2	0	3	4	2.5
Conocephalus longipennis (Orthoptera: Tettigonidae)	2	5	0	0	1	4	2
Araneus inustus (Araneae: Araneidae)	76	8	31	46	85	145	65.17
Lycosa pseudoannulata (Araneae: Lycosidae)	4	5	4	0	9	3	4.17
Oxyopes javanus (Araneae: Oxyopidae)	6	2	0	1	1	6	2.67
Tetragnatha maxillosa (Araneae: Tetragnathidae)	8	10	24	120	162	53	62.83
Cyrtorhinus lividipennis (Hemiptera: Miridae)	0	1	0	1	2	2	1
Opius sp. (Hymenoptera: Braconidae)	2	0	0	1	9	4	2.67
Paederus fuscipes (Coleoptera: Staphylinidae)	1	11	0	0	2	0	2.33
Xanthopimpla flavolineata (Hymenoptera: Ichneumonidae)	0	0	0	0	0	0	0
Anaxipha longipennis (Orthoptera: Gryllidae)	2	0	0	1	0	0	0.5
Brachymeria sp. (Hymenoptera: Chalcididae)	0	0	0	0	0	0	0
Argyrophylax nigrotibialis (Diptera: Tachinidae)	0	0	0	1	1	0	0.33
Average	7.93	8.33	4.53	13	23.33	16.27	
Pest							
Nephotettix virescens	23	6	0	11	51	32	20.5
N. nigropictus	3	2	0	3	31	18	9.5
Recilia dorsalis	1	4	0	1	0	1	1.17
Average	7.5	3.75	0	4.75	24.25	17.25	

Table 2. Population density of pest insects and natural enemies during the dry season from May to October 2020

Insect species	The dry season in the month of						Average
	5	6	7	8	9	10	
Natural enemies							
Agriocnemis pygmaea (Odonata: Coenagrionidae)	12	1	15	33	4	20	14.17
Menochilus sexmaculatus (Coleoptera: Coccinellidae)	10	0	3	7	9	27	9.33
Ophionea nigrofasciata (Coleoptera: Carabidae)	5	0	0	0	3	17	4.17
Conocephalus longipennis (Orthoptera: Tettigonidae)	5	10	1	2	0	11	4.83
Araneus inustus (Araneae: Araneidae)	44	52	112	70	84	135	82.83
Lycosa pseudoannulata (Araneae: Lycosidae)	5	0	4	2	3	2	2.67
Oxyopes javanus (Araneae: Oxyopidae)	5	1	1	0	1	0	1.33
Tetragnatha maxillosa (Araneae: Tetragnathidae)	18	7	90	109	32	4	43.33
Cyrtorhinus lividipennis (Hemiptera: Miridae)	7	1	0	1	2	0	1.83
Opius sp. (Hymenoptera: Braconidae)	6	0	1	1	2	5	2.5
Paederus fuscipes (Coleoptera: Staphylinidae)	5	0	0	4	2	2	2.17
Xanthopimpla flavolineata (Hymenoptera: Ichneumonidae)	7	1	0	0	2	3	2.17
Anaxipha longipennis (Orthoptera: Gryllidae)	6	0	0	0	0	2	1.33
Brachymeria sp. (Hymenoptera: Chalcididae)	7	0	0	0	0	0	1.17
Argyrophylax nigrotibialis (Diptera: Tachinidae)	5	0	0	0	0	0	0.83
Average	9.80	4.87	15.13	15.27	9.60	15.20	
Pest							
Nephotettix virescens	8	12	15	101	30	16	30.33
N. nigropictus	5	5	2	11	5	5	5.5
Recilia dorsalis	4	0	0	0	1	5	1.67
Average	5.66	5.66	5.66	37.33	12	8.66	

There were 15 types of natural enemies observed using a swing net. The dominant natural enemies included *Araneus inustus*, *Tetragnatha maxillosa*, *Agriocnemis pygmaea*, and *Menochilus sexmaculatus*. The highest natural enemy population occurred in March, April, and August. The presence of natural enemies at the research site was discovered in the first week of observation in November 2019. In the first week of observation, three types of natural enemies were found: *Agriocnemis pygmaea*, *Monochilus sexmaculatus*, *Araneus inustus*, and *Tetragnatha maxillosa*. It is in line with Senoaji and Praptana (2015), where *Agriocnemis pygmaea*, *Araneus inustus*, *Tetragnatha maxillosa*, *Oxyopes javanus*, *Lycosa pseudoannulata* are natural enemies found earlier than other types of natural enemies.

In general, an increase in vector populations is followed by an increase in natural enemy populations (Temaja et al. 2022). The high vector population in March and August

was followed by an increase in natural enemy populations in the same month. Similar findings were reported by Selvam et al. (2021). They found that the presence of natural enemies increased dramatically due to a rise in green planthoppers' imago and nymph populations. Meanwhile, increasing the population of natural enemies could suppress the population of vector insects (Listihani et al. 2023). In March and August, a high vector population was followed by a high natural enemy population. It then led to a decrease in the vector population in April, September, and October. The abundance and diversity of natural enemies affect the population of green planthoppers (insect vectors) in rice plantations (Horgan et al. 2019).

According to Suzuki et al. (1992), rice plants are considered threatened if there are five rice hills of tungro-symptomatic plants per 1000 rice hills or if the tungro disease incidence is 0.5% at the age of 2 WAP. At the observation site in September, the incidence of tungro disease in the Taichung Native 1 (TN1) rice variety was extremely high, reaching 29.38% (Figure 5). So, rice plants in this research are considered threatened. One reason was that the Taichung Native 1 (TN1) variety had no history of crossing with resistant parents. Virus-resistant varieties can be grouped into V0-V4 based on resistant parents (Bunawan et al. 2014). Planting resistant varieties are crucial to avoid damage and yield loss; Sutrawati et al. (2021) stated that three rice varieties, namely Ciherang, Inpari 30, and Sentani, were resistant to tungro disease in Bengkulu, Indonesia, and showed a very low incidence of tungro disease. However, this variety is susceptible to brown planthopper and stunt virus attacks in rice plants (Listihani et al. 2022a).

Moreover, rice plants are susceptible to green planthoppers during the vegetative phase, from the nursery to the formation of maximum tillers 35 days after planting (Suzuki et al. 1992). High-N fertilization also affects the development of green planthoppers (Kim et al. 2019). According to Jabeen et al. (2017), RTBV and RTSV viruses are commonly found in phloem tissue, and transmission of the tungro virus occurs simultaneously with the feeding activity of green planthoppers in rice fields. Roshan and Raju (2017) discovered that *N. virescens* absorbed food liquid from phloem tissue more than from xylem tissue. As a source of energy for plant growth, the phloem tissue transports photosynthetic assimilation products from the leaves to all plant parts.

According to Jabeen et al. (2017), phloem tissue contains more albumin compounds, carbohydrates, and other organic salts as a source of its life cycle's growth and development. Green planthoppers have a high dispersal ability with a semi-persistent acquisition-feeding period. The virus is in the mouth apparatus (stylet). It is infective for 10-100 hours after the vector sucks the diseased plant and acquires the virus without viral replication in the insect body. Hence, the population density of vector insects propagates the tungro virus effectively.

It is thought that the high population of green planthoppers in this area was due to the non-simultaneous rice planting in the region. The rice plants in the observation plots were a week younger than those in the surrounding rice fields. It is suspected that green planthoppers migrated from the rice planting area, which had entered the generative phase, to the rice planting area just in the vegetative phase. According to Bunawan et al. (2014), simultaneously planting rice will affect the breeding period of green planthoppers and reduce their presence on rice plants.

Moreover, by controlling the insect vector population and utilizing rice varieties resistant to planthoppers and tungro virus, it is possible to control tungro disease. Therefore, planting with tungro virus-resistant varieties and green planthoppers is the most effective method of controlling tungro (Bunawan et al. 2014; Kim et al. 2019; Anand et al. 2022).

In conclusion, there were three types of tungro vector insects (N. *virescens*, *N. nigropictus*, and *R. dorsalis*) and 15 types of natural enemies in Lanrang, South Sulawesi. Among the three types of insect vectors, *N. virescens* was the dominant species. During the rainy season, the dominant vector insect reached its population peak in March, while during the dry season, the population peak of the dominant vector insect occurred in August. The population of *N. virescens* and the incidence of tungro disease were higher in the dry season than in the wet season, reaching 182 individuals and 123%, respectively. An increase followed the increase in the vector population in the natural enemy population. The high vector population sizes in March and August were followed by increases in the population of natural enemies, resulting in a fall in the vector population in April, September, and October.

ACKNOWLEDGEMENTS

The authors thank the *DIPA Lolit Tungro* for supporting this research. We would like to thank Mimi Sutrawati, Hermanu Triwidodo, and the anonymous reviewers for reviewing this manuscript.

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