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INFLUENCE OF NPK FERTILISER ON POPULATIONS OF THE WHITEFLY VECTOR AND INCIDENCE OF CASSAVA MOSAIC VIRUS DISEASE

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ABSTRACT

The influence of NPK fertiliser on the symptoms and spread of cassava mosaic virus disease (CMD) and on populations of the whitefly vector (*Bemisia tabaci*) was investigated in Uganda using three cassava varieties: Migyera (CMD-resistant), Nase 2 (tolerant) and Ebwanatereka (highly susceptible) in 1995-96 and 1996-97 planting seasons. In each season NPK fertiliser significantly (P<0.05) increased the incidence of CMD and led to earlier infection and spread of the disease for varieties Migyera and Nase 2 than in unfertilised control plots, whereas for variety Ebwanatereka no significant differences in infection and disease spread were observed for the control and plots that received NPK application. Adult whitefly populations per shoot were increased significantly (P<0.05) by NPK fertiliser on Nase 2 and Ebwanatereka in 1995-96 and on Ebwanatereka in 1996-97, although the increases were not significantly different. Application of NPK fertiliser did not significantly influence the population of whiteflies on variety Migyera in either experiment. Similarly, NPK fertiliser application did not influence CMD symptom severity for all varieties in either season. These results indicate that NPK fertiliser application is not satisfactory strategy for facilitating the control of CMD.

Key Words: Bemisia tabaci, disease progress, Manihot esculentum

RÉSUMÉ

L'influence de l'engrais NPK sur le symptôme et la progression de la mosaïque du manioc (CMD) et sur la population de *Bemisia tabaci* était evaluee en Ouganda utilisant trois variétés du manioc: Migyera (résistant au CMD), Nase 2 (tolérant) et Ebwanatereka (tres susceptible) pour les saisons pluviales 1995-96 et 1996-97. Dans chaque saison l'engrais NPK a augmenté significativement l'incidence du CMD et a conduit a une infection précose et une progression de la maladie sur les variétés Migyera et Nase 2 comparées aux terrains no-traites par l'engrais. Par contre, Ebwanatereka n'a pas montré des différences significatives en terme d'infection et de progression comparé aux parcelles no-traitées et traitées. La population de Bemisia tabaci par rejet était significativement augmentée (P<0.05) par l'engrais NPK sur Nase 2 et Ebwanatereka en 1995-96 et sur Ebwanatereka en 1996-97, même si les augmentations n'ont pas été significativement différentes. L'application de l'engrais NPK n'a pas influencé significativement la population de Bemisia tabaci sur la variété Migyera dans les différentes expériences. De faÇcon similaire, l'application de l'engrais NPK n'a pas influencé la sévérité du symptôme de CMD pour toutes les variétés pour les différentes saisons. Ces résultats indiquent que l'application de l'engrais NPK n'est pas une stratégies satisfaisante pour le contrôle du CMD.

Mots Clés: Bemisia tabaci, progression d'une maladie, Manihot esculentum

INTRODUCTION

Soil fertility influences the incidence and spread of many plant pathogens and can be manipulated to facilitate the control of some fungal, bacterial and viral diseases (Horst, 1990). The incidence of some fungal (Perrenound, 1977) and bacterial (Mackenzie, 1981) diseases is considerably reduced by increased soil fertility, whereas for others it is increased. Generally, however, the incidence and severity of virus diseases is enhanced with increased levels of plant nutrition (Bawden, 1960). For cassava mosaic disease (CMD), which is caused by whitefly-borne viruses (family: *Geminiviridae*; genus: *Begomovirus*), Arraudeau (1987) recommended a reduction in amounts of nitrogen fertiliser and an increase of potassium to limit the effects of the disease. Ogbe *et al.* (1993) suggested application of a balanced NPK fertiliser to cassava varieties susceptible to CMD, to ameliorate the effects of the

disease. In Uganda, it has been generally observed that areas (eastern and northeastern) most severely affected by CMD in the early years of the recent epidemic are characterised by soils of low fertility and an inconsistent rainfall pattern (Otim-Nape, 1987). It is not clear, however, whether this low fertility status enhanced the epidemic. The study reported here assessed the influence of NPK fertiliser application on the spread of CMD and populations of the whitefly vector (*Bemisia tabaci*) on three cassava varieties having different levels of resistance to the disease.

MATERIALS AND METHODS

Experimental site. Experiments were planted in May 1995 and October 1996, at Namulonge Agricultural and Animal Production Research Institute (NAARI), 28 km north of Kampala, Namulonge is located in Wakiso district of central Uganda, in the humid lake Victoria Crescent zone. The area has a bimodal rainfall, with peaks in March or April in the first rainy season and in October or November in the second season.

Experimental materials and design. Three cassava varieties: Migyera (TMS 30572) which is CMD-resistant, Nase 2 (TMS 30337), tolerant and a highly susceptible local cultivar, Ebwanatereka were grown. Hardwood stem cuttings were collected from healthy plants and were used as planting material. The experiment had a split-plot randomised block design with three replicates. Cassava varieties and fertiliser treatments were arranged in main and sub-plots, respectively. Sub-plots measured 9 x 9 m and there were unplanted gaps of 1.5 m between plots and 2 m between replicates. Planting was done at a spacing of 1m x 1m (10,000 plants/ha) using stem cuttings, 15 to 20 cm long. Applications of 250 kg ha⁻¹ of NPK (46:19:60) fertiliser were broadcast one month after planting (MAP) in the fertiliser treated plots. No fertiliser was applied in the control sub-plots.

Whitefly populations. Whitefly populations were monitored starting one (1) MAP and was continued throughout the growing season. Whiteflies were counted on the underside of the five top-most expanded apical leaves of a representative shoot on each sampled plant. Each leaf was held by the petiole and gently turned upside down to count the number of adults. Counting was done mainly in the relatively cool periods of daylight, between 7.00 and 10.00 a.m., or between 4.00 and 6.00 p.m., when the insects are less active than at other times of the day (Fishpool *et al.*, 1987; Otim-Nape, 1993). Whitefly populations were expressed as the mean number of adults per representative shoot per plant.

Disease assessmement. Cassava mosaic disease incidence and severity were recorded monthly for all plants from 1 MAP to 8 MAP. Disease incidence was assessed as the proportion or percentage of plants with CMD symptoms on a scale of 0-1 (P) or 0-100 (%) (Fargette, 1985). Disease symptom severity was scored using a scale of increasing severity from 1 (no symptoms) to 5 (very severe symptoms) (Hahn *et al.*, 1980; Otim-Nape, 1993). Scores for symptomless plants were omitted when calculating the mean severity for each variety and treatment.

Statistical analysis of data and test of hypothesis. Data on CMD incidence, severity and adult whitefly populations were tested for homogeneity of variance before analyses. CMD incidences were transformed to arcsine values as described by Gomez and Gomez (1984). Adult whitefly numbers per shoot (n) were transformed to square root values of n + 0.5 to allow for the zero counts obtained in some months. The transformed data were then subjected to analysis of variance (ANOVA) using the MSTATC statistical package (Freed *et al.*, 1988).

RESULTS

Adult whitefly populations. Adult whitefly populations per shoot were greater in the first than in the second trial. No significant differences among varieties were observed in whitefly populations in either season. Significantly more whiteflies (P<0.05) were observed in sub-plots with than in those without fertiliser at 3 and 5 MAP in Nase 2 (Fig. 1b) and at 2, 3 and 4 MAP in Ebwanatereka in 1995-96 (Fig. 1c). In 1996-97 this trend was apparent only in Ebwanatereka, 5 MAP (Fig. 1f). However, whitefly populations were significantly (P<0.05) less in fertilised than the unfertilised plots at 4 MAP in Nase 2 (Fig. 1b). Furthermore, although significant, the differences in whitefly populations between the fertilised and unfertilised plots were relatively small. Application of fertiliser did not influence populations of adult whiteflies on Migyera at any stage of growth in either season (Fig. 1a and 1d).

For each variety and experiment adult whitefly populations differed significantly (P = 0.001) with crop age (Fig. 1). Infestation by immigrant adults started immediately after sprouting and the populations increased to maxima 3 or 4 MAP in 1995-96 and 4 or 5 MAP in 1996-97. Populations then declined progressively as the crops matured. Similar population trends were apparent on all three varieties in each season (Fig. 1). Single peaks occurred with all three varieties in 1995-96, whereas two peaks were observed on each variety in 1996-97, when a smaller peak 2

MAP was followed by a somewhat larger peak 4 or 5 MAP. The double peaks occurred 2 and 5 MAP on Migyera and Nase 2, and 2 and 4 MAP on Ebwanatereka in both sub-plots with and those without fertiliser (Figs. 1d, 1e and 1f). Overall, there was a higher (P<0.05) adult whitefly population during the early (1 to 4 MAP) than at the late stages of growth (5 to 8 MAP) in 1995-96, but not in 1996-97(Fig. 1).

Incidence of cassava mosaic disease (CMD). Cassava mosaic disease incidence progressed less rapidly in 1995-96 (Fig. 2a, 2b, 2c) than in 1996-97 (Fig. 2d, 2e, 2f) planting seasons. Moreover, the time to 50% incidence in Nase 2 and Ebwanatereka was longer in 1995-96 than in 1996-97. Overall, the three varieties differed significantly (P<0.05) in final disease incidence in each season. Over 90% of plants of Ebwanatereka were affected, whereas incidences were less (80%) for Nase 2 and much less (30%) in Migyera.

Application of NPK significantly (P<0.05) increased the incidence of CMD in Migyera, 5 MAP and at the final observation date (8 MAP) in 1995-96 (Fig. 2a) and in Nase 2 in both seasons 3, 5, 6 MAP (Fig. 2b) and 7 MAP (Fig. 2e). No significant differences were detected in Ebwanatereka in either season (Fig. 2c and 2f) and almost all plants in sub-plots with and those without fertilisers were affected 8 MAP.

Disease progress differed significantly (P<0.05) among varieties. In 1995-96, cassava mosaic disease was first observed in Nase 2 and Ebwanatereka 1 MAP and the incidences in these varieties increased rapidly to reach maxima at 6 or 7 MAP in fertilised and 7 or 8 MAP in the unfertilised sub-plots (Fig. 2b and 2c). Disease progress in Migyera was relatively slow and the incidence remained consistently low, even at the final observation 8 MAP when less than half the plants were affected (Fig. 2a). Similar trends were observed in 1996-97 when the incidence first exceeded 50% in Ebwanatereka only 2-3 MAP (Fig. 2f) and in Nase 2 at 3-4 MAP (Fig. 2e). The final incidence in Migyera was slightly less than in 1995-96 and the differences between fertilised and unfertilised sub-plots were not significant (Fig. 2a). Unlike Migyera and Ebwanatereka, a decline in CMD incidence was observed 7-8 MAP in Nase 2 in sub-plots receiving fertiliser in 1995-96 (Fig. 2b) and in sub-plots with and without fertiliser in 1996-97 (Fig. 2e).

Cassava mosaic disease severity. Cassava mosaic disease symptoms were more severe in 1995-96 than in 1996-97 for Migyera (Fig. 3a and 3d) and Nase 2 (Fig. 3b and 3e). Symptom severity increased progressively in Ebwanatereka to reach the maximum possible score of 5.0 in both fertilised and unfertilised plots 7 or 8 MAP in both seasons (Fig. 3c and 3f). The symptoms in Nase 2 and Migyera were relatively inconspicuous in both seasons, although there was a slight intensification of symptoms in Migyera in October 1995 (5 MAP) and in Nase 2 in December 1995 (7 MAP), which was followed by a decline (Fig. 3a and 3b). In 1996-97 no appreciable change in symptom severity occurred in Migyera or Nase 2, except for a slight increase in Nase 2 in February 1997 (4 MAP) (Fig. 3d and 3e).

Varieties differed significantly (P = 0.001) in CMD symptom severity at the final observation 8 MAP in both seasons. Symptoms were much more severe in Ebwanatereka (5.0) than in Nase 2 (2.3) or Migyera (2.5) in both seasons. Application of fertilisers did not significantly influence symptom severity for any variety in either season (Fig. 3).

DISCUSSION

The influence of NPK fertiliser application on the spread of CMD and populations of the whitefly vector (*Bemisia tabaci*) was investigated on three cassava varieties having different levels of resistance to the disease. There was considerable spread of CMD to all the three varieties, including the resistant Migyera in both seasons. This indicates that Namulonge was at the time of the study an area of high CMD inoculum pressure, whereas in previous studies conducted between 1991 and 1995, little or no spread occurred, even to the highly susceptible varieties Ebwanatereka and Bao (Otim-Nape, 1993; Byabakama *et al.*, 1997; Otim-Nape *et al.*, 1998). The sudden change in the overall epidemiological situation was due to the onset of the severe pandemic of CMD, which has progressed across much of Uganda in recent years and into western Kenya, north-western Tanzania (Otim-Nape *et al.*, 2000) and Rwanda (Legg *et al.*, 2001).

Application of NPK fertiliser increased the incidence of CMD in all three varieties, the magnitude of the increase being associated with the resistance of the variety to the disease. The significant early spread and increase in CMD incidence in plots with fertiliser for Nase 2 and Migyera is notable, since the two are some of the most widely spread improved varieties, especially in areas where they were distributed to control the epidemic of severe CMD. However, the observation on no increase in CMD incidence due to fertiliser at the final observation date in the highly susceptible variety Ebwanatereka is probably because of disease 'saturation'.

The lack of any significant influence of NPK fertiliser on the severity of CMD symptoms in any of the varieties is contrary to findings by Mollard (1987), who reported that NPK fertiliser enhances CMD symptom expression.

Moreover, Arraudeau (1987) recommended a reduction in amounts of nitrogen fertiliser and an increase of potassium to limit the effects of CMD, while Ogbe *et al.* (1993) suggested the use of a balanced NPK fertiliser to cassava varieties susceptible to CMD, to ameliorate the effects of the disease on the crop. It is, however, notable that the symptoms of some viral diseases like *Tobacco mosaic virus* were not found to be influenced by increased mineral nutrient (nitrogen) supply to the host plant (Kassanis, 1957). Therefore, in order to verify the influence of fertiliser on CMD symptom severity, other studies should consider to explore the role of the individual nutrients: nitrogen, phosphorus and potassium and/or their combinations.

The marked decline in CMD incidence and symptom severity in the final stages of the trials in both plots with and without fertiliser, as observed in Nase 2 in 1995-96 and 1996-97 and in symptom severity in Migyera in 1996-97, are features of CMD-resistant varieties and commonly referred to as recovery or symptom remission (Otim-Nape *et al.*, 1995). This phenomenon was not considered when calculating monthly mean severity scores for Nase 2 and Migyera. The mean severity scores were based solely on plants expressing symptoms at the time of observation, which could have overestimated symptom expression and underestimated the extent to which recovery occurred in the two varieties.

Although the populations of adult whiteflies per shoot were significantly increased by NPK fertiliser on variety Ebwanatereka and Nase 2, the differences were small, but may have been greater had the effects of fertiliser on plant size and shoot number also been considered.

It is generally observed that eastern and northeastern Uganda, which were the areas most severely affected by CMD in the early years of the severe epidemic were (Otim-Nape, 1987), and are still characterised by soils of low fertility and an inconsistent rainfall pattern. In contrast, however, the results of this study suggest that the generally low fertility status of soils in these areas is unlikely to have contributed to the CMD epidemic that has occurred there. Indeed, the evidence obtained indicates that CMD spreads less rapidly in low than in high fertility conditions. Moreover, there is another explanation for the epidemic and the increase in symptom severity that occurred as they are known to be associated with the occurrence of a novel recombinant virus, *East African cassava mosaic virus* - Uganda (EACMV-Ug) (Zhou *et al.*, 1997; Deng *et al.*, 1997; Harrison *et al.*, 1997; Pita *et al.*, 2001), derived from two African cassava mosaic geminiviruses: - *African cassava mosaic virus* (ACMV) and *East African cassava mosaic virus* (ACMV) and/or a change in the vector (Legg *et al.*, 2002).

The role of nitrogen, phosphorus and potassium in enhancing virus replication and inter-cellular movement merits investigation. Analysis of CMD-infected leaf tissues to determine the virus titre in the leaves from plants with and those without fertiliser would also be important in furthering an understanding of nutrient effects. These issues should be considered in future studies.

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Figure 1. Adult whitefly populations in each of three cassava varieties: Migyera (a & d), Nase 2 (b & e) and Ebwanatereka (c & f) in plots with and without NPK fertiliser in 1995-96 (a, b, c) and 1996-97 (d, e, f). Bars show standard errors. Ja=January, F=February, Mh=March, A=April, My=May, Jn=June, Jy=July, Au=August, S=September, O=October, N=November, D=December.

Figure 2. Disease progress curves of cassava mosaic disease incidence for each of three cassava varieties: Migyera (a & d), Nase 2 (b & e) and Ebwanatereka (c & f) in plots with and without NPK fertiliser in 1995-96 (a, b, c) and 1996-97 (d, e, f). Bars show

standard errors of means. Ja=January, F=February, M=March, A=April, My=May, Jn=June, Jy=July, Au=August, S=September, O=October, N=November, D=December.

Figure 3. Severity of cassava mosaic disease in each of three cassava varieties: Migyera (a & d), Nase 2 (b & e) and Ebwanatereka (c & f) in plots with and without NPK fertiliser in 1995-96 (a, b, c) and 1996-97 (d, e, f). Bars show standard errors of means. CMD severity scale ranging from 1=No disease symptoms to 5=Very severe symptoms. Ja=January, F=February, Mh=March, A=April, My=May, Jn=June, Jy=July, Au=August, S=September, O=October, N=November, D=December.