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Evaluation of Sweet Potato Cultivars for Differences in *Cylas puncticollis* (Curculionidae: Brentidae) Damage in South Western Cameroon

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***Abstract* – Sweet potato sometimes dubbed "a crop for the poor" is a food security crop for smallholder farmers in Cameroon. However, its increased production is constrained by the sweet potato weevil, *Cylas puncticollis* (Curculionidae: Brentidae), a serious sweet potato pest throughout Sub-Saharan Africa. A study was therefore conducted to screen 18 indigenous and exotic sweet potato cultivars for their responses to *Cylas puncticollis* through laboratory free-choice and no-choice oviposition as well as host suitability for development bioassays. Correlation and regression analysis for the free-choice bioassay revealed that dry matter content was positively correlated to number of eggs laid (r = 0.55). For the no choice bioassay, there was a positive correlation (r = 0.67) between number of feeding punctures and number of eggs laid. A significantly higher number of eggs were laid on sweet potato cultivars with higher dry matter content compared to those with lower dry matter content. These findings support the hypothesis that a female will choose those hosts for oviposition on which larvae perform best. Storage roots with high dry matter content probably had appropriate food reserves to support adequate offspring development. In the free-choice test, the indigenous sweet potato cultivars were slightly preferred for oviposion and feeding by *C. puncticollis* more than the exotic ones while in the no-choice food and oviposition tests, the cultivars IRAD 048 had the highest number of feeding punctures followed by Buea local white and Kekem in that order. For developmental bioassays, a higher number of weevil adults emerged from the indigenous sweet potato cultivars between 36-45 days compared to the exotic cultivars. None of the sweet potato cultivars tested was completely resistant to *Cylas sp*. However the exotic cultivars were less susceptible to *Cylas* feeding and led to fewer adults emergence from them.**

***Keywords* – Bioassay, Choice, Cultivars, Food Preference, No-Choice, Oviposition, Sweet Potato Weevils.**

I. Introduction

Sweet potato weevils (SPW) are a major constraint to sweet potato (*Ipomoea batatas* L.) production worldwide [8]. In Africa, *Cylas puncticollis* Boheman and *C. brunneus* F. are the major pest species [9], whereas in America and Asia, *C. formicarius* is most prevalent [11]. All three *Cylas* species attack sweet potato both in the field and during storage causing substantial losses of up to 60 to 100% [19]. The female weevil lays eggs in cavities excavated in vines and/or accessible storage roots and the ensuing larvae feed and tunnel into the infested plant tissues. Even low levels of weevil feeding reduce storage roots quality since it induces the production of unpalatable bitter tasting terpenoids that render the slightly damaged roots unpalatable [20]. The infested storage roots are often riddled with cavities, spongy in appearance and dark in color. The weevils also mine the vines of the plant, causing them to darken, crack or collapse [2]. The cryptic nature of the most destructive stage of the weevils, the larvae which tunnel the vines, crowns and storage roots render them difficult to control [22].

One possible sustainable management approach of these weevils is host plant resistance. The use of resistant cultivars is one of the most appropriate ways to control sweet potato weevils because it is more effective, easy to be adopted by resource poor farmers and safe for the environment. However, cultivars with reliable levels of resistance are not yet available [21]. Host plant insect resistance in sweet potato has been documented in the literature [4], [11], [12] but remains non-existent in commercially acceptable cultivars despite years of research and breeding. [10] demonstrated that weevil resistance existed in several breeding lines from the USDA, U.S. Progress in breeding weevil-resistant cultivars has been slow due to inconsistent resistance displayed by the genotypes across different areas. One East African genotype, New Kawogo showed promise as a source of resistance [21], [15], indicating that a wider survey of other local cultivars and landraces may deliver more useful genetic material for breeding. Given that the *Cylas* weevils are also a severe pest in stored sweet potatoes, it is therefore also important to screen cultivars for antibiosis. The objective of this study is therefore to test and identify exotic and local sweet potato cultivars in Cameroon that show some level of resistance/tolerance to *Cylas puncticollis* through laboratory bioassays.

II. Materials and Methods

Study Site

All sweet potato cultivars used in the studies were harvested from previously established fields at the Teaching and Research Farm of the University of Buea in the South-West Region of Cameroon. Buea is situated at 4° 9' 34" north, and 9° 14' 12" south. Buea has a tropical equatorial climate with two major seasons consisting of a wet season between March and November and a dry season from November to March. Average temperatures range from 18 – 29^{0}C with an average annual precipitation of 4,030 mm. The soils are rich volcanic soils very suitable for agricultural activities.

Weevil stock culture and test plants

Feral *Cylas puncticollis* adults were collected from infested sweet potato storage roots (unknown cultivars) from sweet potato farms in Buea and its environs. A stock culture of the weevils was maintained on storage roots of a known weevil susceptible sweet potato variety, (Buea local white) in a 15 L plastic bucket. The bucket was covered with plastic mesh held in place with a rubber band and kept in the laboratory at ambient conditions of 25 $\pm$ 5^{0}C and 70 $\pm$ 10% R.H. There were five buckets in total. Once bi-weekly, fresh storage roots were put in the buckets to replace the old ones which were removed and kept in separate closed containers for daily observations to collect all neonate adults that emerged for use in the various resistance screening bioassays.

The following eleven local sweet potato cultivars, IRAD 112, Tib 1, Bambue orange, Mbouda, Sanchou, Bokito, IRAD o48, Bafia, Tib 2, Kekem, Buea local white and seven exotics namely Jowel 56638, Jowel 44031, Zapello, Tainung, Jonathan, SPK00 kakamega and North Carolina were used in the study. Vines of each cultivar were cut at lengths of 50 cm and planted on beds at an equidistance of 50 cm with the middle portion buried about 10cm deep into the soil. Each bed was 30 cm high, 2 m wide by 5m long and 50 cm apart. Normal agricultural practices of weeding and cultivation (i.e. earthing up) were used. To ensure a continuous supply of the storage roots, plantings were done at monthly intervals.

Bioassays

Storage roots of the 18 different sweet potato cultivars were tested for their suitabilities for *Cylas spp*. oviposition in two different tests, namely; free-choice and no choice bioassays in multi-well tissue culture plates. In addition, free- and no-choice developmental bioassays were also conducted to test the suitability of each cultivar for weevil development.

Free- and no-choice oviposition bioassays

Sweet potato storage roots of each cultivar were harvested, gently washed with tap water and allowed under the sun to dry for 30 minutes. Using a sharp knife, the storage roots were split longitudinally and cores were then produced from the storage roots using a 1.5cm diameter cork borer. The cores were inserted into wells of tissue culture plates so that only the periderms were exposed to the insects. The diameters of the cores were equal to those of the wells thus providing a close fit. For the free choice bioassay, storage root cores from the storage roots of all the sweet potato cultivars were inserted into the different wells of the plate for the insects to freely make a choice while in the no-choice test, cores from a single cultivar were put in the wells where the insect had no choice but the meal provided. The plates used for the experiment were flat bottom multi-well tissue culture plates with 24 wells and having low evaporation lids (Beckton Dickenson Labware).

Adult female weevils of approximately 1 to 14 days old were used in these bioassays to ensure maximum fecundity before the weevils die. The weevils were sexed based on sexual dimorphism of the antenna. The antennae of the males are straight while those of the female are round or club-shaped. Also the females are often also slightly bigger in size than the males. The weevils were starved for three hours prior to using them in the bioassays. Twenty five weevils were put into each multi-well plate and allowed for 48 hours after which the numbers of feeding punctures as well as the number of eggs per root core were counted under a stereomicroscope. The experiment was replicated four times. The feeding punctures were distinguished from oviposition sites by their greater depth and the absence of a fecal plug [1].

Plate 1: Multi-well tissue culture plates with storage root cores from various sweet potatoes cultivars

Free-choice weevil development test

In this test, 18 appropriately labeled storage roots each from a different sweet potato cultivar were put in a 15L plastic bucket and then covered with a plastic mesh held in place with a rubber band. Then, 1-14-day old 200 female adult weevils from the stock culture were released at the center of the container to allow the weevils freely oviposit on any storage root of their choice. The experiment was replicated four times. The set up was kept at ambient conditions for 48 hrs to give the weevils enough time to oviposit on the storage roots. Thereafter, the storage roots were removed from the container and each placed in a different appropriately labeled container and covered as described above. Care was taken not to transfer the storage roots together with the adult insects. The storage roots were then kept in the laboratory under ambient conditions and observed daily for adult emergence till 65 days after infestation when the experiment was terminated. On each day of observation, all emerged adults from each container were removed, counted and then released in the stock culture rearing facility. At the end of the experiments,

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cumulative daily adult as well as the total number of adults emerged was calculated for each sweet potato cultivar.

No-choice development test

In the second test, 18 storage roots each from the different cultivars were still used. However, in this case, equal numbers of weevil eggs were artificially introduced into each storage roots to study the suitability of each sweet potato cultivar for weevil development. Under the magnification of a stereomicroscope, a sharp scapula was used to remove the brown fecal material oviposition plug (often covering the oviposition punctures of *Cylas* weevils) and 50 1-4 day old *Cylas* eggs transferred into a fine incision in each storage root and left to hatch under ambient laboratory conditions. After 25 days of storage, each container was opened daily and the number of emerged adults counted and removed; this continued up to 65 days of storage when the experiment was terminated. There were four replications for each sweet potato cultivar.

Statistical analysis

Correlation and regression analysis was used to verify the relationships between dry matter content and feeding as well as oviposition punctures. This was also used to test the relationship between oviposition and feeding. Analysis of variance was used to test for significance on oviposition and feeding damage of the different sweet potato clones.

III. Results

In both the choice and no choice tests, the adult weevils fed on the external surfaces of roots producing circular feeding punctures which were distinguished from oviposition sites by their greater depth and the absence of a fecal plug. In the free-choice test, the highest numbers of feeding punctures were observed in the cultivar IRAD 1112 which had a soft texture while the lowest was recorded for the North Carolina with a hard flesh texture. For eggs laid, the highest numbers were laid on IRAD 048 which had a yellow flesh colour and hard texture followed by Mbouda and Sanchou both with light yellow and very hard flesh texture (Table 1). The Zapello cultivar had the highest dry matter content, followed by Jowel 56638, IRAD 048 and Mbouda in that order (Table 2)

Table 1: Characteristics of different sweet potato cultivars.

VARIETY	Skin Colour	Flesh Colour	Texture
IRAD 1112	Cream white	Light yellow	Soft
Tib1	Cream white	Light yellow	moderate
Jowel 56638	Orange (Dark)	Light orange	hard
Zapello	Orange (Light)	Yellow	Very hard
Bambui Orange	Orange	Dark Orange	Moderate
Mbouda	Cream White	Light Yellow	Very Hard
Tainung	Light Orange	Dark Orange	Moderate
Jonathan	Light Brown	Light Orange	Hard
SPK00 Kakamega	Pink	Orange	Hard
Jowel 44031	Orange	Light Orange	Hard
North Carolina	Pink	Cream White	Hard
Sanchou	Cream White	Light yellow	Very hard
Bokito 1	Cream White	Cream White	Moderate
IRAD 048	Cream White	Yellow	Very hard
Bafia 2	Orange	Orange	Moderate
Tib2	Cream White	Light Yellow	Moderate
Kekem 1	Cream White	Cream White	Hard
Buea local white	Cream white	Cream White	Soft

Table 2: Free choice food and oviposition preference tests (mean ±SE) of the different sweet potato cultivars

VARIETY	% Dry Matter Content	Feeding Punctures	Eggs
IRAD 1112	22.9±1.4	18.5±3.1	9.3±2.9
Tib1	22±2.6	13.7±4.2	15.2±4.1
Jowel 56638	33.5±1.4	18.2±3.4	11.7±3.1
Zapello	34.6±1.7	14±3.2	17.1±2.8
Bambui Orange	26.3±2.8	15.2±2.5	14.9±3.9
Mbouda	33.1±1.4	16.3±3.8	18.6±2.8
Tainung	23.4±1.6	14.5±4.1	8.2±2.1
Jonathan	25.2±1.4	11.4±2.3	13.4±2.1
SPK00 Kakamega	24.9±1.4	10.2±2.4	7.2±2.6
Jowel 44031	27.4±1.3	18.2±5.1	14.2±3.3
North Carolina	28.0±1.6	8.9±2.8	9.2±2.4

Sanchou	28.0±0.4	12.1±4.5	18.6±3.6
Bokito 1	24.2.8±1.9	15.2±3.1	14.1±4.3
IRAD 048	33.2±0.28	12.2±3.2	19.4±4.9
Bafia 2	27.5±1.0	17.3±4.6	14.5±3.9
Tib2	26.9±2.6	15.2±4.8	15.3±5.6
Kekem 1	32.6±2.4	16.6±5.7	16.7±4.2
Buea local white	22.1±1.5	18.2±4.3	12.2±3.6

All hard to very hard textured cultivars had relatively high dry matter content showing that the textures of the storage roots were directly proportional to the percentage dry matter content.

Correlation and regression analysis for free choice bioassay showed no correlation between dry matter content and feeding punctures (Fig. 1) but a positive correlation (r: 0.55) between dry matter content and egg deposit (Fig. 2) while feeding punctures and eggs laid were not related (Fig 3).

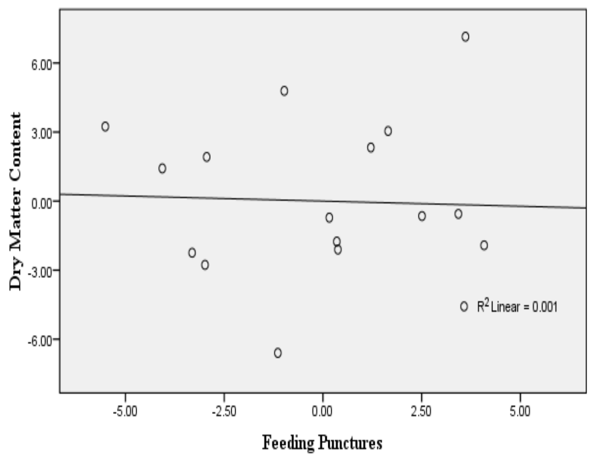

Fig.1. Dry matter content and Feeding Punctures

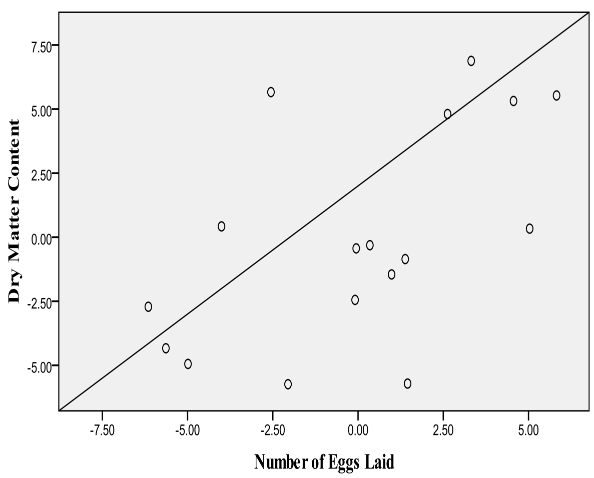

Fig.2. Dry matter content and eggs laid

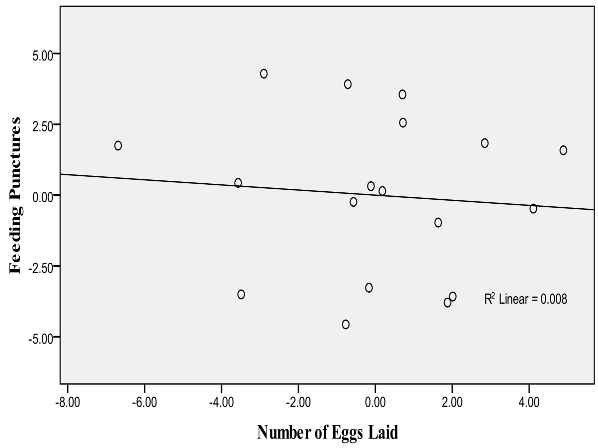

Fig.3. Feeding punctures and eggs laid

Overall, in the free-choice test, the indigenous sweet potato cultivars were slightly preferred for oviposion and feeding by *Cylas puncticollis* more than the exotic ones (Fig. 4).

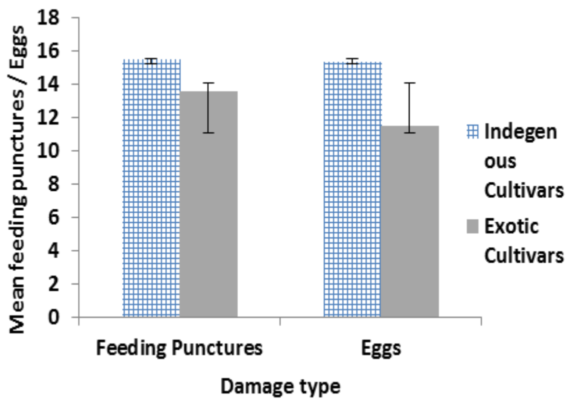

Fig.4. Relative preference for exotic and indigenous cultivars in free choice bioassay.

In the no-choice food and oviposition tests, the cultivars IRAD 048 had the highest number of feeding punctures followed by Buea local white and Kekem in that order. For the oviposition tests, the highest numbers of eggs were laid on Buea local white followed by IRAD 048 (Table 3).

Table 3: No choice food preference test (mean ±SE) with different sweet potato varieties and characteristics.

Variety	Feeding Punctures	Eggs
IRAD 1112	15.2±4.3	10.4±2.7
Tib1	12.7±5.1	12.3±3.1
Jowel 56638	21.4±5.2	10.7±2.4
Zapello	11±2.4	11.5±3.8
Bambui Orange	16.1±3.2	13.9±3.2
Mbouda	13.2±3.6	12.5±3.9
Tainung	15.6±4.1	9.8±2.5
Jonathan	14.3±3.1	12.3±3.1
SPK00 Kakamega	11.2±3.2	8.3±3.5
Jowel 44031	16.5±6.2	12.2±4.2
North Carolina	7.8±2.6	7.25±2.1
Sanchou	20.3±3.7	16.5±3.1
Bokito	14.2±4.1	13.2±3.2
IRAD 048	24.6±4.4	17.8±4.2
Bafia	15.3±3.7	12.6±3.9
Tib2	13.3±4.2	15.5±5.3
Kekem	23.4±5.1	12.7±3.4
Buea local white	23.5±3.2	19.7±2.5

In the no-choice bioassay, there was a positive correlation (r= 0.67) between number of feeding punctures and number of eggs laid (Fig. 5) but no relationship between dry matter content and feeding punctures (Fig. 6) as well as dry matter content and number of eggs laid (fig. 7) .

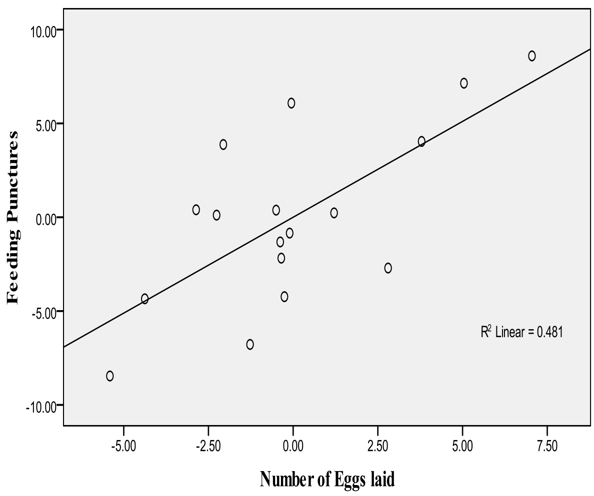

Fig.5. Plot for feeding punctures and number of eggs

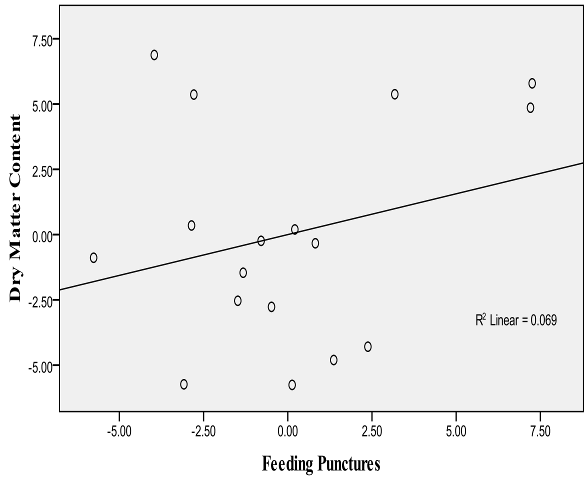

Fig.6. Plot for dry matter content and feeding punctures in no-choice.

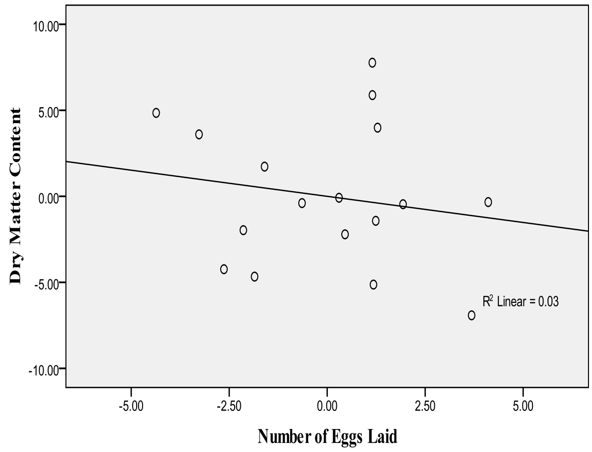

Fig.7. Plot for dry matter and number of eggs in no-choice test.

Generally, *C. puncticollis* slightly preferred feeding and laying eggs on the indigenous sweet potato cultivars over the exotics in the no-choice tests (fig. 8).

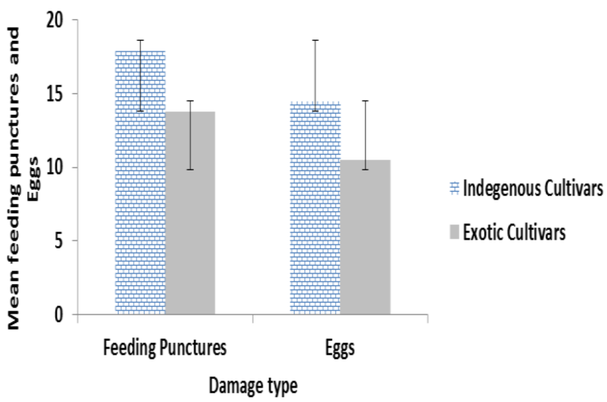

Fig.8. Relative preference for exotic and indigenous cultivars in no choice bioassay.

In the free-choice weevil development bioassay, a higher number of weevil adults emerged from the indigenous sweet potato cultivars between 36-45 days compared to the exotic cultivars. Within this period, the cultivars SPK00 Kakamega, Jowel 44031, North Carolina, Tib1 and IRAD 048 had < I adult that emerged from each cultivar (Table 3). Most of the adult weevils emerged between 46-55 days irrespective of the sweet potato cultivar. The lowest total number of weevils emerged from the cultivar North Carolina followed by SPK00 Kakamega then Jonathan all with orange flesh and hard texture. The highest numbers of adults were obtained from IRAD 112 followed by Buea local white both having soft texture and white to cream white flesh. Overall, higher total numbers of weevils emerged from the indigenous more than the exotic cultivars (Table 4).

Table 4: Mean number (mean±SE) of *Cylas puncticollis* adults that emerged from the various sweet potato cultivars at different periods in the free-choice development test.

	Days of emergence				
Sweet potato cultivars	25-35 days	36-45 days	46-55 days	56-65 days	Total
IRAD 1112	0.0±0.0	8.2±3.2	15.4±3.4	2.1±0.5	25.7
Tib1	0.0±0.0	4.2±1.6	11.5±2.4	0.0±0.0	15.7
Jowel 56638	0.0±0.0	3.1±1.4	9.3±1.9	0.5±0.06	12.9
Zapello	0.0±0.0	4.3±1.6	12.2±3.2	0.0±0.0	16.5
Bambui orange	0.0±0.0	6.5±2.3	8.5±2.2	1.2±0.4	16.2
Mbouda	0.0±0.0	5.2±2.1	10.2±3.6	0.0±0.0	15.4
Tainung	0.0±0.0	1.4±0.5	9.3±2.6	0.0±0.0	10.7
Jonathan	0.0±0.0	1.2±0.5	7.4±1.5	0.0±0.0	8.6
SPK00 Kakamega	0.0±0.0	0.5±0.06	6.2±2.4	1.6±0.5	8.3
Jowel 44031	0.0±0.0	0.5±0.1	8.6±2.0	0.0±0.0	9.1
North Carolina	0.0±0.0	0.0±0.0	5.2±1.4	0.0±0.0	5.2
Sanchou	0.0±0.0	2.5±.08	12.1±3.1		14.6
Bokito	0.0±0.0	3.8±1.1	7.6±2.1	0.0±0.0	11.4
IRAD 048	0.0±0.0	0.7±0.1	10.1±2.6	0.8±0.05	11.6
Bafia	0.0±0.0	3.1±1.5	11.2±3.6	0.0±0.0	14.3
TiB2	0.0±0.0	0.5±0.09	8.2.2±1.8	0.0±0.0	8.7
Kekem	0.0±0.0	1.4±0.4	7.8±2.2	0.0±0.0	9.2
Buea local white	0.0±0.0	3.4±1.3	15.9±4.2	3.1±1.1	23.1

The highest percentage of adults emerged from Buea Local white, followed by IRAD 112 and then Kekem 1 while the least recovery was from Jonathan, followed by Jowel and SPK00 Kakamega (Figure 9); each of these last three cultivars had a hard texture an orange flesh. Fewer adults emerged from the exotic cultivars compared to the indigenous ones.

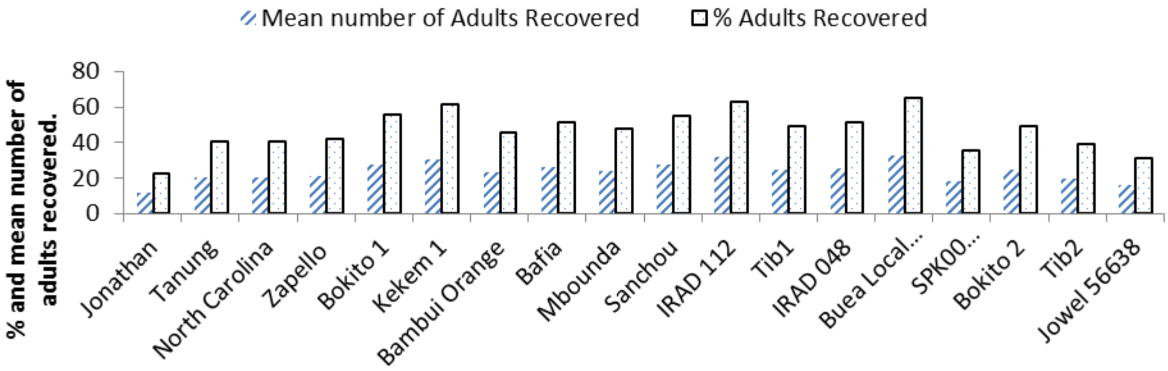

Fig.9. Mean number of Cylas adults and percentage recovered from storage roots artificially infested with eggs.

IV. Discussions

In the free choice bioassay, IRAD 1112 and Buea local white both with soft flesh had the highest number of feeding punctures while the hard fleshed IRAD 048, Mbouda and Zapello had the highest oviposition punctures. These storage roots with hard flesh (high dry matter content) had a positive correlation with eggs laid. Apparently, the insects prefer to lay eggs on harder storage roots with high dry matter content though they also fed on

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those with lower dry matter content when allowed to choose freely. Probably storage roots with high dry matter content served as superior sources of nutrients for egg development and oviposition in these weevils. However, when the insects had no alternative food choice, they laid on the available storage roots on which they feed giving a positive correlation **(**r=0.67) between feeding punctures and eggs laid. This confirms that eggs are laid at the feeding site as observed in previous studies [17] In the free-choice oviposition test, storage roots of sweet potato cultivars with higher dry matter content tended to be preferred for oviposition probably because they provided the larvae with adequate food reserves and could therefore sustain their development long enough. This corroborates the insects' optimal oviposition theory [17], [14] that a female will choose those hosts for oviposition on which larvae perform best, as this will maximize her own fitness.

The local sweet potato cultivars were slightly preferred for feeding in the free-choice bioassays probably because the weevils differentiate the local cultivars from the foreign. Long periods of previous exposures and hence adaptation on the local cultivars may provide an explanation for the preference for and performance on the indigenous sweet potatoes over exotic once since experience or learning may affect host choice of phytophagous insects [6], [7]. Insects that have been in contact with a particular host may show a biased preference towards that host.

In the free-choice weevil development test, adults emerged from the Buea Local sweet potato cultivar from days 25 to 65 probably because the weevils had adapted to feed on this indigenous variety better than the imported ones . The first experience of these weevils with stimuli associated with indigenous host will lead to induced preference for this host. The slight preference of the weevils for the indigenous cultivars may therefore be as a result of this feeding pre-adaptation and/or superior nutritional quality of these cultivars for the weevils. North Carolina with its hard texture and orange flesh on the contrary had the lowest number of adult emergence probably because it did not provide adequate appropriate nutrients for the insect development. In most of the cultivars with hard textures, the majority of the weevils emerged late (46-55 days). Nutritional quality and secondary metabolites of host plants can affect whether a particular host plant species is used by an herbivorous insect population or species [5]. Therefore differences in nutritional profiles of the various sweet potato cultivars may have influenced the preferences of the weevils**.** Orange –fleshed sweet potatoes for example are excellent sources of provitamin A and Vitamin C while purple cultivars are loaded with anti-oxidants and this distinctive rich colour is given by the phytochemical anthocyanins. Anthocyanins exhibit greater antioxidant activity than either vitamin C or vitamin E [13]. This quality diet variability may have instead been detrimental to the weevil development thus making cultivars with orange flesh storage roots to prolong the insect development periods.

V. Conclusion

The different sweet potato cultivars had varied levels of feeding damage when *Cylas puncticollis* adults were offered free food and ovipositon choices. However, when confined or had no choice, they equally fed and laid eggs on the available storage roots offered. This implies that none of the sweet potato cultivars tested was completely resistant to *Cylas sp*. However the exotic varieties were less susceptible to *Cylas* feeding and led to fewer adults emergence from them. Other factors including physical and physiological attributes which may make the roots more accessible to damage by the pest may also play a role in the levels of attack of the *Cylas sp.*

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