



Diversity and agronomic impact of rice stem borer at Nkolbisson, Yaoundé-Cameroon (Central Africa)

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Abstract

Today, rice has become one of the most consumed cereals in Africa in general and Cameroon in particular. The low domestic production imposes a strong import that accentuates the country's trade imbalance. To reduce this issue of external dependency, the national government is encouraging domestic production. However, producer's efforts are inhibited by various constraints among which the pest attacks in general and in particular the stem borers. Data collection was based on sampling and incubation of white rice panicles in an experimental plots set up at Nkolbisson, Yaoundé. The susceptibility of rice varieties to different pest species was evaluated by comparing the frequency of pest species on their infested panicles. Among the NERICA rice lines, NERICA 3 had the greatest percentage of attacks panicles that was 41.96 % while NERICA 9 and 13 had the less percentage of attacks panicles that was 14.29 % for both of them. A total of three pest species were identified from the panicles incubated, *Orseolia oryzivora*, *Chilo zacconius* and *Diopsis apicalis*, *O. oryzivora* being the most frequent. NERICA 3 with 41.96% of incubated panicles appears to be the most susceptible variety, while the Japanese's Akitakomachi and Fukuhibiki showed no attack. These results are discussed in relation to the total sugar content and polyphenol in different rice varieties studied.

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Introduction

Rice is the second worldwide cereal after maize in terms of cultivated area (153 million ha in 2004) and the quantity produced (608 Mt in 2004), with an average yield of 4.0 t / ha that mask wide disparities between regions (World Rice Statistics, 2005). It is, however, the first cereal in human consumption (Courtois, 2007), as it is the staple food of more than 2.5 billion people in developing countries, with annual consumption exceeding 100 kg / capita in some countries. Rice is a monocotyledon plant of the Poaceae family. It belongs to the Oryzaes tribe which includes 7 to 16 genres according to classifications (Schalbroeck, 2001 and Courtois, 2007). The genus *Oryza* comprises 24 species, out of which two are cultivated *Oryza sativa* L. with a global distribution and *Oryza glaberrima* Steud, confined to West Africa (IRRI, 2005).

From cross breeding, that aimed to combine the productivity of Asian species *O. sativa* and boldness of African species *O. glaberrima*, various varieties of new rice for Africa or NERICA has been produced (WARDA, 2003). Among them, the upland lines responds favourably to a large ecological conditions (Akintayo *et al.*, 2008). It was obtained by interspecific crosses. However, upland rice production is generally submitted to various diseases and pest's constraints, including insects stem borers. The Diopsidea or stalked flies are Dipterans whose larvae or maggots are very harmful to rice plants (Heinrichs and Alberto, 2004). They are present in all rice ecosystems of intertropical Africa (Brenière, 1983), but preferentially in shady moist ones such as the lowlands (Olalekan, 2002) and irrigated rice (Heinrichs and Alberto, 2004). Lô (2010) reported that in Haute-Casamance rice ecosystems in Senegal, insect community found on the ground is about 4.9% constituted by borers (*Chilo zacconius* Bleszynski, 1970, *Chilo diffusilineus* Bleszynski, 1970 and *Orseolia oryzivora* Harris and Gagné, 1982). Their adults do not eat rice, but their larvae are located in the stems where they feed and caused serious damages. Indeed, at a stage of rice growth where young tissues are not yet protected by the silica, many

individuals of early instars introduced themselves between the sheath and the stem before entering the rod through a hole drilled by one of them Lô (2010). Early attacks caused drying of the tiller, a phenomenon known as the "dead heart"; when attacks occurred at the flowering stage it induced the dryness of the panicle and the phenomenon is known as the "white panicle" (Lô, 2010). In heavily infested farms, these damages can lead to a yield loss ranging from 60% to 100% (Heinrichs and Alberto, 2004). African rice gall midge, *O. oryzivora* is a major pest of rice encountered in twenty countries of sub-Saharan Africa (Williams *et al.*, 2002). According to Umeh and Joshi (1993) and Nacro *et al.* (1995), these gall midges are parasitized by the micro-hymenopteran parasitic wasps *Platygaster diplosisae* Risbec, 1956a (Hymenoptera, Platygasteridae) and *Aprostocetus procerae* Risbec, 1956 (Hymenoptera, Eulophidae). Studies conducted in West Africa by Ba *et al.* (2004) and Williams *et al.* (1999) report that alternative host plants, of the rice gall midge belonged exclusively to the genus *Oryza*; on these plants larvae may also host these parasitoids (Williams *et al.*, 1999). Attacks of the stem borer Lepidoptera larvae at the reproductive stage (white panicles) induce crop weight loss, but no significant reduction in the number of panicles (Polaszek and Delvare, 2000). Late attacks (at heading stage) are the most harmful, because during the vegetative stage, the plants are still able to produce tillers and replace damaged ones. Without consideration of season, the diversity and dynamics of stem borers are still unknown in the centre region of Cameroon in upland areas as well as lowlands. Moreover, there is no available information on the susceptibility of the various rice varieties. The study of the susceptibility of rice varieties to different species of borers would enable a variety selection in order to limit the damage of these insects. In order to set up appropriate control strategies against these pests, the present study aims to (i) identify the main rice stem borers, (ii) evaluate the susceptibility of different rice varieties to their attacks.

Materials and methods

Plant material

The plant material consisted of six rice varieties. Four varieties of NERICA rice type (3, 8, 9, 13) (crossing *O. sativa* and *O. glaberima*) and two varieties of the species *Oryza sativa* (Akitakomachi and Fukuhibiki).

Study site

This work was carried out in the Cameroon Centre Region Yaounde precisely at Nkolbisson, (Fig. 1). The study site was located at 03 ° 51' North latitude and 011° 27' East longitude.

The climate in the city of Yaounde is a transitional equatorial climate, characterized by the alternation of two dry seasons and two rainy seasons. It records an average temperature of 23.5°C contrasted between 16 and 31°C seasonally and a total rainfall of 1650 mm per year. The average humidity is about 80% and showed daily variations of 35 to 98%. The most frequent winds are wet and blow toward the Southwest. The vegetation is intertropical type with the predominance of the southern humid forest (Wethe, 2001).

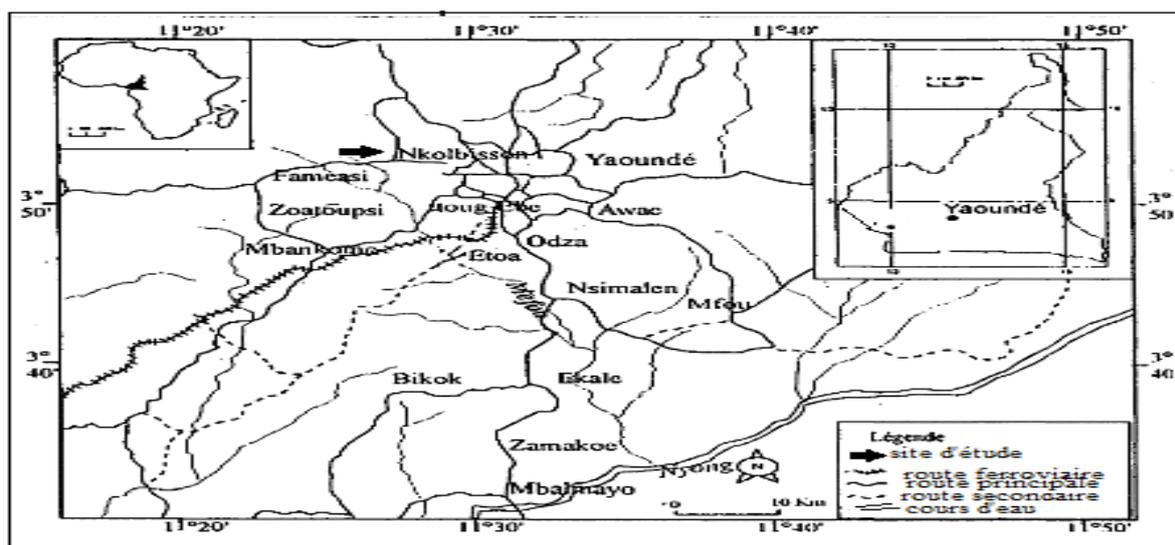


Fig. 1. Location map of the study site (Ngon Ngon, 2007 modified).

Seeding method and crop maintenance

The work was carried out in lowland, on a soil laboured manually. Each rice variety occupied a plot of 30.6 m² (6m x 5.1m). For each variety, the spacing was 30 cm between the lines and 15 cm on the line. The distance between two varieties was 50 cm.

Seedlings were held in April 4, 2014. Weeding was carried out two times during the crop cycle. The first took place 15 days after sowing and the second, a month after the first one. Thinning took place after the first weeding where two vigorous plants were left for a total of 738 seedlings per varieties.

Soil Fertilization

Two weeks after planting, NPK (20-10-10) were applied at a dose of 300 kg / ha; also, when 46% of panicle were formed (that means 60-70 days after

planting according to the cycle of the rice variety), urea was applied at a dose of 65 kg / ha and finally, 35kg / ha of urea to heading / flowering.

Data collection

Each plot was visited at two days interval, from sowing to harvest. During these visits, each plant were carefully examine and those affected (dead heart or white panicles) were collected, stripped of their roots and their leaves, and individually carried to the laboratory for incubations.

Breeding insect larvae

In the laboratory, each panicle was introduced into transparent breeding box (30 cm depth and 5 cm in diameter) provided with 3 to 5 mL of tap water. They were then followed daily up to the emergence of adult insects.

Morphological identification of insect species

The morphological identification of insect was made according dichotomic key provided by Heinrichs (2004).

Analysis of phenols

Phenol analysis followed protocol of Singleton and Rossi, (1965). From this, 500 μ L of diluted extract (1/10 g/mL) or Gallic acid (standard) or 50% ethanol (white) contained in a 10 mL tube, 2 mL of a solution of Folin Ciocalteu 10%. Five minutes later, 2 mL of a freshly prepared solution of sodium carbonate are added to the mixture which is then stirred in a blender before being allowed to stand for 30 min. The absorbance of the colour complex (blue) which is formed is determined at 765 nm against the white. The Gallic acid solutions are prepared at concentrations ranging from 0.25 to 0.03125 mg / mL following a geometric reason 2. The phenol content is derived from the calibration curve and expressed as mg Gallic acid equivalent / 100 g fresh weight.

Analysis of total sugars

Extraction of total sugars

This analysis was done in both fermented and unfermented samples. Fermented and frozen samples were defrosted at room temperature, and then dried in an oven at 60 °C. 0.1 g of each sample type (and non-fermented) were mixed with 30 ml of 80% ethanol contained in a tube provides with a lid. The mixture is boiled in a water bath for 30 min. The Whatman filter paper N° 4 is then used for filtration of the extract obtained. The volume of the collected filtrate was completed to 30 ml with distilled water.

Determination of total sugars

One mL of filtrate, glucose (standard) or distilled water (blank) contained in a test tube of 10 ml, was added on 1 mL of phenol 5% (m/v) and 5 ml of concentrated sulphuric acid. The prepared tubes are kept stirring for 90 min, in a "shaker bath to 30 ° C. The absorbance of the yellow-orange complex which formed was detected at 485 nm. The concentrations of D-glucose from 1 to 10 mg / mL were allowed to

perform the calibration curve from which the contents of total sugars, expressed as mg equivalent glucose/100g dry weight were determined.

The parameters considered were: the number of heart death, the number of white panicles, the stage of development of the variety concerned, yield, flowering dates of the different varieties, insect species that emerged after breeding larvae in the stems and the determination of total sugars and phenols in the stems of different varieties of rice.

The percentage of major pest attacks was obtained by taking the ratio between the white panicles emerged where the species of borers on the total number of white panicles observed within each of the varieties concerned by the study.

Data analysis

Data were analyzed using the Principal Component Analysis (PCA) of XLSTAT 2014.5.02 software version.

Results

Rice heading Stage

The Japanese varieties Akitakomachi and Fukuhibiki presented the fasted growing cycles. They reached 10% of heading 57 and 59 days after sowing, while the NERICA lines took from 69 to 75 days (Fig. 2b).

Diversity of insects of rice stems borers

A total of three stem borer species and one species of parasitic wasp were obtained from incubation of the 112 rice panicles. Among them, the stem borer, *Chilo zacconius*, the less frequent caused least damages (Fig. 2a); *Orseolia oryzivora* caused the most important damages. Akitachomachi and Fukuhibiki varieties showed no white panicle. NERICA 3 is the most susceptible to borers. *Platygaster oryzae* was a parasitoid of *Orseolia oryzivora*.

Percentage of adult stem borer species that emerged from the white panicle

A total of 112 affected panicles were collected from the four NERICA rice line cultivated. NERICA 3, with

41.9% of the affected panicles appeared as the most susceptible, followed respectively by NERICA 8 (29.46%), NERICA 9 and 13 with 14.29% each) (Table I). The two varieties of the Japanese species showed no attacked. Considering the frequency of adults that

emerged from incubations, *O. oryzivora* (with 12 out of the 19 adults obtained) was the most represented, followed by *Chilo zacconius* and *Diopsis apicalis* (Table I). The highest frequency of the most harmful pest was noted on NERICA 3.

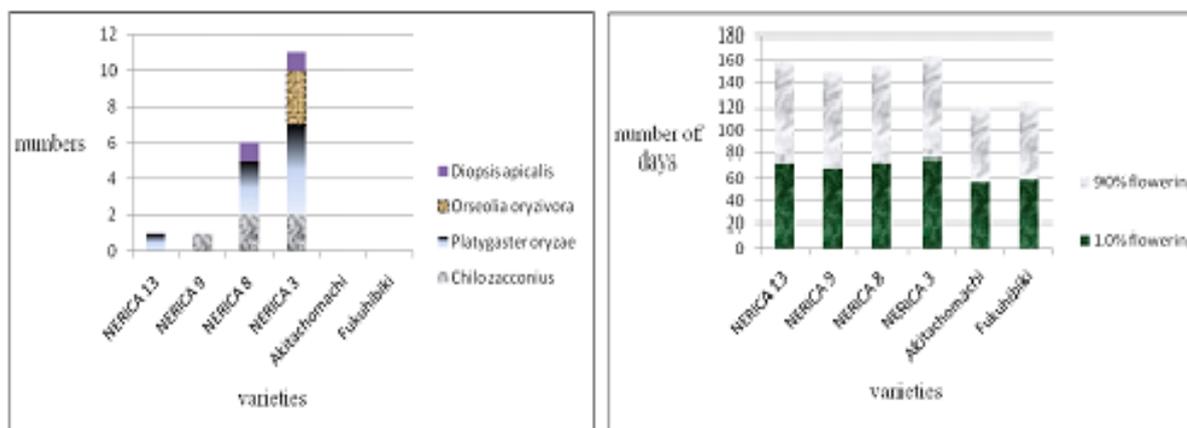


Fig. 2. (a) Number of the adult stems borers emerged according to the variety; (b) Number of days to 10% flowering.

Optical densities obtained after determination of sugars and polyphenol

The Japanese varieties are those that have the lowest total sugars rate with 1.95 and 1.09 respectively for the Akitakomachi and Fukuhibiki. In contrast Akitakomachi presented the highest rate of polyphenols (Table II). Concerning African varieties, NERICA 3 has the highest sugar rate, 7.88 followed by NERICA 8 (5.07) and; NERICA 9 and 8 have substantially the same rate of total sugars. Fukuhibiki has the lowest rate of total phenols with an optical density of 95.43. Table II. Optical densities of total sugars and phenols.

Principal Component Analysis (PCA)

The insect pests *Diopsis apicalis* and *Orseolia oryzivora* were more closely related to NERICA 3. This NERICA line appeared to be more susceptible to these pests (Fig. 3a). Fukuhibiki, Akitakomachi and NERICA 13 Varieties are very far from these insect pest species. *Chilo zacconius* is halfway between NERICA 8 and NERICA 9 varieties. These varieties are sensitive to *C. zacconius*. The rates of total phenols were very important in the stems of NERICA 13 varieties and Akitakomachi (Fig. 3a). Insect pest

species are very distant from the rate of total phenols but close to NERICA 3 and NERICA 8 (Fig. 3b). Fukuhibiki and NERICA 9 have smaller phenols rate. Polyphenols which constitute an intrinsic defence substance in the plant reflect the strength of NERICA 13 and Akitakomachi to protect against stem borers. NERICA 3 presented the most important rate of sugar. It is therefore, close to the total sugar content of rice stem and *Orseolia oryzivora* pest species. The stems of Japanese varieties are far from the insect pest species and presented the smallest rate of total sugars. The insect pest *Chilo zacconius* is isolated; it is not attracted by the high total sugar level (Fig. 3c) while *Orseolia oryzivora* prefers high sugar levels. Fukuhibiki, Akitakomachi, NERICA 9 and NERICA 13 varieties are far from the 10 % flowering stage as from *Diopsis* sp. (Fig. 3d). The species *O. oryzivora* and *C. zacconius* are distant from early flowering (Fig. 3e). *Diopsis apicalis* is very present in the early flowering of the NERICA 3 varieties and NERICA 8, so this borer attacks at the vegetative stage of the plant. The flowering period started from 10% up to 90% flowering. This flowering duration is close to *Orseolia oryzivora*, NERICA 3 and 13 (Fig. 3e), this could justify the sensitivity of NERICA 3 to the rice gall

midge. Late flowering rice varieties are therefore exposed to *O. oryzivora* in contrary, *D. apicalis* and *C. zacconius* are not related to the flowering duration.

Discussion

The extent of damages caused by stem borers on different developmental stages of the NERICA lines

showed that all are susceptible to stem borers attacks, and that the extent of damage varies according to NERICA line and the pest species. Damage observed (white panicle) was the result of feeding activity of Diptera and Lepidoptera larvae in the stem by the larvae of borers (Stoll, 2002).

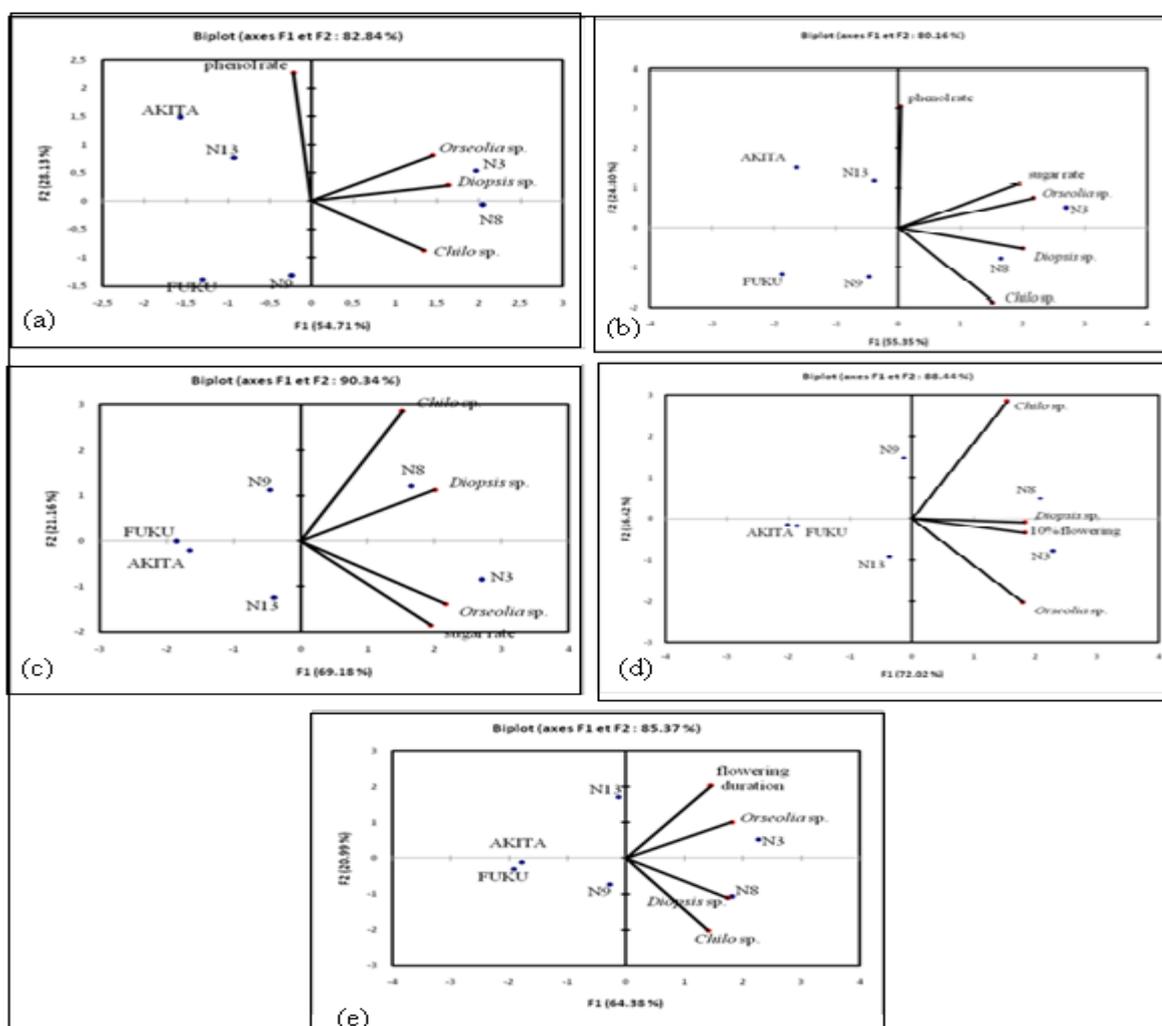


Fig. 3. (a) Study of the relationship between the rate of phenols, the rice stem borer species and rice varieties, (b): The relationship between the rate of total sugars, polyphenol, stem borer species and rice varieties, (c): relationship between the rate of total sugars, the stem borer species and rice varieties, (d): Relationship between Early flowering stage, the stem borer species and rice varieties, (e): Relationship between the flowering duration, the stem borer species and rice varieties.

The Japanese varieties, Akitakomachi and Fukihibiki showed no “death heart” neither “white panicle”; this could be explained either by the fact that they present a low total sugar rate or the fact that they are resistant to the borers’ species. From the NERICA 13 and NERICA 9 stems emerged two borer species; *Orseolia*

oryzivora Harris and Gagné, 1982 represented by its parasitoid *Platygaster diplosisae* and *Chilo zacconius* Blez (Lepidoptera). NERICA 13 appeared as a resistant variety; this may be explained by the high rate of poly phenols in its stems. Compared to the three other varieties, NERICA 3 was a late flowering

variety. Late flowering plant varieties are known to be more susceptible to pest insects as their long life cycles made them to be exposed to stem borer for a longer period. The value of total sugars content of stems was higher in NERICA 3, which had the highest number of white panicles. The rate of total phenols was higher within the Akitakomachi variety and it has not presented aborted panicles. This could be the reason why Akitakomachi is resistant to stem borers. Consequently, plant susceptibility may be related either to the total sugar or polyphenol content of stems. These results corroborate those of Vijaykumar *et al.* (2009) who argue that the total sugars rate is not related to the resistance of rice varieties on the contrary, high levels of sugars contribute to the susceptibility of the plant regarding the stem borers; as NERICA 3, which has the highest sugar levels is the most sensitive.

From the white panicles harvested emerged respectively two species of Diptera stem borers *O. oryzivora* and *D. apicalis* and the Lepidoptera stem borer *C. zacconius*. These results are not in conformity with those of Ondo Ovono *et al.* (2014) who stated that “white panicles” in rice were caused by Lepidoptera larvae and that Diptera stem borers were encountered in the NERICA rice variety only during the vegetative stage. In our data, attacks of the Lepidoptera stem borers spread over all rice growth stages. Then, they concluded that Lepidoptera stem borers were the main constraint of (caused of dead hearth and white panicles) NERICA varieties cultivated in Franceville at Gabon (Ondo Ovono *et al.*, 2014). This statement was similar to that of Brink and Belay (2006). In our results African rice gall midge seems to be the most harmful stem borer. *Oryza sativa* is known as the preferred host plant of these insect pests, with more than 15% of galls recorded in the wet season; its damage is negligible in the dry season (Dakouo *et al.*, 2004). However, further study are needed to clarify whether this is related or not to environmental conditions. It is suggested that Lepidoptera stem borer prefer the dry season which spans from the months of August to December while Diptera stem borer may prefer the rainy season which

lasts from March to July.

Deuse and Appert (1988) on one hand and Olalekan (2002) on the other reported that the tillering stage was more favourable to the proliferation of flies stalk. However, no insect species emerged from the dead hearts tillers harvested. On the field, rice tillers maintained their softness up to heading. This may explain why the Diopsid population settled, multiply and become sedentary causing as many dead hearts as white panicles (Bijlmakers and Verhoek, 1995).

Conclusion

A total of three pest species, including two flies *Orseolia oryzivora* and *Diopsis apicalis* and a moth, *Chilo zacconius* were recorded from rice tillers incubated. *O. oryzivora* appeared as the most harmful species. NERICA 3 was the most susceptible variety among the NERICA Akitakomachi and Fukuhibiki showed no attack.) Because of its long cycle, its high sugar rate and its low polyphenols rate, NERICA 3 was the most susceptible variety. However, the high larval mortality rate recorded does not allow a fine determination of stem borer species impact on rice yield in our plots. Further studies, based on the dissection of panicles collected and identification of pest species from the larva or maggot would allowed improving this conclusions.

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