



Pathogenicity of indigenous entomopathogenic nematodes from Benin against mango fruit fly (*Bactrocera dorsalis*) under laboratory conditions

Anique Godjo^{a,b,*}, Lionel Zadjé^a, Wilfrida Decraemer^c, Anne Willems^b, Leonard Afouda^a

^a Fac. Agronomy, University of Parakou, 02 B.P.1003, Parakou, Benin

^b Dept. Biochemistry and Microbiology, Fac. Sciences, Ghent University, K. L. Ledeganckstraat 35, 9000 Gent, Belgium

^c Dept. Biology, Fac. Sciences, Ghent University, K. L. Ledeganckstraat 35, 9000 Gent, Belgium

ARTICLE INFO

Keywords:

Biological control

Tephritid

Heterorhabditis taysearae

Steinernema sp.

Mangifera indica L.

ABSTRACT

Bactrocera dorsalis fruit fly is the economically most significant tephritid pest species on Mango, *Mangifera indica* L., in Benin, and entomopathogenic nematodes (EPNs) represent good candidates for its control in the soil. In this study, the susceptibility of larvae and pupae of *B. dorsalis* to 12 EPN isolates originating from Benin was investigated. The effect of nematode concentrations (20, 50, 100, 200 and 300 Infective Juveniles (IJs)/*B. dorsalis* larva) and of different substrate moisture content (10, 15, 20, 25 and 30% v/w) on *B. dorsalis* mortality at the larval stage was studied. Also, the reproduction potential inside *B. dorsalis* larvae was assessed. Our results revealed that the susceptibility of *B. dorsalis* larvae was significantly different among the 12 tested nematode isolates with *H. taysearae* isolate Azohoue2 causing the greatest insect mortality (96.09 ± 1.44%). The lowest insect mortality (7.03 ± 4.43%) was recorded with *Steinernema* sp. strain Bembereke. Significant differences in insect mortality were recorded when EPNs were applied at varying IJs concentrations. A concentration of 100 nematodes of either *H. taysearae* Azohoue2 or *H. taysearae* Hessa1 per *B. dorsalis* larva was enough to kill at least 90% of *B. dorsalis* larvae. Larvae were less susceptible to nematodes at higher moisture content (25% and 30%). In addition, pupae were less susceptible to nematodes than larvae. Furthermore, the tested nematode isolates were able to reproduce inside *B. dorsalis* third instar larva with the *Heterorhabditis* isolates giving the greatest multiplication rate (59577.2 IJs ± 14307.41).

1. Introduction

Mango (*Mangifera indica* L., Anacardiaceae) is one of the most important tropical fruits produced in West Africa, a region most favorable for fruit production and export (Vannière et al., 2004; Gerbaud, 2007; Vayssières et al., 2009a). Mango fruit constitutes a very important source of nutrition for rural populations living in northern Benin (Vayssières et al., 2008). In Africa and particularly in Benin, the production of this fruit is confronted with several problems including quality loss due to fruit flies (Tephritidae, Diptera), especially *Ceratitis capitata*, *Ceratitis cosyra* and *B. dorsalis* (Vayssières et al., 2009b). The latter, formerly known as *Bactrocera invadens* (Schutze et al., 2014), is the most important pest causing serious damage in orchards of mango as well as in other important tropical fruit crops including guava and citrus (Goergen et al., 2011; Vayssières et al., 2009b). Chemical applications have been used as traditional methods to control these fruit flies for many years. For example, Spinosad GF-120 (Spinosad + foodstuff attractant) and Proteus 170 O-TEQ (Thiaclopride + Deltamethrine) showed great performance for control of flies (Vayssières et al., 2009a;

N'Depo et al., 2015). However, the environmental side-effects have led to interest in other, environmental friendly, cost effective and locally available control strategies to enhance mango production and export. In this respect, several control methods have recently been developed including the sterile insect technique (Clarke et al., 2011) and the biological control based on the use of weaver ants, *Oecophylla smaragdina* and *Oecophylla longinoda*, (Anato et al., 2015; Offenberg et al., 2013; Wargui et al., 2015). Unfortunately, the latter method is associated with some constraints as the ants delay the labor during harvest and are responsible for small black spots left on the fruit (Sinzogan et al., 2008).

EPNs of the genera *Steinernema* (Panagrolaimomorpha: Steinernematidae) and *Heterorhabditis* (Rhabditomorpha: Heterorhabditidae) are effective biocontrol agents (Grewal et al., 2005). They have been found in most countries and are successfully used to control many insect pests around the world (Ehlers, 2001). Several strains of *Heterorhabditis taysearae*, *Heterorhabditis indica* and *Steinernema* sp. have been isolated from Benin and all demonstrated a cruiser type insect search strategy (Zadjé et al., 2014b). *H. taysearae*

* Corresponding author at: Dept. Biochemistry and Microbiology, Fac. Sciences, Ghent University, K. L. Ledeganckstraat 35, 9000 Gent, Belgium.
E-mail address: TognisseAnique.Godjo@UGent.be (A. Godjo).

<http://dx.doi.org/10.1016/j.biocontrol.2017.10.009>

Received 1 January 2017; Received in revised form 12 October 2017; Accepted 17 October 2017

1049-9644/ © 2017 Elsevier Inc. All rights reserved.

Shamseldan et al., 1996, has been recently considered as a senior synonym of *Heterorhabditis sonorensis* Stock et al., 2009 by Hunt and Subbotin (2016).

The Infective Juvenile (IJ) represents the only free-living developmental stage of EPNs that occurs naturally in the soil. They are symbiotically associated with bacteria of the family Enterobacteriaceae which belong to the genera *Xenorhabdus* (*Steinernema*) or *Photorhabdus* (*Heterorhabditis*) (Ciche et al., 2006). IJs of both genera *Steinernema* and *Heterorhabditis* can infect the insect larvae via body openings such as anus, mouth or spiracles (Campbell and Lewis, 2002). In addition to these ways of penetrating the insect host, *Heterorhabditis* species are able to actively enter the hemocoel through the host cuticle by the use of their additional dorsal tooth to perforate the inter-segmental membrane of the cuticle (Bedding and Molyneux, 1982; Griffin et al., 2005). Inside the host they release intestinal bacteria into the insect hemocoel. These bacteria reproduce and produce metabolites that kill the insect within 1–2 days (Dowds and Peters, 2002) and serve at the same time as food source for the nematode. An effective sustainable *B. dorsalis* management approach could be the use of EPNs to control insect pests at soil-borne stages of the insect life cycle. Indeed, the late larval instar of *B. dorsalis* leaves the infested fruit and falls on the ground where it burrows in the top 4 cm of the soil prior to pupating after a short dispersal period (Hou et al., 2006). Adult flies emerge from pupae after 1–2 weeks (longer in cool conditions). This offers an opportunity to EPN IJs present in the soil to invade *B. dorsalis* larvae or pupae even if the exposure time to the larvae is relatively short. Many studies have been conducted on the Mediterranean fruit fly *Ceratitis capitata* (Gazit et al., 2000; Lindegren and Vail, 1986; Lindegren et al., 1990; Malan and Manrakhan, 2009; Minas et al., 2016; Poinar and Hislop, 1981), the Queensland fruit fly *Bactrocera tryoni* (Froggatt) (Langford et al., 2014), the cherry fruit fly *Rhagoletis cerasi* L. (Herz et al., 2006), *Bactrocera oleae* (Sirjani et al., 2009), *Bactrocera cucurbitae*, *B. dorsalis* (Lindegren and Vail, 1986) and the Natal fruit fly *Ceratitis rosa* (Malan and Manrakhan, 2009) and have demonstrated that the flies were highly susceptible to *Steinernema* and *Heterorhabditis* nematodes.

Based on these previous studies and their known biocontrol abilities, EPN of the families Heterorhabditidae and Steinernematidae in association with their symbiotic bacteria *Photorhabdus* and *Xenorhabdus* respectively, are considered to be promising biocontrol candidates against *B. dorsalis* on mango trees in Benin.

Several studies have revealed that indigenous EPNs are well adapted to local environmental conditions and therefore considered as good biological agents to control insect pests (Bedding, 1990; Grewal et al., 1994; Noujeim et al., 2015; Zadji et al., 2014b). To our knowledge, the susceptibility of *B. dorsalis* to Beninese EPNs has not yet been investigated. The current study is one of a series anticipated for the implementation of cost-effective *B. dorsalis* management using EPNs in mango orchards in Benin. It aimed to: (i) investigate the occurrence of EPNs in mango orchards in northern Benin, (ii) identify the recovered EPN isolates, (iii) test their pathogenicity against mango fruit fly (*B. dorsalis*) under laboratory conditions. Specifically, 12 EPN isolates from Benin were screened for their virulence against the third instar larvae of *B. dorsalis* and the most virulent isolates were selected to investigate the susceptibility of larvae and pupae of *B. dorsalis* under different abiotic laboratory conditions.

2. Materials and methods

2.1. Source of insects

B. dorsalis used in this study were obtained from laboratory rearing initiated from *B. dorsalis* pupae provided by IITA-Benin (International Institute of Tropical Agriculture-Benin). The original colony of *B. dorsalis* used at the IITA-Benin institute was established from naturally infested mango fruits collected in Northern Benin. Flies were fed with a mixture of brown sugar and yeast extract at 3:1 proportion (Vayssières

et al., 2015). Cages were supplied with water. Ripened papaya fruits were exposed to 10 day old female flies to allow them laying eggs into the ripened papaya used as host. The infested papaya was incubated at 28 °C and 60–80% relative humidity (RH) during 7 days, after which the third instar of *B. dorsalis* larvae started to exit the fruit. We used in our assays the third instar larvae collected approximately 1 h after they had jumped from infested papaya to pupate. Larvae that were not used in assays were left in sand with 10% humidity to pupate and become adults within approximately eight days.

2.2. Source of nematodes

Most of the nematodes used in this study were provided by the Laboratoire de Phytotechnie, d'Amélioration et de Protection des Plantes (LaPAPP), Benin. They were collected from soil in several vegetations (Table 1) in Benin (Zadji et al., 2013). Other nematodes were newly collected from a local soil sampling (January–February 2015) exclusively in several mango orchards located in northern Benin. Seventy soil samples in total were collected from fourteen mango orchards (each at least 1 hectare of area) selected at random in eight villages of Parakou, Borgou department located in northern Benin. In each orchard, 5 samples of approximately 1.5 kg each were taken randomly at ≤ 15 cm depth. Each soil sample was individually processed for nematode extraction using the *Galleria mellonella* (Lepidoptera, Piralidae) baiting method (Bedding and Akhurst, 1975) and white trap (White, 1927). Pathogenicity of the isolated nematodes was confirmed by re-infesting fresh *G. mellonella* larvae as described above and newly emerged IJs collected from white trap were kept at 13 °C for further study.

The nematode species, sample number, origin, vegetation and accession numbers of all EPN isolates included in this study are presented in Table 1. Nematodes used for the assays were acclimated for 2 h at room temperature (25 °C) after removal from incubator (13 °C) to help them adjust to the new temperature and allow better performance. Nematode viability (based on their movement) was checked under a stereomicroscope. The concentrations of nematodes were calculated by volumetric dilutions in tap water using the formula of Navon and Ascher (2000).

2.3. Nematode identification

The identity of most of the nematode isolates provided by the LaPAPP laboratory was described by Zadji et al. (2013). However, new nematode isolates retrieved from soil samples collected in mango orchards were identified in this study (Table 1).

2.3.1. Molecular identification

For each nematode isolate, DNA was extracted from a single specimen in an Eppendorf tube (250 µl) containing 1 µl of double distilled water. Ten µl of 0.05 N NaOH was added plus 1 µl of 4.5% Tween 20 solution (Janssen et al., 2016). The tube was heated at 95 °C for 15 min and cooled at room temperature prior to storage at 4 °C for use within next month or at –20 °C for later use. The ITS region was amplified and sequenced using the primers pair AB28 (ATATGCTTAAGTTCAGCGG-GT) and TW81 (GTTTCCTAGGTGAACCTGC). ITS sequences were aligned with their closest BLAST search matches (obtained from GenBank database) using ClustalW Multiple alignment. Afterwards, a phylogenetic tree was generated in Mega-6 software using the Neighbor-Joining method (Saitou and Nei, 1987). *Caenorhabditis elegans* EU131007 was used as outgroup.

2.3.2. Morphological/morphometric identification

Light microscopic pictures were taken using a Soft Imaging System GmbH (Cell'D software, Münster-Germany) connected to an Olympus BX51 microscope. The same system was used to measure 20 IJs and, 20 F1 males of the studied nematode strains. Juveniles were heat killed

Download English Version:

<https://daneshyari.com/en/article/8877750>

Download Persian Version:

<https://daneshyari.com/article/8877750>

[Daneshyari.com](https://daneshyari.com)