# EFFECT OF PHENOLIC ACID OF SOME ADVANCED SWEETPOTATO (*Ipomoea batatas* (L.) Lam) BREEDING LINES AT PRE-RELEASE STAGE ON *Cylas* spp. INFESTATION AT NYANYA, NIGERIA.

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## Abstract

*The effect of phenolic acid on susceptibility of some advanced sweetpotato (Ipomoea batatas (L.) Lam) breeding lines to Cylas spp. was investigated at Nyanya, Nigeria. Sweetpotato genotypes were planted in a randomized complete block design (RCBD) with three replicates at the location. Results of Cylas infestation assessment showed that variation in susceptibility did exist among lines. Whereas ANOVA established significant difference (P<0.01) in levels of infestation expressed as percentage clean yield, a non-significant difference was observed in extent of individual root damage expressed as Cylas mean scores. Sound roots were picked at random and transported to the laboratory for phenolic acid determination. Total phenolic acid which was expressed as chlorogenic acid equivalent (CAE)/mg dry weight was found to differ considerably among breeding lines. The orange-fleshed line Centennial (2.05 mg/g dry weight) had the highest total phenolic acid while the white-fleshed line NRSP/05/3B (0.22 mg/g dry weight) had the lowest content. Also, separation of methanol extract of sweetpotato roots using silica-gel thin layer chromatography showed that only caffeic acid was present in two breeding lines – NRSP/05/022 and NRSP/05/1B. The large differences found in such a small germplasm collection suggest that selecting or breeding sweetpotato with high phenolic acid is possible. Further investigation was done to identify relationships among 16 variable traits using correlation analysis. Correlation studies showed significant relationship (P<0.05, P<0.001) between total phenolic acid and four variable traits. A highly significant relationship (P<0.001) between total phenolic acid and Cylas mean scores ($r^2$=0.43) indicate potential biological activity on Cylas spp. infestation thereby suggesting that total phenolic acid and indeed type of phenolic acid is associated with Cylas susceptibility among these breeding lines.*

**Keywords:** effect, phenolic acid, advanced sweetpotato breeding lines, *Cylas* spp.

## INTRODUCTION

Sweetpotato weevils (SPW) of the genus *Cylas* (Coleoptera: Apionidae) are the most important insect pests world-wide of sweetpotato, [*Ipomoea batatas* (L.) Lam] (Jansson and Raman, 1991). *Cylas formicarius* F. occurs throughout the Americas and Asia, while *C. puncticollis* Boheman and *C. brunneus* Fabricius are exclusively African species (CAB International, 1993). The cryptic feeding habits of the larvae and the nocturnal activity of the adults make detection and control of infestations difficult, while varieties of sweetpotato having significant levels of resistance to weevils have not yet been developed. Host plant resistance will however provide a pivotal role in the management of insect pests (Rajasekhara Rao, 2002).

Extensive studies have been done on the role of root chemistry of storage roots in the management of sweet potato weevil. Recent analyses showed that the levels of resin glycosides and caffeic acid varied between sweetpotato genotypes and within genotypes among years and areas of production (Harrison *et al.*, 2003), and have also shown insecticidal activities (Jackson and Peterson, 2000). This may indicate a relationship between the quantity of these two compounds and the antibiosis of sweetpotato. Host plant nutritional parameters are also believed to affect incidence of sweetpotato weevil. The relationship between potash and silica in sweetpotato stems was negatively

correlated with *C. formicarius* infestation (Singh *et al.,* 1993). Nitrogen and potassium was also reported to have influenced the storage root-surface chemistry of sweet potato genotypes which had a bearing on resistance to sweet potato weevil (Marti *et al.*, 1993). Methanol extracts of external and internal tissue of sweet potato (cv. Centennial, Jewel, Regal and Resisto) genotypes showed differences in quantities of carbohydrates, organic acids and chlorogenic acid (3-O-caffeoylquinic acid). With the exception of malic acid, the concentration of carbohydrates and organic acids did not correlate with cultivar susceptibility to the sweetpotato weevil (Son *et al.,* 1990).

Phenolic acids are hydroxylated derivatives of benzoic and cinnamic acids. The most common hydroxycinnamic acid derivatives are *p*-coumaric, caffeic, chlorogenic and ferulic acids which frequently occur in food as simple esters with quinic acid or glucose (Matilla and Kumpulainen, 2002). Sweetpotato phenolics were first isolated by Rudkin and Nelson (1947), who identified chlorogenic acid and related compounds. Caffeic acid, and the caffeoylquinic acid derivatives, chlorogenic and isochlorogenic acids, were found to have accumulated in wounded tissue or in response to infection by the black rot fungus, *Ceratosystis fimbriata* Ell. and Halst (Uritani and Miyano, 1955). The concentration of chlorogenic acid can increase tremendously when plants are attacked by viruses, bacteria, fungi (Farkas and Kiraly, 1962) nematodes, insects (Dowd and Vega, 1996), or in response to various abiotic stresses such as mechanical damage (Kojima and Kondo, 1985), ultraviolet radiation (Lott, 1960), and toxins such as mercuric chloride (Uritani *et al*., 1960). Although marked increases occur after attack, reports of direct evidence linking chlorogenic acid with pest resistance are sporadic (Ellis, 1998). Growth of gypsy moth (*Lymantria dispar* L.) larvae was slowed by 3,5-dicaffeoylquinnic acid (DCQA) at concentrations as low as 10 µg/g; however, the cabbage looper (*Trichoplusia ni* Hubner) was not affected at concentrations of 100 µg/g (Beninger *et al.,* 2004). The same authors demonstrated that the photosynthesis and growth of *Lemna gibba* L. was reduced by 3,5- DCQA at 100 µg/g. Yeast, molds, and bacteria were inhibited by 1,3- DCQA, 3,5-DCQA, and 4,5-DCQA (Zhu *et al.,* 2004).

Previous studies have therefore reported significant cultivar differences in phenolic compounds with the suggestion that they may be significantly associated with susceptibility to sweetpotato weevil. The advanced sweetpotato breeding lines at pre-release stage in Nigeria have never been analyzed for phenolic acids. This study was therefore carried out to determine the phenolic acid content of these breeding lines and to investigate if any relationship exists between phenolic acid and *Cylas* spp. susceptibility.

## MATERIALS AND METHODS

### Experimental site/Field design

In September 2011, all fifteen genotypes (12 breeding lines + 3 controls) were planted in a randomized complete block design with three replicates of each genotype at National Root Crops Research Institute Nyanya sub-station, Abuja at longitude 09˚04'N and Latitude 07˚37'E, with an elevation of 426m above sea level. The plot measured 3×3m and consisted of three ridges 1m apart per plot. Each plot represented a treatment. Weeding was done manually at four weeks after planting (WAP). Nitrogen, phosphorus and potassium fertilizers 15:15:15 were applied at the rate of 400kg/ha (i.e. 120g per ridge of 3m) at 4 WAP after weeding rouging out was done at 7 WAP. The plots were allowed to be naturally infested with *Cylas* spp. (Hahn, 1979).

### Data Collection

At 4 months maturity, roots were harvested and the following observable traits recorded for each location:

- Stand count at harvest
- Physical characteristics of the sweetpotato genotypes
- Foliage yield weight per plot

- Weight and number of size categorized roots per plot:
    1. Mean marketable (roots>150g) root weight (kg/plot)
    2. Mean unmarketable root weight (kg/plot)
    3. Mean number of marketable roots per plot
    4. Mean number of unmarketable roots per plot
- Assessment of *Cylas* spp. infestation, as described below:

**Method A:**
Nondestructive damage scoring of infestation levels. Roots from each plot were separated into different categories depending on the percentage of the external surface showing *Cylas* spp. damage. A five-point score similar to that used by Stathers *et al.* (2003) was used where: 1=All tubers clean of Cylas weevil damage across the plot; 2≤25% of each tuber in the plot damaged; 3=26-50% of each tuber in the plot damaged; 4=51-75% of each tuber in the plot damaged; 5≥75% of each of the tubers in a plot damaged by *Cylas* weevil. The mean root score was calculated for each plot (Stathers *et al.*, 2003).

**Method B:** percentage (marketable) infested roots (by number). Using the same data obtained for method A, the percentage number of roots with any *Cylas* spp. damage was calculated. This was only measured for marketable roots.

$$\text{i.e.} \quad \frac{\text{Number of marketable roots infested by } \textit{Cylas}}{\text{Total number of marketable roots}} \times 100$$

**Method C:** percentage clean (marketable) yield (by weight). The percentage weight of roots that were clean (category 1 – uninfested) was calculated. This was done for only the marketable roots

$$\text{i.e.} \quad \frac{\text{Weight of uninfested marketable roots}}{\text{Total number of marketable roots}} \times 100$$

**Phenolic Acids Determination**
**Tissue preparation**
Sound sweetpotato roots from field experiment at Nyanya were selected at random and dried. The air-dried roots were powdered and the resulting sweetpotato flour bottled for further required analysis (Friend, 1985).

**Extraction of phenolic compounds**
3g of powdered tissue was measured and placed into a porous cellulose thimble. The thimble was then placed into the extraction chamber of a soxhlet apparatus and extracted with about 150 ml of 80% methanol. The solvents containing the extracts were evaporated over a water bath at 70$^{o}$C until about 1ml of sample was left. This was stored at room temperature until needed. 0.5ml of the extract was placed in a 15ml centrifuge tube and 8ml of 80% methanol was added to it. The tubes were capped and immersed in a water bath at 80˚C for 10 minutes. After vigorously shaking the heated samples for 30 seconds, the tubes were cooled and centrifuged at 4 000 rpm for 15 minutes. The clear supernatant was decanted and brought to a final volume of 10ml using 80% methanol and analyzed for total phenolic content (Walter and Purcell, 1979).

**Total phenolic content determination**
Total phenolic content was determined using a modification of the Folin-Denis method (Swain and Hillis, 1959). Exactly 0.5ml of supernatant was placed in a 25ml test tube and mixed with 8ml of distilled water (buffer) followed by the addition of 0.5ml of Folin-Denis reagent. After 3 minutes, 1ml of 1N $Na_2CO_3$ was added and the solution was allowed to stand for 2

hours at ambient temperature (21˚C). Absorbance of the resulting blue complex was measured at 750nm using a UV-VIS spectrophotometer (752N, Lemfield Medical England). The total phenolic content was expressed as mg chlorogenic acid equivalent (CAE)/g dry weight.

**Individual phenolic content**

Phenolic acids separation and identification was done using the thin layer chromatography method (Friend, 1985). Spots of extract were loaded on the starting line of 5×10 cm silica gel thin layer chromatography plates (Silica gel 60 $F_{254}$, Merck) with the aid of capillary tubes. The plates were then developed with acetic acid and chloroform in the ratio of 3:2 v/v. The solvent front was allowed to run to 8 cm from the starting line. The plates were then dried and examined under UV light before and after spraying the plates with $FeCl_3$ which was used as locating reagent. Absorbing bands were marked and their Retention factor ($R_F$) value calculated. Two standard phenolic compounds – chlorogenic acid and caffeic acid were selected to compare with the extracted sample. Spots of these standardized phenolic compounds (obtained from Sigma Aldrich Co. Ltd) were loaded on a separate plate and run in the solvent as earlier described to obtain their $R_F$ values

$$R_F \text{ value} = \frac{\text{Distance of solute from origin}}{\text{Distance of solvent from the origin}}$$

**Data Analysis**

ANOVA was done using SPSS version 12.0 to establish whether differences observed between genotype means were significant or not. Means were separated using Duncan Multiple Range Test at 5% level of significance. Correlation analyses were done using Pearson's product moment to investigate the relationship among the variables.

## RESULTS

**Assessment of *Cylas* infestation**

Using three methods as described by Stathers *et al.* (2003), two distinguished between clean and infested roots while one took into consideration the extent of individual root damage. The result of these three methods is shown in Table 1. Whereas no significant difference in genotype effect was observed in extent of individual root damage expressed as *Cylas* mean scores (Method A), significant difference was observed in levels of infestation expressed as percentage clean yield (Method C).

**Total phenolic acid determination**

The data obtained from total phenolic acid determination showed that significant difference existed among breeding lines at $P \leq 0.05$ level of significance (Table 2). A wide range of 0.22 – 2.05 mg/g dry weight was observed. Breeding lines differed considerably in total phenolic acid content. An orange-fleshed line Centennial was known to have the highest total phenolic acid of 2.05 mg/g dry weight followed by another orange-fleshed line, CIP 440293 (1.97 mg/g dry weight). The least total phenolic acid content was observed in the white-fleshed line, NRSP/05/3B (0.22 mg/g dry weight). Total phenolic acid of two orange fleshed lines - CIP 199034.1 (1.47 mg/g dry weight) and that of Ex-Oyunga (1.52 mg/g dry weight) were not significantly different at 5% level of significance. Also the total phenolic content of three white-fleshed lines – NRSP/05/10D (0.86 mg/g dry weight), NRSP/05/7C (0.81 mg/g dry weight) and NRSP/05/1B (0.84 mg/g dry weight) were not significantly different. All other genotypes were significantly different at $P<0.05$ level of significance.

**Individual Phenolic Acid Determination**

The result of the thin layer chromatography for screening phenolic acids in methanol extract of sweetpotato roots is shown in Table 3. The chromatogram showed higher sensitivity of standards used leading to very visible blue spots in normal light which turned darker after spray with 2% $FeCl_3$. Their appearance in UV light produced blue fluorescence with dark absorbing bands.

The result of other samples in UV light and after spray with $FeCl_3$ is also shown in Table 3. With the exception of NRSP/05/022 and

NRSP/05/1B, no other genotypes produced spots that were visible in normal light. All spots produced however gave a characteristic blue fluorescence in UV light. The colour intensity of the spots produced also varied in comparison with the standards from pale to dark blue.

The $R_F$ values of both standards - chlorogenic and caffeic acids were also calculated and result given as 0.55 and 0.76 respectively. Precise calculation of $R_F$ values of other genotypes was compared to the standards. The $R_F$ value of 0.53 was found in all the genotypes except NRSP 05/1B which produced only 0.55. Only NRSP/05/022 and TIS 87/0087 produced spots with $R_F$ values of 0.76.

**Correlation Analysis**

Correlation analysis of 16 variable traits of the 15 genotypes (12 breeding lines + 3 check varieties) at Nyanya showed that there were significant associations ($P \leq 0.05$) among variables (Table 4).

Significant relationship was observed between *Cylas* mean scores and root number ($r^2=0.84$), root yield ($r^2=0.63$), marketable number ($r^2=0.67$), marketable weight ($r^2=0.58$), total phenolic acid ($r^2=0.43$), stand count ($r^2=0.31$) and clean number ($r^2=0.50$). While both root number ($r^2=0.31$) and marketable number ($r^2=0.27$) were significantly associated with percentage clean weight at $P \leq 0.05$ level of significance (Table 4). Total phenolic acid showed positive and significant correlation with *Cylas* mean scores ($r^2=0.43$), root number ($r^2=0.36$), root yield ($r^2=0.27$) and unmarketable root weight ($r^2=0.30$).

Other traits exhibited both positive and negative significant associations among themselves. Root number showed positive association with all the other traits except foliage yield. Percentage infested yield was negatively correlated with foliage yield ($r^2=-0.39$) which was significant at $P \leq 0.01$ level of significance.

Table 1: Result of assessment of *Cylas* infestation of fresh roots of some sweetepotato genotypes at Nyanya

| Genotype | Marketable yield ($tha^{-1}$) | *Cylas* Mean Scores (1-5) | % Mean infested roots | % Clean yield (by weight) |
|---|---|---|---|---|
| NRSP/05/7C | $3.77^{cd}$ | $2.13^{a}$ | $93.06^{d}$ | $26.05^{b}$ |
| NRSP/05/5A | $1.00^{d}$ | $2.75^{a}$ | $93.33^{d}$ | $5.57^{b}$ |
| NRSP/05/10D | $5.47^{c}$ | $2.01^{a}$ | $57.61^{b}$ | $31.78^{b}$ |
| NRSP/05/1B | $0.50^{d}$ | $3.00^{a}$ | $92.16^{d}$ | $4.17^{b}$ |
| NRSP/05/3D | $2.40^{cd}$ | $2.50^{a}$ | $70.79^{bc}$ | $20.04^{b}$ |
| NRSP/05/3B | $0.15^{d}$ | $2.00^{a}$ | $25^{a}$ | $33.33^{b}$ |
| CIP 440163 | $3.60^{cd}$ | $2.23^{a}$ | $75.56^{bc}$ | $19.07^{b}$ |
| CIP 440293 | $10.40^{ab}$ | $2.30^{a}$ | $85.50^{bc}$ | $11.65^{b}$ |
| CIP 199034.1 | $0.08^{d}$ | $2.50^{a}$ | $0^{a}$ | $0^{b}$ |
| Centennial | $1.17^{d}$ | $2.30^{a}$ | $90.48^{d}$ | $7.58^{b}$ |
| NRSP/05/022 | $12.70^{a}$ | $2.70^{a}$ | $87.52^{bc}$ | $11.19^{b}$ |
| Ex-Oyunga | $0.20^{d}$ | $1.67^{a}$ | $100^{d}$ | $0^{b}$ |
| TIS 87/0087 (check 1) | $1.77^{d}$ | $1.50^{a}$ | $28.33^{a}$ | $76.54^{b}$ |
| Ex-Igbariam (check 2) | $3.20^{cd}$ | $2.77^{a}$ | $92.16^{d}$ | $8.77^{b}$ |
| Buttermilk (local check) | $9.03^{b}$ | $2.30^{a}$ | $77.86^{bc}$ | $26.39^{b}$ |

Means with the same letter are not significantly different at $P \leq 0.05$ level of significance. Each value is a mean of three replicates.

Table 2: Total phenolic acid content of roots of fifteen sweetpotato genotypes obtained from field experiment at Nyanya, Nigeria.

| Genotype | Fresh root colour | Total phenolics* |
|---|---|---|
| NRSP/05/7C | White | 0.81[h] |
| NRSP/05/5A | White | 0.63[i] |
| NRSP/05/10D | White | 0.86[h] |
| NRSP/05/1B | White | 0.84[h] |
| NRSP/05/3D | White | 1.22[e] |
| NRSP/05/3B | White | 0.22[k] |
| CIP 440163 | Orange | 0.42[j] |
| CIP 440293 | Orange | 1.97[b] |
| CIP 199034.1 | Orange | 1.47[d] |
| Centennial | Orange | 2.05[a] |
| NRSP/05/022 | Orange | 1.12[f] |
| Ex-Oyunga | Orange | 1.52[d] |
| TIS 87/0087 (Check 1) | White | 1.81[c] |
| Ex-Igbariam (Check 2) | Yellow | 0.92[g] |
| Buttermilk (Local check) | White | 1.72[d] |

*Data expressed as mg of chlorogenic acid equivalents per g of dry weight. Each mean with the same letters is not significantly different at P≤0.05 level of significance. Each value is a mean of three replicates.

Table 3: Thin-layer chromatography screening of methanol extract of 15 sweetpotato genotypes obtained from field experiment at Nyanya

| Sample | Observation | | Inference | $R_F$ Value |
|---|---|---|---|---|
| | with UV light | with $FeCl_3$ | | |
| Chlorogenic acid solution* | Blue fluorescence with dark absorbing bands | Deep blue spot | Phenolic present | 0.55 |
| Caffeic acid solution* | Blue fluorescence with dark absorbing bands | Deep blue spot | Phenolic present | 0.76 |
| NRSP/05/022 | Blue fluorescence | Deep blue spot | Phenolics present | 0.76, 0.53 |
| Buttermilk | Blue fluorescence | Blue spots | Phenolics present | 0.53, 0.55 |
| CIP 199034.1 | Blue fluorescence | Pale blue spot | Phenolics present | 0.53 |
| Centennial | Blue fluorescence | Not visible | Phenolics present | 0.53, 0.80 |
| CIP 440163 | Blue fluorescence | Blue spot | Phenolics present | 0.53 |
| CIP 440293 | Blue fluorescence | Deep blue spot | Phenolics present | 0.53, 0.40 |
| TIS 87/0087 | Blue fluorescence | Blue spot | Phenolics present | 0.76, 0.53 |
| NRSP/05/1B | Blue fluorescence | Pale blue spot | Phenolics present | 0.55 |
| NRSP/05/5A | Blue fluorescence | Not visible | Phenolics present | 0.40 |
| NRSP/05/3B | Blue fluorescence | Not visible | Phenolics present | 0.53, 0.80 |
| NRSP/05/3D | Blue fluorescence | Not visible | Phenolics present | 0.53 |
| Ex-Oyunga | Blue fluorescence | Not visible | Phenolics present | 0.53 |
| Ex-Igbariam | Blue fluorescence | Pale blue spot | Phenolics present | 0.53, 0.80 |

*standard solutions of some phenolic acid compounds

**Table 10: Correlation coefficients ($r^2$) of 16 variable traits of 15 genotypes obtained from *Cylas* spp. susceptibility trial at Nyanya**

| | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | -0.69**** | -0.05$^{ns}$ | -0.01 $^{ns}$ | 0.21$^{ns}$ | -0.02 $^{ns}$ | 0.02 $^{ns}$ | 0.15 $^{ns}$ | 0.09$^{ns}$ | 0.30* | -0.16 $^{ns}$ | -0.04$^{ns}$ | -0.10 $^{ns}$ | 0.39** | -0.06 $^{ns}$ | -0.08 $^{ns}$ |
| 2 | - | 0.19$^{ns}$ | 0.14$^{ns}$ | -0.39** | 0.17 $^{ns}$ | 0.13$^{ns}$ | -0.20$^{ns}$ | 0.07 $^{ns}$ | -0.15 $^{ns}$ | 0.44** | 0.14 $^{ns}$ | 0.21$^{ns}$ | -0.20 $^{ns}$ | 0.16$^{ns}$ | 0.21$^{ns}$ |
| 3 | | - | 0.90**** | 0.17 $^{ns}$ | 0.94**** | 0.84**** | 0.30* | 0.36* | 0.63**** | 0.31* | 0.84**** | 0.76**** | 0.55**** | 0.70**** | 0.88**** |
| 4 | | | - | 0.11$^{ns}$ | 0.97**** | 0.98**** | 0.22$^{ns}$ | 0.27* | 0.77**** | 0.25 $^{ns}$ | 0.63**** | 0.72**** | 0.72**** | 0.85**** | 0.92**** |
| 5 | | | | - | 0.14 $^{ns}$ | 0.09 $^{ns}$ | 0.27* | 0.06$^{ns}$ | 0.42** | -0.16 $^{ns}$ | -0.21 $^{ns}$ | 0.31* | 0.34* | 0.20 $^{ns}$ | 0.06 $^{ns}$ |
| 6 | | | | | - | 0.95**** | 0.22 $^{ns}$ | 0.27* | 0.74**** | 0.27* | 0.67 **** | 0.72**** | 0.67**** | 0.84**** | 0.96**** |
| 7 | | | | | | - | 0.20 $^{ns}$ | 0.24$^{ns}$ | 0.79**** | 0.24$^{ns}$ | 0.58**** | 0.65**** | 0.75**** | 0.83**** | 0.91**** |
| 8 | | | | | | | - | -0.14 $^{ns}$ | 0.08$^{ns}$ | 0.14 $^{ns}$ | 0.31* | 0.26 $^{ns}$ | 0.10 $^{ns}$ | 0.12 $^{ns}$ | 0.19 $^{ns}$ |
| 9 | | | | | | | | - | 0.25$^{ns}$ | -0.11$^{ns}$ | 0.43*** | 0.18 $^{ns}$ | 0.30* | 0.07$^{ns}$ | 0.27$^{ns}$ |
| 10 | | | | | | | | | - | -0.01$^{ns}$ | 0.50*** | 0.56**** | 0.91**** | 0.69**** | 0.68**** |
| 11 | | | | | | | | | | - | 0.24$^{ns}$ | 0.27$^{ns}$ | -0.01$^{ns}$ | 0.23$^{ns}$ | 0.26$^{ns}$ |
| 12 | | | | | | | | | | | - | 0.73**** | 0.43** | 0.40** | 0.69**** |
| 13 | | | | | | | | | | | | - | 0.42** | 0.74**** | 0.72**** |
| 14 | | | | | | | | | | | | | - | 0.57**** | 0.62**** |
| 15 | | | | | | | | | | | | | | - | 0.82**** |

*significant at P≤0.05; **significant at P≤0.01; ***significant at P≤0.001; ****significant at P≤0.0001; ns=not significant; 1=Clean weight; 2=%infested yield; 3=Root number; 4=Root yield; 5=Foliage yield; 6=Marketable number; 7=Marketable weight; 8=Stand count; 9=Total phenolic; 10=Clean number; 11=%clean weight; 12=*Cylas* scores; 13=Unmarketable number; 14=Unmarketable weight; 15=Infested weight; 16=Infested number.

## DISCUSSION

A wide range in values of total phenolic acid content (0.22 – 2.05 mg/g dry weight) obtained among individual roots indicates that breeding lines differed considerably in total phenolic acid content. The large differences found in such a small germplasm collection suggest that selecting or breeding sweetpotato genotypes with high total phenolic acid content is possible. Due to the many human health benefits attributed to the compounds, this may be a worthwhile objective. An orange-fleshed line Centennial was known to have the highest total phenolic acid content of 2.05 mg/g dry weight followed by another orange-fleshed line, CIP 440293 (1.97 mg/g dry weight). The least total phenolic acid content was observed in the white-fleshed line, NRSP/05/3B (0.22 mg/g dry weight). This suggests that total phenolic acid may be higher in the orange-fleshed lines than in the white-fleshed lines. This is not the first time a high phenolic acid content is observed in Centennial. Son *et al.* (1991) also reported that Centennial was found to have the highest total phenolic acid content among cultivars analyzed for various root compositions.

Correlation studies enable the breeder to understand the mutual component characters on which selection can be based for genetic improvement. Many economically important traits of plants, usually, are related one to another in one or several ways. Various workers have studied the relationships between different traits in sweetpotato (Son *et al.*, 1991; Singh *et al.*, 1993; Stathers *et al.*, 2003). In a *Cylas* susceptibility trial for example, Son *et al.* (1991) reported that there was correlation between concentrations of malic acid and some sweetpotato genotypes. The results of our study identified many significant relationships among variables. Total phenolic acids correlated with five variables - root number, root yield, marketable number, *Cylas* scores and unmarketable weight indicating that total phenolic acids can be selected for by indirectly selecting for these other traits. Thereby suggesting that this trait may be governed by many genes (polygenic). Also the relationship between total phenolic acids and unmarketable weight suggests that smaller roots may have a higher phenolic content. This corroborates the findings of Padda (2006) who reported higher phenolic acids in immature roots than in older roots. Owing to the nutraceutical properties of phenolic acids, smaller roots may therefore be of higher nutritive value than the more mature roots. Also significant correlation with *Cylas* mean scores is indicative of its influence on sweetpotato susceptibility to *Cylas* infestation. This suggests that an increase in total phenolic acids resulted to an increase in *Cylas* mean scores at Nyanya. Several workers have studied the significance of phenolic acids and their compounds on sweetpotato susceptibility to *Cylas* spp. infestation. Stevenson *et al.* (2009) demonstrated that some phenolic acid compounds that were present in a known resistant variety of sweetpotato were absent in New Kawogo (a susceptible variety). This indicates that type of phenolic acid found in sweetpotato is necessary for susceptibility studies. Separation of methanol extract of these sweetpotato genotypes under study identified only caffeic acid as the phenolic acid present among these breeding lines and cultivars. Caffeic acid was identified in two breeding lines - NRSP/05/3B and NRSP/05/022. These breeding lines were the only ones which recorded higher *Cylas* scores with lower total phenolic acid consistent with what was obtainable in the check variety TIS 87/0087. Our data therefore suggested that the different responses of TIS 87/0087, NRSP/05/3B and NRSP/05/022 may be due to the presence of varied concentrations of caffeic acid in them. The presence of caffeic acid is an indicator for pest resistance (Harrison *et al.*, 2008) which thereby suggests potential activity of caffeic acid on *Cylas* infestation resulting to lower susceptibility of these breeding lines. This is however not the first time caffeic acid has been isolated from sweetpotato. Previous work by Mao *et al.* (2004) and Harrison *et al.* (2008) also identified caffeic acid as the phenolic acid in sweetpotato. Studies of bioassays suggest that they may contribute

directly to sweetpotato allelopathy and defense against pathogens (Son *et al.,* 1991*;* Harrison *et al.,* 2008). The caffeic acid content of these genotypes may also provide protection against opportunistic pathogens that enter through wounds that penetrate the outer tissue.

## CONCLUSION

This study reports inhibitory effect of phenolic acids on *Cylas* spp. infestation on some advanced sweetpotato breeding lines. Caffeic acid which is a known allelopathic inhibitor was the only phenolic acid identified among these breeding lines. Some unidentified phenolic acids were isolated from some breeding lines. Further studies on the type of phenolic compounds present and their concentration levels in these breeding lines is however needed to better understand their effect on susceptibility to *Cylas* infestation.

Even though our data does not show significant differences in *Cylas* mean scores, previous multilocational field trials at different agro-ecologies using the same breeding lines have established significant differences at another location in Nigeria (Abayol *et al.,* 2012). This thereby suggests the possible influence of environmental factors on the total phenolic acid content of these breeding lines. Further studies at different locations are therefore needed to confirm this.

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