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# Temporal dynamics of Napier Grass Stunt Disease as influenced by Napier grass clones and initial inoculum


## ABSTRACT

Napier grass stunt disease is the main biotic factor limiting Napier grass production in the East African region. Its management is, however, hampered inadequate epidemiological information. This study determined the temporal spread of NSD in Napier grass fields. A field experiment was setup at National Crops Resources Research Institute, Namulonge to determine the influence of initial inoculum and clones on the spread of NSD in the field. The experiment was arranged in randomized Complete Block Design, replicated 4 times. The initial inoculum levels used were 0%, 10%, 20% and 30% while the clones included KW4, local and P99, respectively. Napier grass stunt disease incidence data was recorded at 60 days intervals starting 90 days after planting up to 450 days. Napier grass was cut back to a height of 5 cm above ground after each data collection. Gompertz model was found to adequately describe NGSD temporal spread, the basis on which all incidence data was transformed. Results indicate that NGSD symptoms appeared in the field after 150 days after planting. However, NGSD incidence at the time was influenced by initial inoculum levels and type of clone. Plots with higher levels of initial inoculum density reached epidemic levels faster than those without. Disease incidence increased with increase in levels of initial inoculum and time, doubling after every 15.48 to 29.83 days, as such the rate of disease spread is moderate. The disease progress was fastest in clone P99 followed by KW4 and least in local. Final NSD incidence and AUDPC were linearly related with the NSD incidence at the time the disease was first detected; indicating that incidence at NSD detection can be used to predict the final disease and AUDPC in the field. Therefore, deployment of NGSD management measures that reduce initial inoculum is important in control of the disease.


**Keywords:** AUDPC, clone, doubling, gompertz, incidence, rate

## 1. INTRODUCTION

Napier grass stunt disease (NGSD) incited by Phytoplasma of group 16SrXI, '*Ca.* Phytoplasmaoryzae' is the most limiting factor to Napier grass production in the east African region. The disease was detected in Kenya in 1997 (1), Uganda in 2001 (2) and Ethiopia in 2007 (3). Many smallholder farmers have lost up to 100% of their Napier crop and have been forced to reduce the number of animals or purchase fodder from the local market (4).

The primary means of NGSD spread is through introduction of infected cuttings by farmers (5) and or sap-sucking insect vectors belonging to the families Cicadellidae (Leafhoppers) and Delphacidae (planthoppers; Hemiptera) which transmit the phytoplasma in a persistent propagative manner (6). Obura*et al.* (2009) identified a planthopper, *Reciliabanda* (*Maiestasbanda*) as the vector of the phytoplasma causing NGSD in Kenya. The vector is abundant in Napier grass fields in Western Kenya (8), thus the spread of NGSD within field is likely to be fast. Although vectors transmitting phytoplasma that cause NGSD in Uganda are not known, Obura*et al.* (7) found the phytoplasma sequence extracted from the *Reciliabanda* in Kenya to be 100% identical to phytoplasma causing NGSD in Uganda, implying that *Reciliabanda* is the likely NGSD phytoplasma vector in Uganda.

Within field spread of phytoplasma diseases is also influenced by host plant resistance to either the vector that caries the phytoplasma or to the phytoplasma itself or both and varies from one variety to another. For instance, merlot which is less susceptible to infection expressed less

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disease in subsequent years compared to Chardonnay which is highly susceptible to Florescence doree, thus continued to show symptoms and eventually died (9). However, influence of host genotype in the temporal spread of NGSD within Napier grass fields is not documented.

As research into management of NGSD in the region intensifies, there is lack of quantitative information on the epidemiology of the disease against which management strategies would be formed such as; the level of initial inoculum assessed as initial disease population, the rate of NGSD development and the time the pathogen and host populations interact during the cropping period (10; 11) to cause the disease. This information is key in guiding deployment of management tactics targeting either reducing initial inoculum or the rate of spread of the disease or both in order to control the disease. For instance, where the disease has a higher rate of disease spread and a short latent period, sanitation practices are generally not worthwhile in controlling the disease (12). Under such circumstances, it is logical to deploy disease management principles that reduce disease rate sufficiently to a level below which disease management principles reducing initial inoculum will be able to keep the disease risk below economic threshold (13). Therefore, this study quantified the temporal rates of within field spread of NGSD and also determined the influence of Napier grass clones on the spread of NGSD.

## 2. MATERIALS AND METHODS

### 2.1 Field establishment

Field plots were established at National Crops Resources Research Institute, Namulonge, Uganda. The plots measuring 9 m by 9 m were planted with Napier grass clones P99, KW4 and Wild type (local - collected from the bush) spaced at 1 m by 1 m. Disease spreader points were randomly introduced in each plot to provide varying levels of initial inoculums. The levels of initial inoculum (infection) included 0% (no spreader plants), 10%, 20% and 30% (spreader plants). The experiment was setup in a randomized complete block design in four replicates, with plots separated by 4 metres while the replicates were separated by a five metre alley.

### 2.2 Data collection

To test the influence of the different levels of initial inoculum and Napier grass clones on the spread of NGSD, incidence data were collected starting 3 months after planting leaving out spreader plants and outer rows of each plot. This was repeated at two months intervals for 15 months. At the end of each data collection, the plants were cutback to 5 cm above ground and left to sprout again. To confirm the presence of the pathogen, leaf samples were periodically collected from the field and subjected to PCR analysis of DNA using universal primers p1/p6 nested with $R16F_2n$ and $R16R_2n$ (14;15), respectively.

### 2.3 Data analysis

Napier grass stunt disease incidence data for each treatment were plotted against sampling dates to generate disease progress curves. In addition, a graph of rate of change of NGSD incidence over time was plotted to determine the most appropriate population growth model for quantifying temporal rates of NGSD (16). The most appropriate model for estimating the growth parameters (slope and intercept) was assessed by transforming the incidence data with each model and the values regressed against time using simple linear regression (17). The best model was chosen basing on F-statistic, coefficients of determination, standard errors of the estimate for NGSD disease incidence (18) and number of times a model was significant. The

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best model chosen was used to transform data and assess parameter estimates for all treatments. Data was also converted into Area Under Disease Progress Curve (AUDPC) based on Campbell and Madden (19). To compare treatment effects on the spread of NGSD, means of intercepts, slopes, standard error of estimate, area under disease progress curves, time to NGSD epidemic onset (5%) and NGSD doubling time were separated using the least significant difference (LSD) test ($P \leq 0.05$) (SAS Institute). The relationships between NGSD incidence, the time of year NGSD was first detected in a plot, final NGSD incidence, and relative areas under NGSD progress curve were quantified using linear regression.

## 3. RESULTS

Basing on the shape of NGSD progress curve (Fig. 1) and the rate of change of NGSD incidence over time (Fig. 2), monomolecular, exponential, logistic or gompertz models were fit to describe the temporal spread of NGSD. However, evaluation of the rate parameter (slope), coefficient of determination ($R^2$), F statistics, subjective evaluation of standard residuals versus predicted values, standard error of estimate and number of times a model appeared significant further revealed that gompertz model was the best in describing the temporal spread of NGSD (Table 1) and as such chosen for the analysis of NGSD temporal parameters.

The coefficient of determination ($R^2$) of the transformed data provided by gompertz model, with time explained 92.99% to 96.10% (Kw4), 96.04% to 97.92% (local) and 93.15% to 96.19% (P99) increase in NGSD incidence over time (Figs 3A, B, C). The standard errors of estimate (SEEy) generated by gompertz model were significant among clones but not initial inoculum and ranged from 0.067 to 0.196 gompits/day for Kw4, 0.033 to 0.065 gompits/day for the local clone and 0.075 to 0.313 gompits/day for P99 (Table 2).

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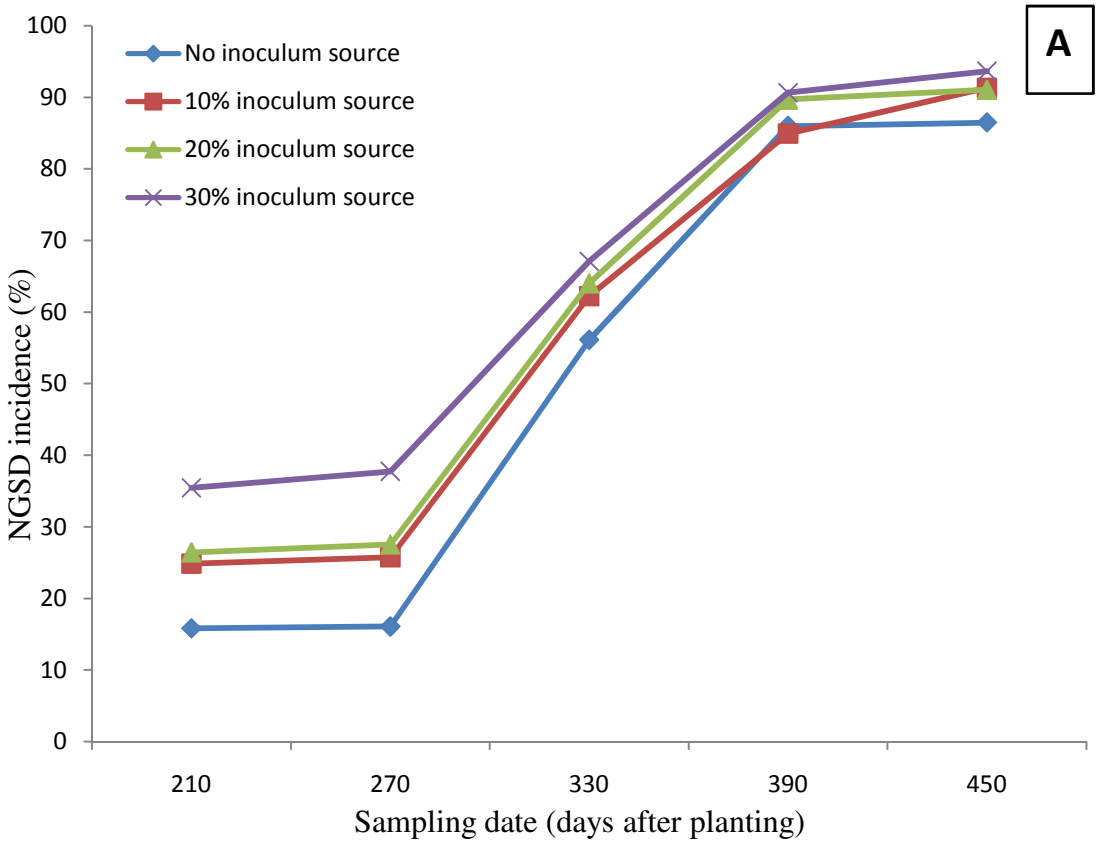


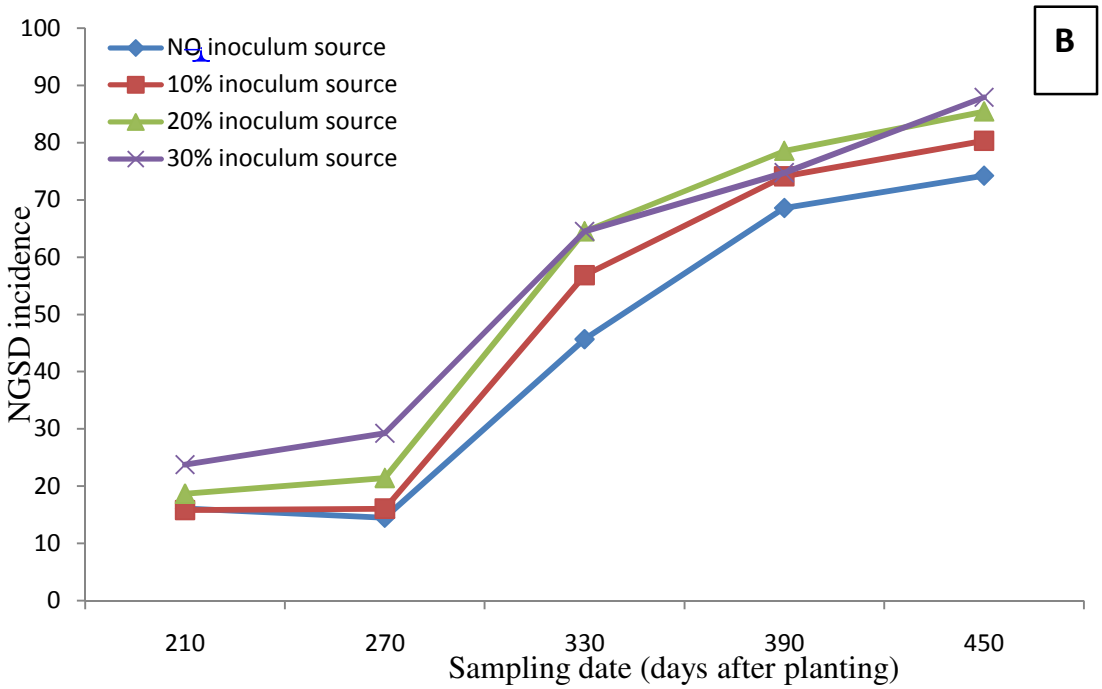

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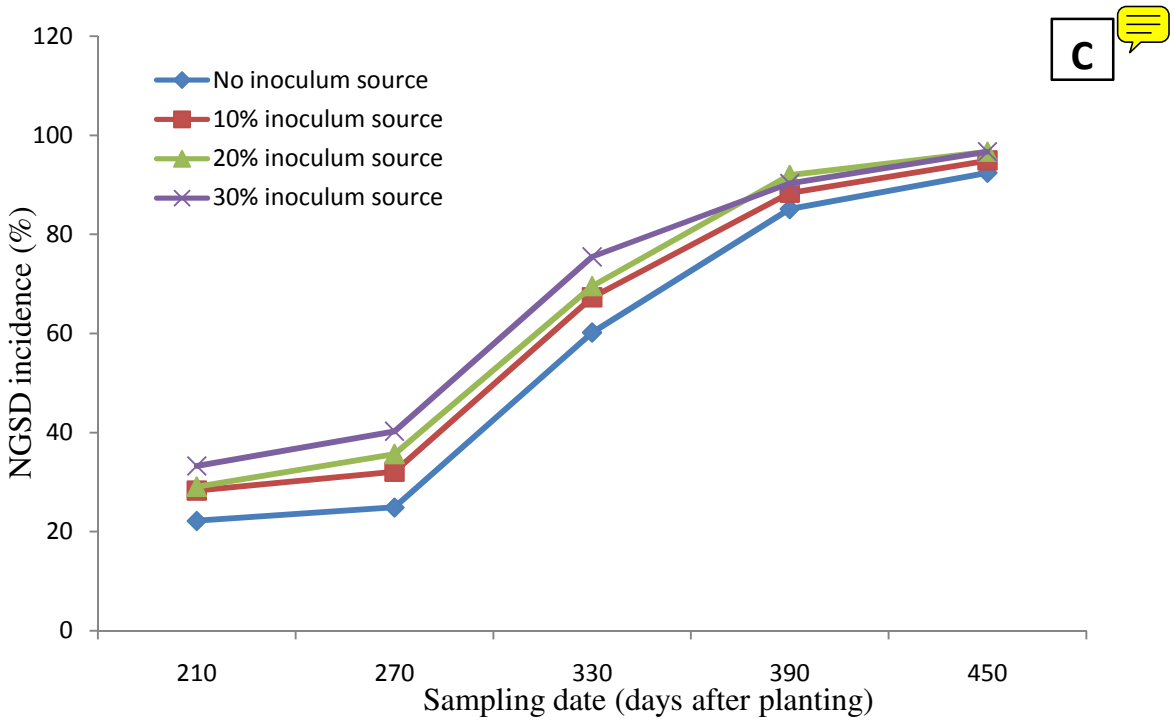


Fig. 1 Mean incidence of Napier stunt disease (NGSD) in Napier grass as ~~influnceed~~ by different levels of inoculm source in Kw4 (A) local (B) and P99 (c) clones over time

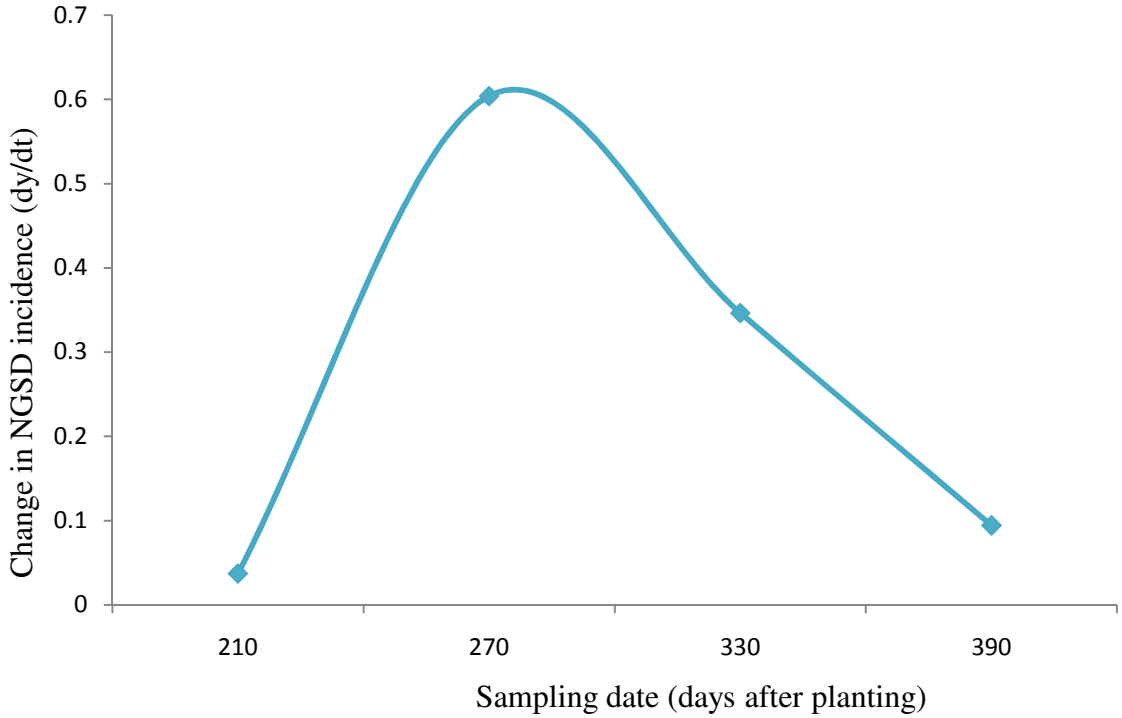

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**Fig.2. Rate of change over time in the mean incidence of Napier stunt disease (NGSD) in Napier grass as influenced by different levels of initial ~~inoculm~~ source and clones**

**Table 1. Summary statistics used in selection of the model for temporal analysis of NGSD as influenced by source of inoculum and clone**

| Model | Linealised Equation y= | Slope (rate) | SEEy | Coefficient of determination ($R^2$) | F start | Number of times model was significant per plot (%) |
|---|---|---|---|---|---|---|
| Exponential | ln(y) | 0.01a | 0.09a | 88.0 | 27 | 62.5 |
| Gompertz | -ln(-ln(y) | 0.01a | 0.13a | 94.0 | 95 | 86.46 |
| Logistic | ln(y(1-y) | 0.02a | 0.21b | 93.0 | 106 | 83.33 |
| Monomolecular | ln(1/1-y) | 0.01a | 0.11a | 89.0 | 36 | 89.58 |

Napier grass stunt disease was first detected in Napier grass plots 210 days after planting in all the clones (Fig. 2) and the level of disease incidence on first detection was significantly influenced by amount of initial inoculum and clone. The NGSD incidence increased with increase in the level of initial inoculum ranging from 5.10 to 20.4 % in the different clones. Napier stunt disease incidence was highest in clone Kw4 followed by P99 and lowest in local, respectively (Table 2).

Napier grass stunt disease epidemic onset (described as the time taken for the disease to reach 5% incidence) did not vary significantly between clones and initial inoculum densities, respectively (Table 2). Plots with the highest initial inoculum (30%) took very short time (181.2 days to 203.0 days) for NGSD to reach epidemic levels compared with those with lower initial inoculum density. However, at this level, days taken by plots that had initial inoculum of 10% and 20% to reach epidemic level were intermediate and not significantly different from other plots. By the time NGSD was detected, its incidence had reached epidemic levels.

The rate of NGSD spread was significantly highest in P99 and lowest in the wild type (local) and increased with increase in the initial inoculum. The rate of increase in NGSD incidence in KW4 ranged from 0.0127 to 0.0143 gompits per day while in P99 it ranged from 0.0141 to 0.0193 gompits per day. In the local clone, it ranged from 0.009 to 0.0107 gompits per day. Overall, NGSD incidence doubled significantly among clones and initial inoculum, respectively; doubling after every 19.65 to 21.02 days (in KW4 clone), 24.67 to 29.63 days (in local clone) and 15.46 to 19.80 days (in P99 clone). Generally, the time taken for incidence to double decreased with increase in the inoculum densities (Table 2). Increase in NGSD incidence was similar in all clones. It was relatively low (below 30%) from the time NGSD was first detected up to the fourth time of data collection and then it increased exponentially. By the time the last data set was recorded (450 days after planting), the NGSD incidence had increased in all the fields by over four times compared to when it was first detected. By this time, the NGSD incidence in plots that had initial inoculum level of 10%, 20% and 30% were equally diseased but significantly higher than in plots that were not inoculated. Clones P99 and KW4 had higher NGSD final incidence compared to the local clone (Table 2; Fig. 4). Area Under Disease Progress Curves (AUDPCs)

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differed significantly among treatments and clones with P99 having a significantly higher values followed by KW4 and least in the local clone. In relation, the level of initial inoculum influenced the AUDPC with 30%, 20% and 10% initial inoculum having a higher AUDPC than plots that were not inoculated (Table 2).

Positive and significant linear relationships between NGSD incidence when it was first detected within Napier grass plots and final incidence were recorded(Fig. 4). Napier grass stunt disease incidence at first detection explained 75.91%, 76.08% and 15.17% variation in the NGSD final incidence in ~~Kw~~4, local and P99 clone, respectively. In relation, NGSD incidence at first detection was linearly related to AUDPC (clone KW4 $R^2$=92.86%; local clone $R^2$=33.36% and clone p99 $R^2$ = 22.86% (Fig. 4).

**Table 1Gompertz model parameters and statistics, and influence of inoculums density on the incidence, epidemic onset, final incidence and Area under disease progress curve**

| Clone | Inoculum levels (%) | Intercept | Slope (rate) | SEEy | $R^2$ | NGSD incidence on first detection (%) | Time taken for the disease to reach 5% incidence (days) | NGSD doubling time (days) | NGSD incidence on last detection (%) | AUDPC |
|---|---|---|---|---|---|---|---|---|---|---|
| ~~kw~~4 | 0 | -3.9a | 0.013c | 0.115ab | 91.5 | 5.1a | 220.6d | 21.0cd | 80.6cd | 13561bc |
| | 10 | -4.0a | 0.014c | 0.067a | 95.3 | 9.7bcd | 206.7bcd | 19.6bc | 88.3efg | 14739def |
| | 20 | -3.9a | 0.014c | 0.196bc | 86.9 | 11.2c | 200.3abc | 19.7bc | 86.2def | 15643f |
| | 30 | -3.8a | 0.014c | 0.19bc | 90.9 | 20.4e | 181.2a | 19.7bc | 90.8fg | 16812g |
| Local | 10 | -3.0a | 0.009a | 0.058a | 90.2 | 7.1ab | 209.2cd | 29.8f | 67.3a | 11047a |
| | 10 | -3.2a | 0.010ab | 0.065a | 90.7 | 6.6ab | 203.bc | 27.0ef | 73.5b | 12924b |
| | 20 | -3.1a | 0.010ab | 0.065a | 92.8 | 7.7ab | 195.2abc | 26.7ef | 75.5bc | 14118cd |
| | 30 | -3.1a | 0.011ab | 0.033a | 96.1 | 12.8cd | 189.3ab | 24.7de | 84.1de | 14300cde |
| P99 | 0 | -4.2a | 0.014c | 0.075a | 95.1 | 8.7abc | 210.1cd | 19.8bc | 89.3eg | 13927c |
| | 10 | -5.0a | 0.017d | 0.103ab | 95.1 | 13.8d | 211.1cd | 16.7ab | 93.4gh | 15080def |
| | 20 | -5.4a | 0.019d | 0.257c | 89.9 | 8.7abc | 221.2d | 13.8a | 95.4h | 15484f |
| | 30 | -4.8a | 0.018d | 0.313d | 87.9 | 13.3d | 203.0bc | 15.5a | 95.4h | 15719f |

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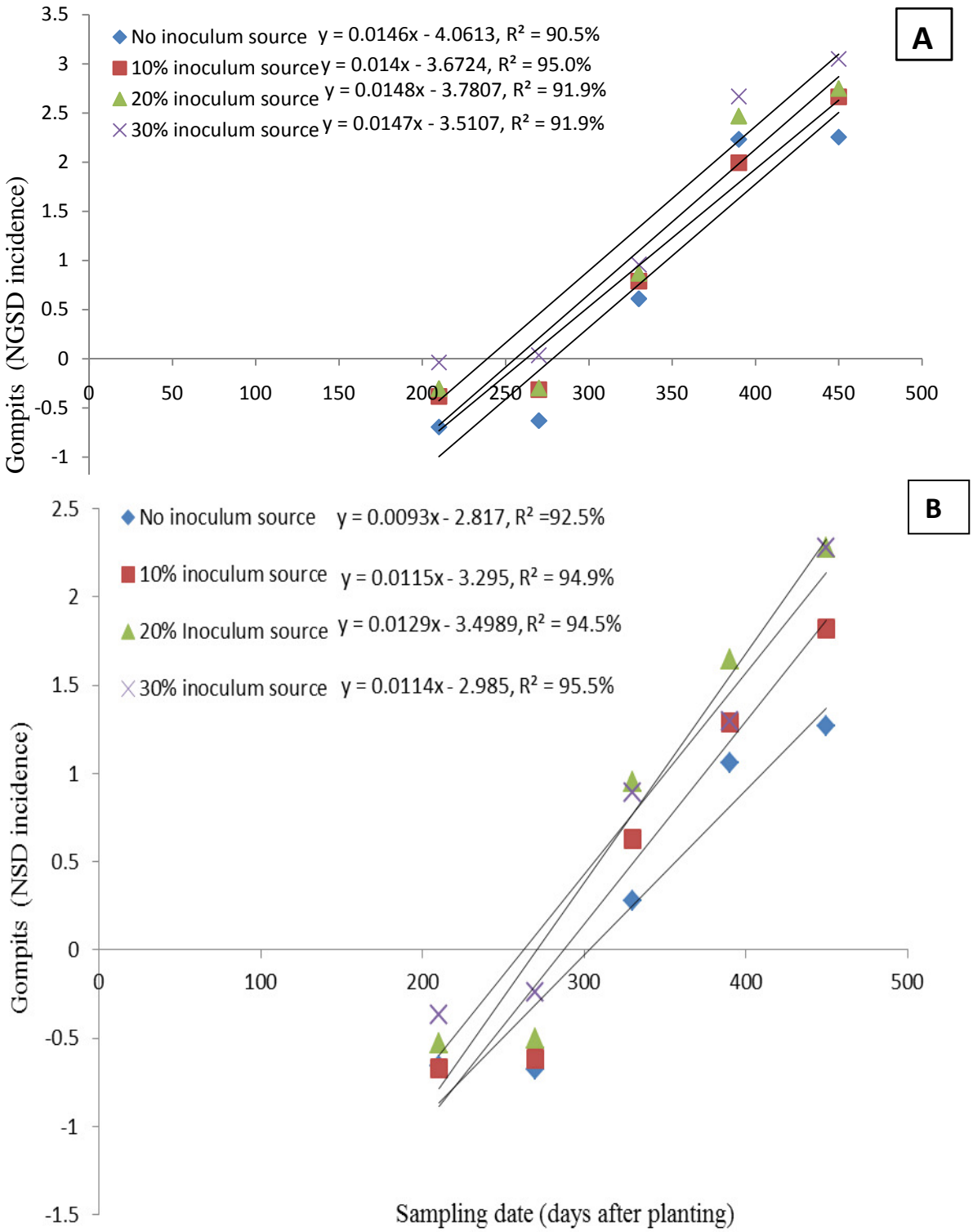

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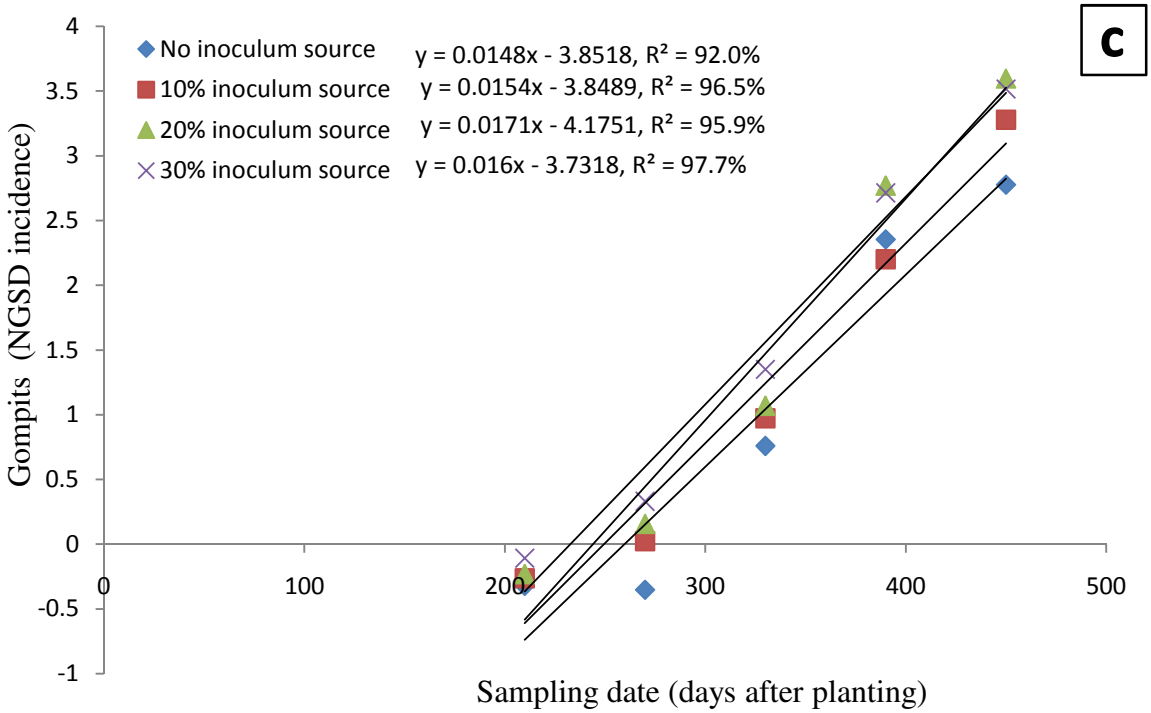
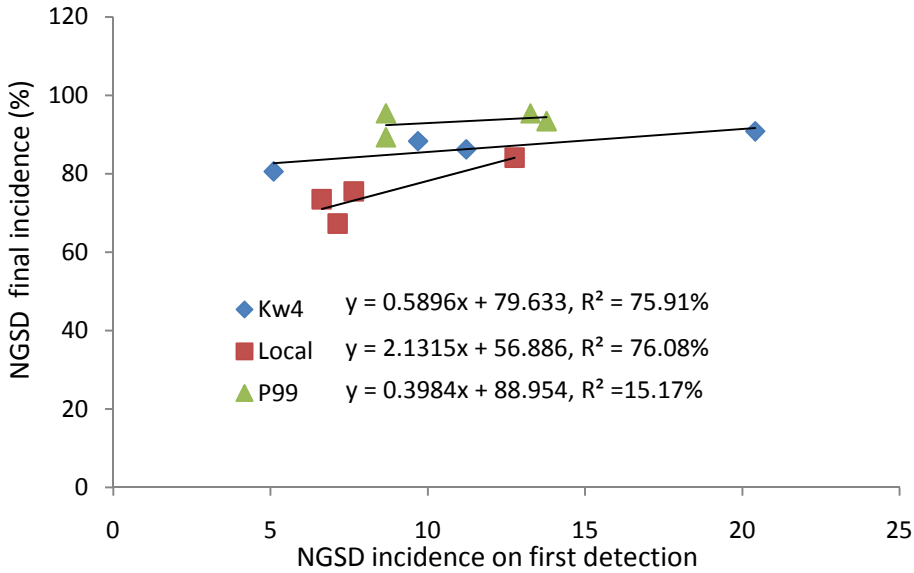



Fig.3. Relationship between gompit NGSD and sampling time on diferent Napier grass clones KW4 (A), local (B) and P99 (C) as influenced by the source of inoculum



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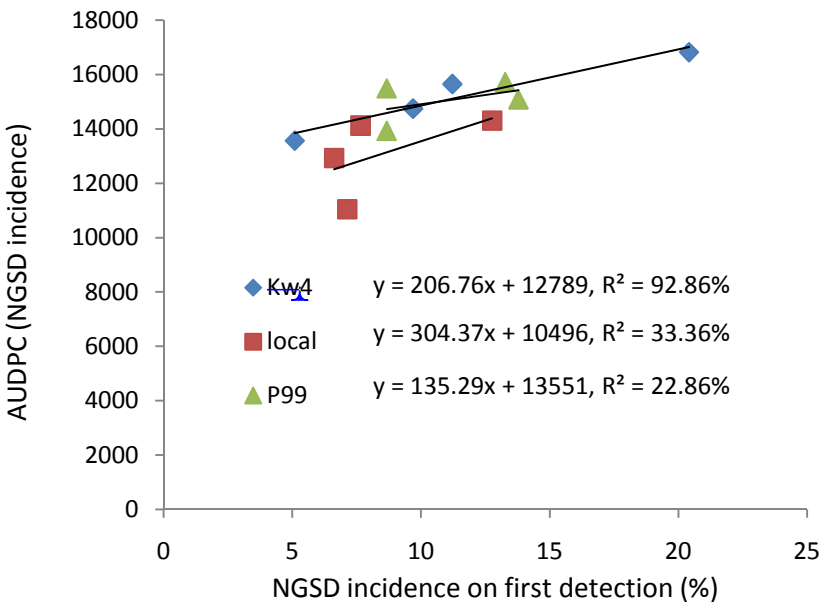


Fig.4. Relationship between NGSD final and NGSD incidence on first detection and, Area Under Disease Progress Curve (AUDPC; NGSD incidence) as influenced by the different Napier clones.

## 4 Discussion

This study examined the temporal spread of NGSD as a basis for development of NGSD management strategies. Gompertz model sufficiently described NGSD progress in the Napier grass fields. This model is usually associated with polycyclic disease epidemics that are characterized by secondary cycles of spread that occur from initial infection *foci*, in the present case being introduced by insect vectors (19; 20; 21) arriving from infected plants.

Generally in phytoplasmapathosystem, host plants develop symptoms at about 7 days after introduction of the phytoplasma by the insect vector, but can take much longer (6- 24 months) depending on the phytoplasma and plant species (23). In Napier grass, it took *Reciliabanda* 30 days of feeding onto an infected Napier grass to acquire phytoplasmatitre high enough to be transmitted to a healthy Napier grass and developed symptoms 120 days after their exposure to an infected vector in the screenhouse (24). In this study, the longer time taken to detect NGSD in the field (about 210 days after planting) could be attributed either to low multiplication rate of the vector, and the long time taken to acquire the phytoplasma and pass it to the healthy Napier grass or the long latent period an infected Napier grass takes to show symptoms or the long time the pathogen takes to multiply in the insect vector(s). The higher NGSD incidence at the time the disease was first detected implies the symptoms could have appeared in the Napier grass fields after 150 but before 210 days after planting, and was not detected as soon as symptoms showed up because of the long sapling interval. Besides, the spread of plant pathogens by insect vector(s) depends on their abundance and inter-plant movements (25; 26). Shinsakuet *al*. (8) noted its abundance in Napier grass fields in Kenya, as such there is a high likelihood that the vector is also present in in the same measure in Uganda, given the close proximity of the two countries. This played a key role in the within field spread of the disease.

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The increase in NGSD final incidence and the high AUDPC could be attributed to the fact that an infected vector retains the capacity to transmit the pathogen for life, giving it an opportunity to continuously infect healthy plants. This is further aggravated by the high mobility of the vector and the practice of cutting back of the Napier grass after every 60 days which could have provided the vectors with more open space to fly to other nearby healthy plants, carrying the phytoplasma with it. Caudwell (27) while working on boisnoir disease observed its rapid progress in young vineyards and attributed it to presence of open soil with sparse vegetation which was highly attractive to the vector *Hyalesthesobsoletus.*

The lower the rate of disease development, the more effective sanitation practices that reduce the initial inoculum become in delaying the epidemic (11). In this study, the rate and time taken for NGSD incidence to double (at least after every 15 days) was moderate, thus representing a low risk scenario. This is in contrast to 5.4 - 6.4 days (high risk scenario) reported by Byamukama *et al*. (28) while working on ~~bean pod mottle virus~~ in soybeans. In this study, it was also realized that NGSD incidence when the disease was first detected within Napier grass fields could predict the final disease incidence and AUDPC. Therefore, deployment of NGSD management tactics which reduce initial inoculum will effectively reduce initial NGSD incidence and NGSD epidemics due to reduction in the rate of spread of the disease in the field (11) and the vector population. These may include rouging of infected plants, weeding to remove alternative host plants, increased fertilization of the soil to boost plant growth, use of tolerant clones and control of the insect vector (5). Stark-urnall and Kast (29) while working on bois noir disease reported appropriate pruning of partially infected vines or cutting of the trunk of systemically infected plants to be effective in facilitating a decrease in disease incidence. However, the above will require that vector population dynamics in relation to disease incidence and severity is fully understood, which information is currently not available.

Deployment of host resistance as a component of integrated disease management acts either as rate reducing resistance – that acts to slow the rate of disease development or as resistance that acts to reduce initial inoculum (11). The local clone has a relatively higher ability to slow down the rate of NGSD spread. However, its inclusion as a component in integrated management of NGSD is not recommended due to its low fodder yield attributes (30). Therefore, deployment of management tactics such as rouging infected plants at first sight, vector control, planting of disease free cuttings, increased soil fertility and weeding would reduce initial inoculum and in the interim control NGSD in Napier grass field.

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