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Sweet potato *Ipomoea batatas* (L.) storage practices used in southern Benin and the use of entomopathogenic nematodes to control sweet potato weevil (*Cylas puncticollis* Boheman) under laboratory conditions

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Abstract
A survey was conducted in 2013 in sweet potato (SP; *Ipomoea batatas* (L.)) fields in Benin to record SP tuber storage practices and assess the efficacy of indigenous isolates of entomopathogenic nematodes (EPN) for the control of SP weevils (*Cylas puncticollis*). The SP tubers werestored in rooms, mounds, stores, or kitchens for 2–28 weeks. Tuber damage severity varied with surveyed site between 38.0% (low damage) and 6.3% (low to moderate damage). Under laboratory conditions, weevil population increased up to 23.53 times inside tubers and tuber weight loss reached 71.64% with maximum tuber powder of 2.86g. Nematodes were foundto efficiently suppress SP weevils inside tubers and to reproduce inside the host giving up to 2193.42 nematodes per insect larvae. Damage to tuber pieces, weevil and EPNprogeny production, and percentage weight loss of tubers varied significantly ($P \leq 0.05$) among EPN isolates. These results showed EPN are promising candidates for successful SP weevil control in Benin.

Keywords: Biological control, entomopathogenic nematode, sweet potato storage practice, sweet potato weevil

1. Introduction
Sweet potato (SP), *Ipomoea batatas* (L.) Lam: Convolvulaceae, is a tuber crop that is grown in tropical and subtropical latitudes around the world [1]. According to Scott *et al.* [1] the importance of SP is likely to increase over the next 20 years.It is currently grown in more than 100 countries as a valuable source of food and an industrial raw material [2]. The crop supplements family income, and therefore strategies to reduce losses to pests and diseases would dramatically boost production and have a positive impact on the livelihoods of millions of poor farmers across sub-Saharan Africa. In Benin, SP was marginalized in research and development programs until recent years [3].

Talekar (unpublished) listed 280 insect pests and 18 mite species associated with SP in the field and in storage. Sweet potato weevils of the genus *Cylas* (Coleoptera: Apionidae) are considered the most important SP pests worldwide. In Africa, *C. puncticollis* Boheman and *C. brunneus* Fabriciusare major production constraints, whereas in America and Asia *C. formicarius elegantulus* summers is the major pest [4]. Weevil infection ranges from 20 to 50% on many farms worldwide and can reach 100% depending on SP variety, especially during dry seasons or storage [5]. Adult SP weevils feed on the tender buds, leaves, vines, and tubers, while the larvae, the most destructive stage, feed and tunnel into mature stems and tubers. As weevils feed on the tubers, they produce a powder that is a mixture of the insects' feces and residue from the tubers (H. Baimey, University of Parakou, 2013, personal observation). Tissue damage leads to thickening, drying, and cracking of the stems and to secondary infection by bacteria and fungi. This makes the tubers unfit for human or animal consumption as the damaged tissues produce terpenes in response to pest attack [6]. The terpenes give the tubers an unpleasant odor and bitter taste, leading to a reduction in their market value [6].

Historically, SP pest management has been a neglected research topic worldwide, especially in the developing world.

The concept and practice of SP integrated management have only recently begun to mature. Some control methods have been tested against SP weevils, such as hazardous synthetic chemical insecticides, plant extracts, early planting, sanitation, resistant varieties, sex pheromones, insect parasitic fungi (*Beauveria bassiana* and *Metarrhizium anisopliae)* and bacteria *(Bacillus thurgiensis*), ants *(Pheidole megacephala* and *Tetramorius guineense*), and entomopathogenic nematodes (EPN; *Heterorhabditis* spp. and *Steinernema* spp.) [3]. However, the cryptic feeding habits of the SP weevil larvae and the nocturnal activity of the adults make detection and control of infestations difficult with most of the above-mentioned methods [5, 7].

Mauléon and Denon [8] observed a decrease in *C. formicarius* populations from 50 at SP planting to 10 and 25,2 months later using a combination of an EPN (*H. indica*) and a sex pheromone [(Z)-3-dodecen-1-ol (E)-2-butenoate] or a sex pheromone alone, respectively, to control the insect population. In Benin, where the pest also impacts SP yields countrywide, its control is mainly based on the use of hazardous pesticides such as Furadan, Oncol Super, Carbofuran, or Regent at pre-planting doses of 4–8kg/ha depending on the pesticide[3]. However, chemical control markedly contributes to agro-ecosystem imbalance, and has non-target effects, especially against natural enemies [9]. Furthermore, the cost of chemical pesticides limits their use by small-scale farmers.

Several studies indicate EPN of the families Heterorhabditidae and Steinernematidae as potential biological control agents against *Cylas* spp. [10]. The nematodes can migrate through the SP rhizosphere in search of pest larvae [11], which are difficult to reach by other control methods. The infective juvenile (IJ) stage of the nematode is the only stage living in the soil searching for the insect host. In contact with the host, the IJ penetrates the later via spiracles, mouth, anus or intersegmental membranes of the cuticle and then enters into the haemocoel of the host [12]. Both *Heterorhabditis* and *Steinernema* nematodes are mutualistically associated with bacteria of the genera *Photorhabdus* and *Xenorhabdus*, respectively [13]. Once inside the haemocoel of the insect host, the nematodes release the symbiotic bacteria which multiply significantly and kill the host within 24 to 48 h. After the death of the host, nematodes continue to feed on the host tissue, mature, and reproduce. When food resources are available, several generations of the nematodes can occur inside the host cadaver. Subsequently, large numbers of IJ are eventually released into the environment to infect new hosts and continue their life cycle [14]. Mannion [11] reported that the EPNs have the ability to exit infected SP weevil cadavers within tubers and to infect new hosts in the soil. Infectivity, defined as the ability of nematodes to cause infection in a target insect, and reproductive capacity, defined as progeny production, vary with EPN isolate and with specific target hosts [11]. The current paper reports the results of a diagnostic survey on weevils affecting SP production in southern Benin and of laboratory experiments conducted to managethe pest population using indigenous EPNs. It represents the start of a series of experiments that need to be conducted to identify sustainable, environmentally safe, and cost-effective methods of control of SP weevils in SP fields in Benin.

2. Materials and Methods

2.1 Survey

Twenty-one SP fields located in seven major SP production localities in southern Benin (Akassato, Azinzonkanmey, Gbefadji, Glo-Djigbe, Sekou, Tokpadome, and Zinvie) were surveyed in September 2013 for damage caused to SP by SP weevils. Three fields were surveyedin each locality. The SP farmers were questioned on local names of farmed cultivars, where tubers were stored, methods used to store them, and the duration of tuber storage. Subsequently, three heaps of around 200 to 300 tubers each were visually assessed for insect damagein each field or in each store. Assessment of damage severity was done based on an arbitrary scale of 0–4,where 0 = clean tuber; 1 = 1–25% of tuber skin showing dry rot symptoms (low level of damage); 2 = 26–50% of tuber skin showing dry rot symptoms (low–moderate level of damage); 3 = 51–75% of tuber skin showing dry rot symptoms (moderate–severe level of damage); 4 = 76–100% of tuber skin showing dry rot symptoms (high level of damage)[15]. Where found, the weevils were collected and identified as *Cylas puncticollis* at the insect museum of the International Institute of Tropical Agriculture, Cotonou station, Benin, by Dr. Goergen Georg.

2.2 Laboratory experiments

Plant material: SP tubers (cultivars ‘Gboado’and ‘Glassodji’) used in the experiments were harvested 4 months after planting ina *Cylas*-free field where SP was grown with no application of pesticides.

Nematodes: Ten indigenous EPNisolates (all *Heterorhabditis sonorensis*) were obtained from the Laboratory of Nematology of the Higher National School of Agronomic Sciences and Techniques of Djougou (ENSTA-Dj). They were extracted in the laboratory from soil samples previously collected in southern and central parts of Benin during diagnostic surveys for EPN conducted in 2012 (see details in Table 1)[16]. Nematode IJ used in the experiments were 5 days old at the time of their use.

Table 1: Information on the origins of indigenous isolates of *H.sonorensis* recovered in southern and central Benin and on types and pH of soil samples from which the nematodes were extracted.

Isolate name	Current name	Origin (village, commune, department)	Tree	Soil type[γ]	Soil pH[γ]
22b	Zoundomey	Zoundomey, Lalo, Couffo	Orange	Sandy	5.9
32b	Azohoue	Azohoue, Tori-Bossito, Atlantique	Mandarin	Sandy loam	6.1
44a	Kemondji	Kemondji, Zakpota, Zou	Orange	Sandy clay	6.5
45a	Zangnanado	Zagnanado, Cove, Zou	Teak	Sandy clay	7.1
5d	Ouere	Adja-Ouerecentre, Plateau	Orange	Sandy	6.0
62b	Djidja	Djidja, Djidja, Zou	Neem	Sandy loam	6.2
63c	Kassehlo	Kassehlo, Djidja, Zou	ALB[§]	Sandy clay	6.5
67d	Avokanzoun	Avokanzoun, Bohicon, Zou	Oil palm	Sandy clay	5.2
80a	Ze	Awokpa, Ze, Atlantique	Banana	Sandy	5.8
9d	Hessa	Hessa, Adjohoun, Oueme	Orange	Sandy	5.1

[γ]Soil type and pH were determined in the laboratory of soil sciences at the University of Abomey-Calavi, Benin. [§]African locust bean.

The insect: Populations of SP weevils (*C. puncticollis*) used in the experiments were initially reared from single males and single females taken from a SP field located in Azinzonkanmey village in the commune of Kpomasse, Southern Benin. The insects were permanently reared on *Cylas*-free SP tubers and were identified as *C. puncticollis* by their morphological features. Fresh adult insects were used in the experiments.

a) Damage caused by sweetpotato weevils to storage tubers

Two sizesof *Cylas*-free SP tubers (*ca*. 150 g and 300 g) of the cultivars 'Gboado' and 'Glassodji', were used. They were individually placed in 2.5-l plastic containers. Twenty SP weevil adults (15 females and 5males) were transferred into each container and placed on the tubers. Control tubers received no weevils. Containers were arranged in a completely randomized design and replicated 10 times. Tubers were exposed to weevils for 1, 4, 8, 12, or 16 days, after which the weevils were removed from the containers. Thirty days after transferring the weevils into the containers, powder from SP tubers was collected using a brush and was weighed. Live and dead adult weevils were collected and counted separately. Tubers were carefully dissected and weevil larvae collected and counted. The total final weevil population was calculated as the sum of populations of adults and larvae collected for each treatment (i.e. daysof tuber exposure to weevils) and was used to calculate the proportion of each category of weevil (i.e. live and dead adults and larvae). Increase in weevil population was calculated as the final weevil population divided by the initial weevil population. Fragments of the tubers were weighed and percentage tuber weight loss was calculated. Control tubers were evaluated only for powder weight and percentage weight loss onthe same dayas infected tubers. The experiment was repeated once, with a new batch of fresh adult SP weevils.

b) Nematode infectivity

Cylas-free SP tubers were cut into slices of *ca*. 10 g using a cork borer. The surface of the tuber slices was left to air dry for 4h to minimize growth of microorganisms such as fungi and bacteria. The tuber slices were individually transferred into 9-cm-diameter Petri dishes and eight adult SP weevils (five females and three males) were added to each dish. Petri dishes were then closed and arranged in a completely randomized design replicated five times. After 14 days, the eight adults of SP weevils (alive or dead) were taken from the Petri dishes. The SP tuber slices were inoculated each with EPN (20 nematodes per slice) using a 0.5–10 µl micropipette. Ten EPN isolates (all *H. sonorensis*) were used (see Table1). Some other slices received no weevils and no nematodes (Control 1). Others received the weevils but were not inoculated with nematodes (Control 2). Five days post-inoculation, the powder of SP tuber slicein each Petri dish was collected using a brush and weighed. The SP tuber slices were carefully dissected individually, weighed, and live and dead SP larvae of the weevils observed, removed, and counted. For each treatment, total final weevil population, proportion of live and dead larvae, and percentage tuber weight loss were calculated as described for the experiment on damage caused by weevils to storage tubers. The experiment was repeated oncewith fresh SP weevils and nematodes.

c) Nematode progeny production

To assess nematode progeny production inside larvae of SP weevils, five larvae cadavers were collected per treatment and transferred individually into traps [17]. Three weeks later, nematodes that emerged from the larvae cadavers were collected and their population densities assessed.

d) Statistical data analysis

Experimental data were subjected to an analysis of variance (ANOVA). Nematode population densities were compared using the SAS system, Version 9.2 for Windows 2008[18]and the Student–Newman–Keuls test ($P \leq 0.05$). Nematode population densities were $\log_{10}(x+1)$ transformed prior to analyses to normalize the data. Percentage visual assessment data were normalized using Arcsin[sqrt (x/100)] transformation prior to analyses.

3. Results

Nine local names were given toSP cultivars grown in the surveyed area: 'Amankan', 'Boounbo', 'Dokunwewe', 'Glassodji, 'Gboado', 'Hanman', 'Soamiva','Toffiweli', and 'Weli'.

AllSP farmers stored their harvest in rooms at home (indoor storage). Among them, 33.33% kept also their harvest in the field unharvested (in-ground storage) while 9.52% also kept it in stores, and 4.76% also in kitchens (Fig. 1A). The SP tubers were stored mainly in jute bags (52.38% of farmers). However, 33.33% of farmers stored tuberson the floor, 28.57% in mounds in fields, and 14.29% in baskets (Fig.1B). Harvested tubers can be stored for 2–8 weeks without deterioration (10.00% to 36.67% of farmers); however, when kept in mounds (i.e. unharvested), they can be stored for 12–28weeks (3.33% to 20.00% of farmers) (Fig.1C). Half (50.00%) of the tubers visually assessed looked clean and were considered weevil-free. For infected tubers, percentage infection varied between 38.00% (low damage) and 6.30% (low to moderate damage). No tubers had a high level of damage (Fig. 1D).

The longer the time of exposure of tubers to weevils, the higher the weight of tuber powder. The weight of powder from tubers exposed to weevils for 12 or 16 days (2.71 and 2.86 g, respectively) were similar ($P \leq 0.05$). The same observation was made for 1 and 4days of exposure (1.35 and 1.69 g, respectively). However, the differences between these two groups were significant ($P \leq 0.05$). No powder was observed in containers receiving no weevils (Table 2).

Observations made for the population of live and dead adult weevils reflected those made for SP tuber powder weight. The final weevil population increased with the duration of exposure of tubers to weevils from 147.30 to 470.50. The final weevil populations collected from the tubers exposed to the insects for 1, 4,or 8 days were significantly lower ($P \leq 0.05$) than those collected after 12 or 16 days of exposure (Table 2).

The insect population increased with the duration of exposure of tubers to the weevils from a mean of 7.37 (1 day of exposure) to 23.53 times (16 days of exposure) with significant differences between means (Table 2).

On each assessment date, the proportion of live insect adults was significantly higher ($P \leq 0.05$) than that of dead adults and that of larvae (Table 2).

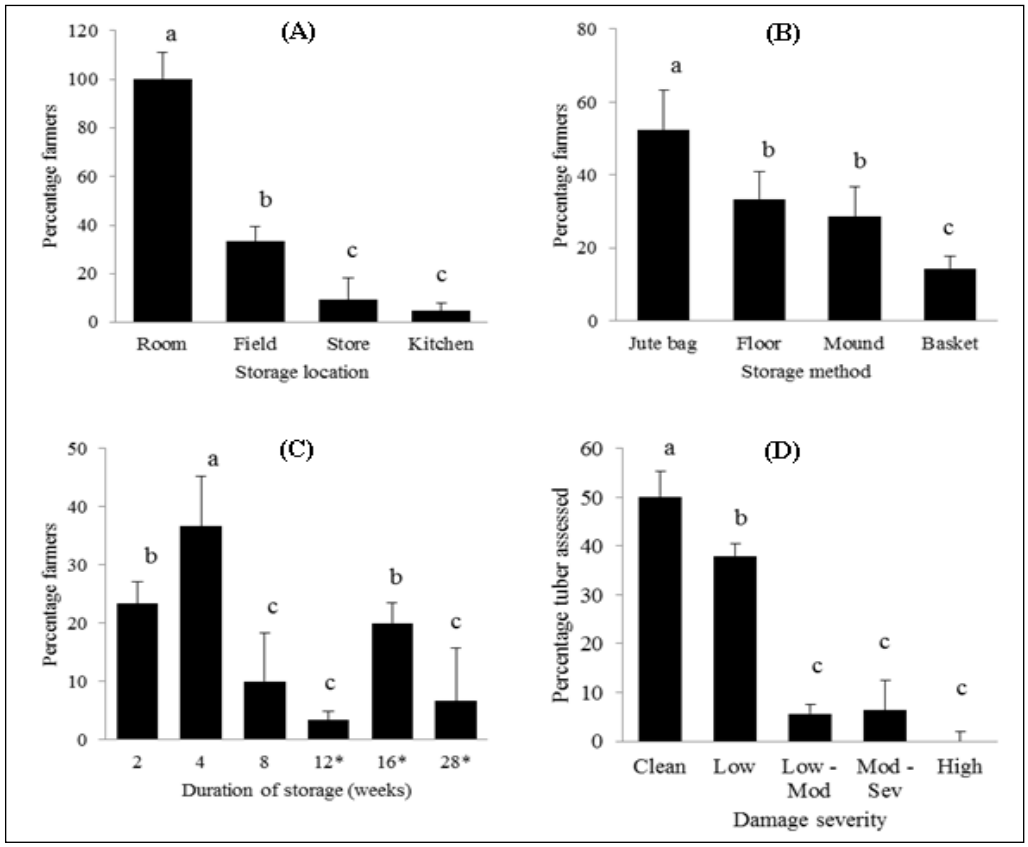

Fig. 1: Locations (A), methods (B) and duration (C) of storage of sweet potato tubers and damage severity (D) caused by sweet potato weevils (*Cylas puncticollis*) to sweet potato tubers observed during a survey conducted in southern Benin in September 2013.

Columns with the same letter in each figure represent non-significant differences according to the least significant difference test ($P \leq 0.05$). Error bars represent standard errors. In Fig. 1C, "" represents numbers of weeks of keeping sweet potato tubers unharvested in the field. Low–Mod and Mod–Sev indicate low–moderate damage and moderate–severe damage, respectively.*

The proportion of dead adults increased from 2.17% to 6.84% as days of tuber exposure to weevils increased. The opposite was found for the proportion of larvae, which decreased from 3.53% to 2.17%. The proportions of live adults were similar after 1 and 4 days of exposure of tubers to weevils but significantly lower than those of live adults after 8, 12, and 16 days of exposure (Table 2).

The weight loss of SP tubers increased with the duration of their exposure to weevils and reached 71.64% after 16 days of exposure. This was significantly different ($P \leq 0.05$) from the 49.01% and 53.64% observed with 1 and 4 days of exposure, respectively. Control tubers lost an average of 40.18% of their initial weight, which was similar to the weight loss observed when roots were exposed to weevils for 1 day, and significantly different from weight losses observed for other treatments ($P \leq 0.05$; Table 2).

Table 2: Damage caused to sweet potato tubers by sweet potato weevils, *Cylas puncticollis*, according to duration of exposure of the tubers to insects and consequent variation in weevil population inside infected tubers.

Days of tuber exposure to weevils	Tuber powder weight (g)	Total final weevil population	Increase in weevil population	Proportion of live adults	Proportion of dead adults	Proportion of larvae	Tuber weight Loss (%)
1	1.35c	147.30c	7.37c	94.30aA	2.17bB	3.53aB	49.01bc
4	1.69bc	167.70c	8.39c	94.45aA	2.27bB	3.28aB	53.64b
8	1.96b	267.40b	13.37b	91.32cA	5.61abB	3.07aB	65.64a
12	2.71a	439.00a	21.95a	92.14bcA	5.97abB	1.89bB	69.94a
16	2.86a	470.50a	23.53a	90.99cA	6.84aB	2.17abB	71.64a
Control	0.00d	-	-	-	-	-	40.18c
Std. Error	0.17	20.16	2.25	8.46	4.11	1.16	3.16

Each mean represents mean value from 150 and 300 g tubers. Means followed by the same lowercase letter in a column are not significantly different according to least significantdifference (LSD) test, ($P \leq 0.05$). For proportion of live adults, dead adults and larvae, means followed by the same capital letter for the same treatment (i.e. day of tuber exposure to weevil) are not significantly different according to the LSD test ($P \leq 0.05$).

The weight of powder from tubers, the total final weevil population, the increase in weevil population and the proportion of dead weevil adults were significantly higher ($P \leq 0.05$) for tubers initially weighing 300g than for tubers weighing 150 g. However, the proportion of live adults and of larvae and also of weight loss of tubers did not vary significantly ($P > 0.05$) with initial tuber weight (Table3).

Table 3: Effect of sweet potato weevil, *Cylas puncticollis*, on sweet potato tubers and reproduction of the weevils inside the tubers.

Tuber weight (g)	Tuber powder weight (g)	Total final weevil population	Increase in weevil population	% Population of live adults	% Population of dead adults	% Population of larvae	% tuber weight loss
150	0.78b	143.70b	7.19b	95.57aA	0.95bB	3.48aB	57.39a
300	2.75a	355.26a	17.76a	90.27aA	7.16aB	2.57aB	59.30a
Std. Error	0.10	15.20	0.80	9.27	2.01	0.20	1.82

Each mean represents mean value from 150 and 300 g tubers. Means followed by the same lowercase letter in a column are not significantly different according to the least significant difference (LSD) test, ($P \leq 0.05$). For proportion of live adults, dead adults and larvae, means followed by the same capital letter for the same treatment (i.e. day of tuber exposure to weevil) are not significantly different according to the LSD test ($P \leq 0.05$).

The weight of powder from tubers varied with nematode isolate from 0.00 g (Control 1) to 0.38 g (Control 2) with significant differences ($P \leq 0.05$) between mean values. In the presence of nematodes, tuber powder weight was similar for isolates 67c, 62b, 9d, 32b, 22b, and 5d (0.20–0.26 g) and significantly lower than that of isolates 45a, 63c, 80a, and 44a (0.27–0.35 g) (Table 4).

The total final weevil population ranged from 10.80 (isolate 9d) to 20.40 (isolate 5d) with significant differences between the two means. Final population of weevils for Control 2 (19.20) was significantly higher than that for nematode isolates 9d and 80a (10.80 and 12.40, respectively). For all nematode isolates and for Control 2, the proportion of live larvae was significantly higher ($P \leq 0.05$) than that of dead larvae. No larvae were dead in Control 2 tuber pieces. The lowest proportion of live larvae was observed for nematode isolates 9d and 22b (51.85% and 53.33%, respectively). These proportions were similar to that of nematode isolate 80a (56.45%). The highest proportion of dead larvae was observed for nematode isolate 9d (48.15%). However, this proportion was similar to those of nematode isolates 22b, 80a, 62b, 67c, and 45a (46.67%, 43.55%, 36.26%, 32.89%, and 32.47%, respectively) (Table 4).

The percentage weight loss of tubers varied with nematode isolate witha lowest mean value of 48.34% observed when tubers were exposed to no weevils (Control 1). This value is significantly lower than that of Control 2, with tubers exposed to weevils (57.24%). The weight loss of tubers was significantly higher in the presence of weevils and nematode isolates 45a, 63c, 67c, 62b, and in Control 2 (58.48%, 55.38%, 54.72%, 54.22%, and 57.24%, respectively) than in the absence of weevils and nematodes (48.34%) (Table4).

Table 4: Effect of 10 isolates of insect parasitic nematodes on sweet potato weevil, *Cylas puncticollis* populations and consequent damage caused to sweet potato tuber pieces by the weevil.

Nematode isolate	Nematode name based on origin	Tuber powder weight (g)	Total final weevil population	Proportion of live larvae	Proportion of dead larvae	Tuber weight loss (%)
45a	Zangnanado	0.34a	15.40b	67.53bA	32.47abB	58.48a
63c	Kassehlo	0.35a	19.20ab	69.79bA	30.21bB	55.38ab
67c	Avokanzoun	0.20c	15.20b	67.11bA	32.89abB	54.72ab
62b	Djidja	0.23bc	18.20ab	63.74bA	36.26abB	54.22ab
9d	Hessa	0.23bc	10.80c	51.85cA	48.15aA	53.56abc
80a	Ze	0.27b	12.40c	56.45bcA	43.55aA	52.86abc
32b	Azohoue	0.23bc	19.20ab	71.88bA	28.13bB	50.64abc
44a	Kemondji	0.27b	15.80b	68.35bA	31.65bB	50.28abc
22b	Zoundomey	0.25bc	15.00b	53.33cA	46.67aA	49.86abc
5d	Ouere	0.26bc	20.40a	68.63bA	31.37bB	48.68abc
Control 1	–	0.00d	-	-	-	48.34c
Control 2	–	0.38a	19.20ab	100.00aA	0.00cB	57.24ab
Std. error		0.06	1.08	2.96	1.18	3.56

The 10 nematode isolates used were all *H. sonorensis*. Control 1 = no weevils and no nematodes applied; Control 2 = weevils applied but no nematodes applied. Means followed by the same lowercase letter in a column are not significantly different according to the least significant difference (LSD) test, ($P \leq 0.05$). For percentages of live and dead larvae, means followed by the same capital letter for the same treatment (i.e. day of tuber exposure to weevil) are not significantly different according to the LSD test ($P \leq 0.05$).

The correlation between 1) the duration of exposure of tuber pieces to weevils and initial tuber piece weight and 2) initial tuber piece weight and percentage tuber piece weight loss were weak and negative (Pearson correlation coefficients, *r* of –0.01 and –0.06, respectively). In other cases, the relationship was strong and positive and *r* reached 0.85 between tuber powder production and weevil multiplication (Table 5).

Table 5: Correlation between duration of exposure of sweet potato tuber pieces to the weevil *Cylas puncticollis*, initial weight of sweet potato tuber pieces exposed to the weevil, percentage weight loss of tuber pieces, weight of powder from tuber pieces, and weevil multiplication.

	Duration of exposure of tubersto weevils	Initial tuber weight	Percentage tuber weight loss	Tuber powder weight
Initial tuber weight	–0.01 (1.00)			
Percentage tuber weight loss	0.72 (0.01)	–0.06 (0.63)		
Tuber powder weight	0.55 (0.01)	0.64 (0.01)	0.50 (0.01)	
Population of weevils[§]	0.71 (0.01)	0.47 (0.01)	0.61 (0.01)	0.85 (0.01)

Values in the table represent Pearson's coefficients of correlation (*r*) and the values in brackets are probabilities (*P*-values). [§]Larvae and adult weevils (alive and dead).

Nematode progeny production inside SP weevil cadavers varied significantly ($P \leq 0.05$) with nematode isolate. The mean population density of nematodes emerging from single

SP weevils ranged from 570.0 with nematode isolate 9d to 2,193.4 with isolate 63c, with a significant difference between those two means ($P \leq 0.05$). Population densities of nematode isolates 62b, 5d, 67c, 45a, 80a, and 32b were similar ($P \leq 0.05$) and ranged between 1,006.8 and 1,483.0 (Fig. 2).

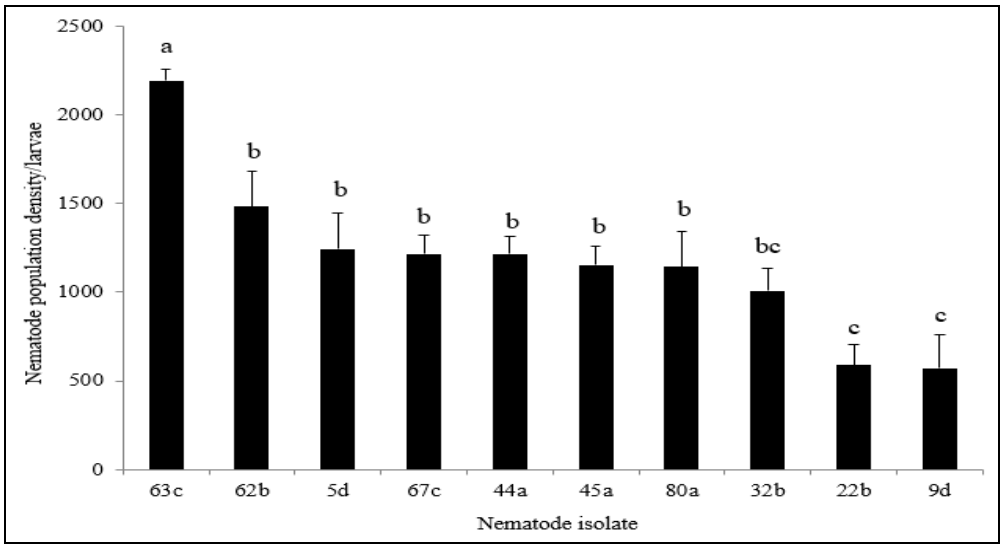

Fig 2: Progeny production of 10 indigenous insect parasitic nematodes in cadavers of larvae of sweet potato weevil, *Cylas puncticollis* under laboratory conditions.

Columns with the same letter represent mean nematode population densities that do not differ significantly according to the least significant difference (LSD) test, $P \leq 0.05$. Error bars represent standard errors.

4. Discussion

Our study indicated that in the survey area, indoor storage of SP tubers is mostly used by farmers, followed by in-ground storage, and tubers are kept in different containers or locations after harvest, i.e. in jute bags, in baskets, or left on the floor. Devereau [19] additionally observed pit storage and Mutandwa and Gadzirayi [20] clamp storage, which all extend the storage time of SP tubers to some degree. These various storage places might affect SP tuber quality and storage length differently. With environmental conditions of temperature and humidity varying during storage with storage method and location, the extent of eventual tuber infection by *Cylas* spp.will vary. Teye [21] indicated that when inappropriate SP storage technology is used, weevil infection aggravates the problems of high post-harvest losses to farmers, especially during glut seasons when the roots are in abundant supply. This explains why the successful preservation of harvested storage tubers for extended periods is a major problem for SP farmers, sellers, and consumers. To optimize crop usage, it is therefore necessary to find ways to store harvested tubers for extended periods. Under such conditions SP tubers can be stored for 5–12 months rather than the normal 2–3 months. In our survey area, SP farmers indicated that SP tubers can be stored without deterioration for prolonged periods of 12–28 weeks only when the tubers are left in the mounds unharvested.

As the current study did not focus on the effect of different containers on SP tuber physiology and infection by SP weevils in fields and stores, we cannot formulate conclusions on the comparative advantages or disadvantages of the containers observed during our survey. Studies are necessary that focus on suitable storage structures for limiting weevil infection and increasing the shelf-life of SP tubers in the surveyed area.

Infection of SP by weevils in Benin was previously reported by MAEP [3] without a precise description of the insect species. Our study revealed the presence of *C. puncticollis* associated with SP in Benin. However, the low infection level of tubers observed during our survey might be related to the survey period (the start of the dry season) and the age of the tubers evaluated. The survey period might also have affected the results on the 50% of tubers visually assessed during the survey which looked clean and were recorded as weevil-free. Smit *et al*. [5] reported that weevil infection is mainly noticeable during dry seasons when weevils infect SP tubers through cracks in the soil and especially during storage when it can reach 100% depending on SP variety. Considering the weevil life cycle, during which they spend more than 1 month inside tubers or vines (from egg to adult), the surveyed tubers would have to have been monitored for some time (around2–3 weeks extra) to check for eventual emergence of adult weevils. This could probably increase the percentage of infected tubers.

During feeding, the insects create galleries inside the tubers that increase the area exposed to air. This means that the tuber subsequently dries out and loses weight. Damage to SP tubers by weevils was shown by the production of powder. The weight of powder produced increased with the duration of exposure of the tubers to weevils, due to the increase in insect population and consequent quantity of food consumed. The results observed for control tubers are an indication that SP tuber weight loss was not only dueto weevil infection. Under our study conditions, weight loss could be attributed to physiological factors caused by mechanisms such as transpiration (water loss), respiration (dry matter loss), sun scorch (tissue degradation), greening (toxin formation), and starch inversion (increasing transpiration and respiration) [22]. Our results showed a 40.18% weight loss of control tubers, which was not significantly different ($P \leq 0.05$) from that of tubers exposed to weevils for 1 day. This indicates that significant weight loss in infected tubers, compared with that of uninfected tubers, can be observed only if infection in treated tubers is of sufficient duration.

Denon and Mauléon [23] reported EPN to be the organisms with the greatest potential for the control of *Cylas* spp. The nematode isolates used in our experiments originated from various types of vegetation and soil textures, and soil pH [24].

These nematode isolates were efficient in suppressing *C. puncticollis* populations inside SP tubers and were able to reproduce inside the insect larvae. Furthermore, we observed that virulence and nematode reproduction inside the insect host varied by nematode isolate, despite them all belonging to the same species (*H. sonorensis*). As a consequence, percentage weight loss of tubers varied with nematode isolate.

5. Conclusion

This study investigated SP tuber storage practices used in southern Benin and assessed the effect of indigenous EPNs on SP weevils under laboratory conditions. The present study observed that several methods are used to store SP tubers, such as indoor storage, in-ground storage, and storage in stores or in kitchens. Some survey sites were infested by SP weevils, *C.puncticollis*, which caused substantial damage to stored SP tubers. Complementary studies are necessary to assess the effect of recorded storage practices on the severity of damage caused to the stored tubers by the weevils. This study showed that, under laboratory conditions, there was a positive correlation between the time of exposure of SP tubers to SP weevils and the damage caused to the tubers, weevil population increase, and tuber weight loss. The values of these parameters were also affected by the initial size of SP tubers. The indigenous EPN isolates used in this study proved to be efficient in locating and killing SP weevils inside the tubers. This led to reduced damage to SP tubers by the weevils. However, the effect of the nematodes on the weevil population and also the progeny production of the nematodes inside the hosts depended on the nematode isolate. Some EPN isolates were able to kill their hosts and to multiply sufficiently inside them. This is an indication that some Benin EPN isolates can be used as an environmentally safe control method against *C. puncticollis.* Greenhouse and multi-location field experiments are needed to confirm these results and to select the best performing nematode isolates for use by SP farmers.

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7. References

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