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The potential of crop rotation in the management of bacterial wilt of potato in southwestern Uganda

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Abstract

Bacterial wilt (BW) of potato *(Solanum tuberosum)* caused by *Ralstonia solanacearum*, is a major constraint to potato production in southwestern Uganda. It is a recalcitrant disease and one of the plausable management options is crop rotation. However, the suitable crops to be incorporated in a crop rotation regime with a view to reduce BW inoculum levels have not been systematically identified. Studies to identify crop(s) that would in the subsequent season delay on-set of wilt, reduce wilt incidence, lower latent infection, reduce *Ralstonia solanacearum* populations in the soil and increase tuber yield were conducted at Kachwekano Agricultural Research and Development Centre. Trials were carried out for three consecutive seasons during 2000A, 2000B, and 2001A in wilt-infested plots using the randomised complete block design. In 2000A and 2001A all study plots were under potato. The rotation crops (*Sorghum bicolor, Triticum aestivum, Zea mays, Eleusine carocana, Ipomea batatas L., Daucus carota, Phaseolus vulgaris* and *Solanum tuberosum* L.) were planted in the same plots during 2000B. Rotation with *Sorghum bicolor, Triticum aestivum, Zea mays, Eleusine carocana,* delayed on-set of wilt incidence, reduced wilt incidence and latent wilt infestation, and gave higher marketable tuber yield than the other rotation crops evaluated in the study. One-season rotation crops improved the shelf-life of consumption potatoes but not of seed tubers because of latent infections that were detected after subjecting the tubers to incubation at high temperature and indirect enzyme-linked immunosorbent assay on nitrocellulose membrane (NCM-ELISA). Using any one of the evaluated rotation crops was assessed to reduce BW inoculum and was thus better than continuous cultivation of potato.

Key words: Marketable yield, one-season rotation crops, *Ralstonia solanacearum*, *Solanum tuberosum*, wilt incidence

Introduction

Bacterial wilt (BW) of potato (*Solanum tuberosum* L.) caused by *Ralstonia solanacearum* E. F. Smith (*Rs*) is an important seed and soil-borne disease in south western and other potato growing areas of Uganda. In south western Uganda, potato is the number one cash crop and the fourth important food crop (Adipala, 1999; Lemaga *et al.*, 2001). Most households survive on less than one hectare while others depend on rented land for cultivation. The farming community comprises of 70% subsistence growers who practice intensive cropping without any form of organised rotation, and continuous cultivation of potato is common resulting in increased incidence of various diseases, particularly bacterial wilt (Adipala *et al.*, 2000; Tusiime *et al.*, 2000). Thus, application of crop rotation as a bacterial wilt control strategy is limited, but can be enhanced by selection of multipurpose crops that can be used in a crop rotation regime because there are crops that support substantial amounts of the bacterium in their rhizosphere and others that aggressively suppress it in a relatively short time (Terblanche and De Villiers, 1999). Lemaga *et al.* (2001) and Hayward (1994a) recommend crop rotation as a component of integrated disease management (IDM). Potato grown in the highlands is only attacked by race 3 biovar 2-A, whose host range is restricted to potato and tomato, but also remains latent in a wide range of host weeds (Hayward, 1994b). Due to the pathogen's exceptional ability to survive in soil and the rhizosphere of some non-host plants, it renders most single management practices ineffective in controlling the disease. In this study therefore, use of clean planting material, application of fertilisers and control of other diseases and pests were used in combination with selected rotation crops to investigate their potential for BW management. The goal was to identify effective rotation options that are suitable for the control of bacterial wilt within the existing farming systems in south western Uganda. The specific objective was to identify multipurpose crops that are appropriate for a crop rotation regime that would in the subsequent season delay on-set of wilt, reduce wilt incidence, lower latent infection, reduce *Ralstonia solanacearum* populations in the soil and increase tuber size and yield.

Materials and methods

The research focused on *Ralstonia solanacearum*, race 3, biovar 2-A, prevalent on herbaceous weeds, potato tubers and other host crops in Kachwekano (Tusiime *et al.*, 2000; Lemaga *et al.*, 2001). Studies were conducted between 2000 and 2001, at Kachwekano Agricultural Research and Development Centre (01° 16'S, 29° 57'E) located at an altitude of 2200 metres. The experiments were conducted for three consecutive seasons during 2000A, 2000B and 2001A, in bacterial wilt-infested plots, measuring 6 m x 4 m with 1 m alley between plots, laid out in a randomised complete block design with three replicates.

Prior to planting the 2000A experiment, the disease pressure in the selected wilt-infested field was further increased by planting a bacterial wilt susceptible tomato variety (money-maker) for 6 weeks. Tomato plants that did not wilt within 28 days after planting (DAP) were artificially inoculated by stem-puncture technique, using a pure culture of *R. solanacearum* bacterial ooze that had been isolated from tubers infected and cultured in the laboratory on the modified Kelman's growth medium (Priou *et al.*, 1998). After all tomato plants wilted, their remains were chopped into very small pieces and spread evenly in the field to ensure uniform inoculum level in the field (Tusiime *et al.*, 2000; Lemaga *et al.*, 2001)

During 2000A potato was grown in all experimental plots to simulate the shortened rotation existing in the south-western Uganda and to create uniform field conditions. The crops were observed and data on pathogenicity soil sterility and incubation tests taken. In addition data on BW disease incidence and tuber yields were taken. During 2000B, the same experimental plots were planted to *Sorghum bicolor*, *Triticum aestivum*, *Zea mays*, *Eleusine coracana*, *Ipomea batatas* L., *Daucus carota*, *Phaseolus vulgaris* and *Solanum tuberosum* L., food crops commonly grown in the highlands of southwestern Uganda. The following one-season rotation sequences: potato-sorghum-potato, potato-finger millet-potato, potato-carrots-potato, potato-beans-potato, potato-sweetpotato-potato, potato-maize-potato, potato-wheat-potato, potato-potato-potato, were used and the varieties were as follows: sorghum-Kenya, finger millet-local (Entachweka), carrots-Nantes coreless, beans-K20, sweetpotato-local (Magabari), maize-Longe 1, wheat-Chiriku, potato-Victoria.

Potato, sweetpotato, maize and sorghum were spaced at 30 cm within rows and 75 cm between rows. Carrots, wheat and finger millet were spaced at 5 cm within rows and 30 cm between rows. Beans were spaced at 15 cm within rows and 30 cm between rows. The potato variety was selected because it is popular with the farmers due to its moderate tolerance to bacterial wilt and good marketability. During 2001A season, potato was again grown in all the study plots to test the effect of the rotation crops on the subsequent potato crop. Data were collected on percentage of wilted plants on a weekly basis starting from the on-set wilt of symptoms. Plants that were partially or completely wilted were all considered wilted and staked to avoid confusion in the subsequent recording and also to avoid missing out the plants that died off early in the season. Final wilt incidence for each treatment was calculated as percentage of total number of plants emerged.

The initial soil fertility of the field experimental plots was determined, and the necessary nutrients were added using the following inorganic fertilisers: urea, single super phosphate and muriate of potash according to Buekema *et al.*, 1990 and Lemaga *et al.*, 2001. The most common pests and diseases i.e., potato aphids and late blight were controlled using Ambush super (Karate) Lambda cyhalothrin 2.5 % and Ridomilgold (MZ), 63.5 WP Mancozeb 56 % + Metalaxyl 7.5 %, respectively. During 2000A and 2001A the experimental crops were sprayed twice and during 2000B they were sprayed thrice using both the insecticide and the fungicide stated above following the manufacturers recommended rates. All rotation crops were managed by following the recommended agronomic practices.

Tubers were harvested when 75% of the plants reached senescence. Weights of ware tubers, seed size and bacterial wilt infected tubers were recorded separately at harvest (Lemaga *et al.*, 2001). Indicator plant /pathogenicity test was used to test *R. solanacearum* inoculum levels in the soil (Priou *et al.*, 1999) before planting 2000A trials and after each season. The tomato variety, money maker was used for the tests. About 8 kg of infested soil was collected from the rhizosphere of the different rotation crops; soil collected from each plot was mixed thoroughly before loading it into plastic trays measuring30 cm by 60 cm. From each field plot, two tray loads were collected and in each tray 50 viable seeds were planted. The wilted tomato plants were recorded weekly and percentage wilt was calculated based on the number of tomatoes emerged.

Three different methods were used to determine latent infection in tubers and these were: sterile soil tests, incubation at high temperature for six weeks and indirect enzyme-linked immunosorbent assay on nitrocellulose membrane (NCM-ELISA) tests. From each field plot, 120 tubers externally symptom-less were collected, wrapped separately, and allowed to cure at Kachwekano for 2-3 weeks. For each test, 40 tubers were used and were divided into replicate samples for the first two tests and four replicate samples for the last test. Incubated tubers were subjected to 29° C for 45 days. Tubers were observed for bacterial wilt ooze weekly during the first four weeks and daily during the last two weeks. Tubers used for the sterile soil tests were stored for three months to induce sprouts and were then planted in the sterile soil medium loaded in plastic trays of 30 cm by 60 cm and watered with cool boiled rain water. After two weeks, the emerged plants were observed for wilt symptoms on daily basis. In both cases, tubers that showed bacterial wilt symptoms were removed, recorded and disposed-of carefully (Tusiime *et al.*, 1996a). At the end of 21 and 45 days the remaining tubers were cut into two and ring discolouration was observed and recorded. The average percentage of bacterial wilt latent infection was calculated.

The third set of 40 externally symptom-less tubers was subjected to indirect enzyme-linked immunosorbent assay on nitrocellulose membrane (NCM-ELISA) tests. Tubers were washed using tap water, rinsed in distilled water and dipped in 5 % sodium hypochlorite (NaOCl) solution before the extraction (Priou *et al.*, 1998). The knife, the cuticle remover, and tweezers were flame sterilised. A thin slice was cut from the stolon-end and the cuticle remover was used to collect not more than 0.5 g per tuber. Four replicate samples were used and each comprised of 10 tubers. The tuber pieces for each replicate were collected in a plastic bag and weighed before adding the sterile citrate extraction buffer on the basis of 2 millilitres per gram of tissue and kept separately. The potato tissue was crushed using a pestle to facilitate extraction of *R. solanacearum.* To avoid oxidation, the plastic bags containing the samples were kept on ice for not more than an hour. The samples of the tuber extracts (0.5 ml) were enriched right away, with 0.5 ml of selective modified Kelman's medium without Tetrazolium chloride (SMSA) according to Priou *et al.* (1998). The enriched broth was incubated for 48 hours before loading the samples on the nitrocellulose membrane. Latent infection in stems and root segments of the rotation crops was also determined following a similar procedure.

All the data collected were subjected to analysis of variance using Genstat 4.23 release statistical package. Percentage values were transformed into $\log_{10}$ before analysis.

Results

Data collected during the shortened season simulation are presented in Table 1. The baseline information revealed that BW inoculum is prevalent in the soils of the study area. The effects of one-season rotation crops on the subsequent potato crop were as shown in Table 2. *Sorghum bicolor*, *Triticum aestivum*, *Zea mays*, *Eleusine carocana* crops grown during 2000B season had a significant effect on the subsequent potato crop by delaying on-set of wilt for longer periods than *Ipomea batatas* L., *Daucus carota*, *Phaseolus vulgaris* and *Solanum tuberosum* L. repeat. They also reduced wilt incidence and latent infection, increased tuber size and yield, and improved the shelf-life of ware potato. Incubation at high temperature more efficiently detected latent *R. solanacearum* infection than indirect enzyme-linked immunosorbent assay on nitrocellulose membrane (NCM-ELISA) or sterile soil medium method. During 2000A and 2000B NCM-ELISA method did not detect latent *R. solanacearum*, but during 2001A, latent infection was detected in tubers from plots that had been under *Ipomea batatas* L., *Daucus carota*, and *Phaseolus vulgaris*. The tubers harvested from *Solanum tuberosum* L repeat plots had clear bacterial ooze symptoms, whereas the tubers from plots previously

Table 1. Baseline information for bacterial wilt of the shortened crop rotation in south western Uganda at Kachwekano during first rains 2000.

Treatment	Pathogenicity test %		On-set of wilt (days)	Wilt incidence (%)	Sterile soil test (%)	Incubation test (%)	Ware potato yield (t ha^{-1})	Seed tuber yield (t ha^{-1})	Diseased tuber yield (t ha^{-1})	Total tuber yield (%)
	02/2000	07/2000								
Future sweetpotato plot	62	39	44	35.3	64	72.5	14.1	2.8	1.0	17.8
Future wheat plot	60	37	44	33.7	65	72.5	14.1	2.8	0.8	17.8
Future potato plot	60	36	44	34.7	67	71.5	14.0	2.8	0.8	17.6
Future maize plot	62	35	44	34.7	67	72.5	14.1	2.8	0.9	18.3
Future millet plot	60	36	44	34.7	64	72.5	14.2	2.8	1.0	17.7
Future carrots plot	60	37	44	32.7	69	72.0	14.1	2.7	0.8	17.9
Future sorghum plot	60	36	44	35.3	68	71.5	14.0	2.8	0.9	17.6
Future Phaseolus beans plot	62	39	44	34.7	65	72.5	14.1	2.8	1.0	17.9
$LSD_{(0.5)}$	2.3	4.7	2	4.7	10.8	4.7	0.5	1.0	2.0	0.7
CV%	3.2	11.5	0.01	6	19.1	13.0	2.7	2.3	17.7	2.4

Table 2. Effect of one-season rotation crops on days to on-set of wilt, final wilt incidence, pathogenicity, incubation and sterile soil tests at Kachwekano during 2000B and 2001A.

Treatment	onset of wilt (days)	Wilt incidence (%)	Pathogenicity test (%)		Sterile soil test (%)	Incubation test (%)	Ware potato yield (t ha^{-1})	Seed size yield (t ha^{-1})	Diseased tubers (t ha^{-1})	Total tuber yield (t ha^{-1})
	2001A	2001A	2000B	2001A	2001A	2001A	2001A	2001A	2001A	2001A
Sweetpotato-potato	28	26	16	12	74	60.2	5.6	4.6	2.6	12.8
Wheat-potato	42	6	8	6	55	33.9	9.5	4.5	0.6	14.5
Potato-potato	21(45)	72(20)	88	86	87(100)	70.1	1.1(29.9)	1.0(1.7)	8.3(6.3)	10.5(37.8)
Maize-potato	35	8	10	8	57	40	8.6	3.8	0.1	13.1
Finger millet-potato	35	8	11	10	64	40.2	8.3	3.3	0.7	12.2
Carrots-potato	28	16	17	15	79	60.5	6.3	2.7	4.5	13.5
Sorghum-potato	45	4	7	6	68	30.8	9.8	2.3	0.5	12.6
Beans-potato	28	26	24	22	82	70.7	2.5	2.8	6.4	11.8
$LSD_{(0.05)}$	5.2	9.2	21.3	8.8	9.2	7.5	1.4	1.2	0.2	1.5
CV %	13.3	30.5	19.1	20.7	15.5	13.0	16.1	28.7	22.6	15.7

In the brackets are data for potato rotation crop 2000B.

under *Sorghum bicolor*, *Triticum aestivum*, *Zea mays*, and *Eleusine carocana* tested negative. The stem and root segments of *Sorghum bicolor*, *Triticum aestivum*, *Zea mays*, and *Eleusine carocana* tested negative, whereas those of *Ipomea batatas* L., *Daucus carota*, *Phaseolus vulgaris* and *Solanum tuberosum* tested positive. During 2001A, there was significant statistical difference between latent infection detection using sterile soil medium method and incubation at high temperature, ware potato, seed size tuber and diseased tuber yield and total yield. Tubers harvested from plots previously under *Sorghum bicolor*, *Triticum aestivum*, *Zea mays*, and *Eleusine carocana* were able to store for a longer time without rotting (more than three months) than those harvested from plots that had been under *Ipomea batats* L. and *Daucus carota* (one month) which were in turn stored longer than tubers from plots that had been under *Phaseolus vulgaris* and *Solanum tuberosum* (less than three weeks)

Discussion

Low wilt incidence in the field cannot be relied on as an indictor of low level of the bacterium in the soil as shown by high percentage latent infection obtained after subjecting potato samples collected from plots previously under *Sorghum bicolor*, *Triticum aestivum*, *Zea mays*, and *Eleusine carocana* to incubation under high temperature. Although incubation under high temperature takes longer, it gives higher latent wilt counts and could be carried out by the farmers at their own farms and obtain results. The NCM-ELISA method although very fast in detecting *R. solanacearum* latent infection, requires a wide range of equipment and only technical personnel based at already established research institutions must be available to assist the farmers to determine the health status of their seed tubers. This limits its use to only the rich farmers or non governmental organisations (NGOs) who can afford to transport their seed tuber samples to these locations, thus rendering it un affordable to the poor farmers that form the majority of the farming community in southwestern Uganda. The farmers could also carry out the sterile soil medium method on their own but, it is demanding in terms of containers in which to load sterile soil, collecting the soil, sterilisation equipment and labour for watering.

The effect of *Sorghum bicolor*, *Triticum aestivum*, *Zea mays*, and *Eleusine carocana* one-season rotation on the BW infections of subsequent *Solanum tuberosum* crop indicates the effectiveness of crop rotation measures for the control of BW. On set of wilt was delayed and there was decreased wilt incidence, increased tuber size and yield and prolonged shelf-life of the subsequent potato crop were these crops were planted before the potato. *Ipomea batatas* and *Daucus carota* and the worst effect was from *Phaseolus vulgaris* and *Solanum tuberosum* L. repeat. However growing any of the evaluated crops was better than repeating *Solanum tuberosum* L. and our studies therefore point out that this should be avoided.

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References

Adipala, E. 1999. Potato Production in Uganda. A survey perspective. Makerere University, Kampala.

Adipala, E., Namanda, S., Mukalazi, J., Abalo, G., Kimoone, G. and Hakiza, J.J. 2000. Understanding farmers perception of potato production constraints and responses to yield decline in Uganda. In: Adipala, E., Nampala, P and Osiru, M. (Eds). African Potato Association Conference Proceedings 5:96 -107.

Buekema, H. P. and Van der Zaag, D.E. 1990. Introduction to potato production. Student edition, Publication and Documentation (Pudoc) Wageningen, The Netherlands. pp. 124 - 133.

EPPO /CABI, 1996. Quarantine pest. Data sheets on *Ralstonia solanacearum* 26:1070 - 1081

Hayward, A. C. 1994a. The hosts of *Pseudomonas solanacearum.* In: Hayward, A.C., and G. L. Hartman (Eds.). Bacterial wilt. The disease and its causative agent *Pseudomonas solanacearum*. CAB International, Walliford, UK. pp. 9-24.

Hayward, A. C. 1994b. Systematics and phylogeny of *Pseudomonas solanacearum*, and related bacteria. Pages 123-135. In: Hayward, A.C. and Hartman, G. L. (Eds.). Bacterial wilt. The disease and its causative agent *Pseudomonas solanacearum*. CAB International, Walliford, UK.

Lemaga, B., Kanzikwera, R., Hakiza J.J., Kakuhenzire, R.and Manzi, G. 2001. The effect of crop rotation on bacterial wilt incidence and potato tuber yield. Africa Crop Science Journal. 9 (1): 257 - 266.

Priou, S., Aley, E., Chujoy, B., Lemaga, L. and French, E. 1999. Integrated control of bacterial wilt. CIP slide training series. International Potato Centre (CIP), Lima, Peru.

Priou, S. and Gutarra, L. 1998. NCM-ELISA kit for the detection of *Ralstonia solanacerum* in latently infected potato tubers. Manual of instruction for use. International Potato Centre (CIP). Lima, Peru.

Terblanche J. and De Villiers, D. 1999. Marigolds as biological control for bacterial wilt. African Crop Science Conference Proceedings 4:523-525.

Tusiime, G., Adipala, E., Opio, F. and Bhagsari, A. S. 1996a. Occurrence of *Pseudomonas solanacearum* latent infection in potato tubers and weeds in highland Uganda. African Journal of Plant Protection 6:108-118.

Tusiime, G. and Adipala, E. 2000. Management of bacterial wilt of potato: approaches, limitations and the role of stakeholders. In: Adipala, E., Nampala, P. and Osiru, M. (Eds.). Proceedings Africa Potato Association Conference 5:347-352.