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Full Length Research Paper

Current status of bacterial -speck and -spot diseases of tomato in three tomato-growing regions of Tanzania

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Disease surveys were conducted in Arusha, Dodoma, Iringa and Morogoro regions in Tanzania in 2004 and 2005, to establish the current status of bacterial -speck and -spot diseases of tomato. The surveys also aimed at assessing the effect of cultural practices on the two diseases, with the aim of determining suitable management options. Information was also collected through oral interviews on the sanitary conditions of the fields, land use intensity, source of seeds, and chemical application. Results of the surveys indicated that bacterial -speck and -spot were widespread in farmers' fields throughout the study area. All the farmers interviewed applied pesticides to control tomato diseases. Data collected showed a positive correlation between field weediness and disease occurrence, indicating that field sanitation would be an effective way of reducing the two tomato diseases. There was a positive correlation between tomato variety and disease, and the locally available varieties were all susceptible to bacterial -speck and -spot. Of 104 tomato disease samples collected, 65 were found to be affected by the bacterial speck pathogen (*Pseudomonas syringae* pv. *tomato*), and 39 fruit samples were found to be affected by the bacterial spot organism (*Xanthomonas campestris* pv. *vesicatoria*). Screening of tomato seeds procured from the open market showed that they were not infected with *P. syringae* pv. *tomato* and *X. campestris* pv. *vesicatoria*.

Key words: Bacterial speck, *Pseudomonas syringae* pv. *tomato,* bacterial spot, *Xanthomonas campestris* pv. *vesicatoria,* management options.

INTRODUCTION

Bacterial-speck and -spot diseases of tomato (*Lycopersicon esculentum* Mill.) are caused by *Pseudomonas syringae* pv. *tomato* and *Xanthomonas campestris* pv. *vesicatoria* (Syn. *Xanthomonas vesicatoria*), respectively. Together, they are considered to be the most important foliar diseases of tomato in most production areas (Yu et al., 1995; Blancard, 1997). Although they are caused by different pathogens, the diseases are commonly found together in mixed infections (Delahaut and Stevenson, 2004), causing symptoms that are so similar that they are often confused, one for the other (Cuppels et al., 2006). The diseases attack every part of the tomato plant. Symptoms appear on leaves, flowers, petioles and stems. Bacterial spots on tomato fruits have been reported to cause up to 52% loss of fruit weight (Jones et al., 1986). Bacterial speck lesions on tomato fruits may make them unfit for the market. On tomatoes for processing, bacterial specks are sometimes severe enough to cause considerable grading or loss in quality (Goode and Sasser, 1980). In the field, yield losses due to bacterial speck varied from 75% in plants infected at an early stage of growth to 5% in plants infected later in the season (Yunis et al., 1980).

One of the major sources of the primary inoculum for these diseases is the infected tomato seed (Watterson, 1986; Blancard, 1997). Resistance in the host plant has been reported to be the most effective means of management (Yu et al., 1995; Blancard, 1997). Other methods of managing the diseases include removal of

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plant debris, removal of weeds, rotation and the use of clean (non-infested) seed and transplants (CAB, 2005). Chemical sprays have also been used, but these may not be effective where the weather is favourable for infection (Samodi et al., 1996).

Previous studies have demonstrated the widespread occurrence of bacterial spot in all vegetable-growing areas of Tanzania (Black et al., 2001; Kaaya et al., 2003). Bacterial speck was reportedly not found in the study areas during those previous studies. However, more recent studies by Shenge et al. (2008a) demonstrated that bacterial speck is also now of widespread occurrence in the country. Due to the fact that bacterial -speck and -spot diseases have been reported to often occur together, the two diseases were considered as a disease complex in the present study. In view of the dynamic nature of the two diseases, and their negative impact on tomato production, the current study aimed at establishing the current status of the two diseases in the tomato-producing areas of Tanzania. The second objective, namely; to determine the effect of cultural practices on the two diseases, was informed by the need for effective and sustainable options for managing the diseases in the country.

MATERIALS AND METHODS

Field surveys

Surveys were carried out in farmers' fields in Arusha, Iringa and Morogoro regions, Tanzania in 2004 - 2005 to determine the current status of bacterial -speck and -spot diseases in tomato-producing areas. The surveys covered 75 fields. The locations were selected based on their history of tomato production, and to ensure the representation of different ecological backgrounds.

Within each tomato farm, the incidence of bacterial -speck and -spot diseases was assessed in five 2 x 2 m^2 quadrants, one at each corner and at the centre of the field. An average of 10 plants were assessed along a diagonal within each quadrant. The number of plants within each quadrant showing typical symptoms of bacterial speck and/or bacterial spot on leaves, stems or fruits was recorded. The number of infected plants within the five quadrants was summed up and divided by the number of quadrants to obtain an average for each field. This was used to determine the disease incidence by taking the number of infected plants in each field as a percentage of the total number of plants sampled in that field. Disease severity was scored using the scale of Horsefall and Barret (1945) with modifications, where: 1 = no disease, 2 = 1 - 3% infection, 3 = 5 - 12% infection, 4 = 12 - 25% infection, 5 = 25 - 50% infection and 6 = > 50% infection. Scores of 1 - 2 were classified as low severity, 3 - 4 as moderate severity and 5 - 6 as high severity. The sanitary condition of each field was also evaluated. Other data on land use intensity, use of pesticides and sources of tomato seeds were provided by the farmers through oral interviews.

Tomato fruits bearing typical symptoms of bacterial -speck and -spot were collected from each field. The samples were placed in paper bags and brought to the laboratory where they were washed in tap water to remove soil and debris, and surface-disinfected by rinsing them in 1.0% sodium hypochlorite before dipping them for 3 s in 95% ethanol. They were then rinsed thoroughly in sterile distilled water (SDW) before isolation of the causal bacteria associated with the symptoms.

Bacterial isolation

Tissue segments of about 2 mm^2 were excised from advancing lesion margins on symptomatic tomato fruits. The tissue segments were teased in a few drops of sterile distilled water (SDW), and allowed to stand for 10 - 15 min in a laminar airflow chamber. A loopful of the resulting suspension was streaked onto plates of nutrient agar (NA), incubated for 72 h at 25 - 28°C and observed daily for colony growth. Presumptive colonies of the pathogens were purified by sub-culturing single colonies (Bradbury, 1970). The bacteria were identified as *P. syringae* pv. *tomato* and *X. campestris* pv. *vesicatoria* through biochemical tests, hypersensitivity on tobacco, pathogenicity tests on tomato (*cv. Tanya*) seedlings (Shenge et al., 2007) Biolog, rep-PCR and restriction fragment length polymorphism (RFLP) analysis (Shenge et al., 2008b).

Detection of *P. syringae* pv. tomato and *X. campestris* pv. *vesicatoria* in tomato seeds

Six seed lots from different tomato varieties were purchased from the open market. These were; Rio Grande and Cal J (Pop Vriend Seeds, France), Roma VF and Marglobe (Royal Sluis, Netherlands), Tanya (Tengeru, Tanzania), and Roma VF (East Africa Seeds, Tanzania). From each seed lot, three samples, each consisting of 100 seeds, were planted in 10 cm diameter plastic pots filled with sterile soil. The seeded pots were placed in the screen house in a completely randomised design with three replications and observed for germination. When plants were in the true leaf stage (6 - 7 cm tall), the pots were covered separately with clear polyethylene bags for 36 h to provide high moisture conditions. The pot cultures were held for four weeks. Seedlings were examined for typical bacterial -speck and -spot symptoms on cotyledons and the first true leaves within seven days after emergence or after they were enclosed in the polyethylene bags. The experiment was repeated twice. Data collected were analysed using ANOVA models of MSTATC, and means were separated using LSD test.

RESULTS

Disease surveys

Results of the disease surveys in farmers' fields are summarised in Table 1. The results indicate that bacterial -speck and -spot diseases of tomato are of widespread occurrence in farmers' fields in all the regions and tomato-producing ecological conditions covered in this study. There was no tomato farm surveyed where the diseases were not found (Table 1). Incidence of the diseases ranged between 2 and 80%. Correlation analysis of the data using the statistical package for social scientists (SPSS Release 11.50) revealed that the sanitary condition of the fields correlated positively with disease incidence and severity (Table 1). Tomato fields with high disease scores of 4 and above were among the weedy fields. The highest scores for disease severity (6) were recorded at Mgeta, where the two fields surveyed were both weedy (Table 1). There was a positive correlation (0.268*) between tomato variety and occurrence of bacterial-speck and -spot. However, no correlation existed between land use intensity and disease occurrence. The results also showed that all the tomato farmers inter-

Table 1. Relationship between field history, sanitary condition of fields, source of tomato seeds and the occurrence of bacterial -speck and -spot diseases of tomato in selected villages in Arusha, Iringa and Morogoro regions, Tanzania.

Region	Location/ village	Number of farms sampled (N)	*Land use intensity (years)			Sanitary condition of fields		Source of seeds		No. of farms sprayed with pesticides	Mean Disease Incidence (%)	**Average disease severity score
			1 - 2	3 - 5	≥ 5	Weeded	Unweeded	Number of farms sown with seeds bought from the open market	Number of farms sown with seeds extracted from the previous crop			
Morogoro	Mgeta	2	0	2	0	0	2	2	0	2	72.0	6.0
Arusha	Madira	5	1	3	1	4	1	5	0	5	15.2	1.6
Arusha	Manyire	2	0	2	0	2	0	0	2	2	5.5	1.5
Arusha	Ngarenayuki	16	0	12	4	13	3	14	2	16	13.1	2.3
Morogoro	Mazimbu	3	0	3	0	3	0	3	0	3	7.7	2.0
Iringa	Ilula	46	15	26	5	46	0	46	0	46	8.7	2.5
Iringa	Imalutwa	1	0	1	0	1	0	0	1	1	16.0	2.0
Total		75	16	49	10	69	6	70	5	75		

*Land use intensity = Number of years the field had continuously supported intensive tomato cultivation.
**The disease scores ranged from 1 (no disease) to 6 (more than 50% infection).

viewed applied pesticides as a management strategy for the two bacterial diseases (Table 1). Most of the farmers interviewed (93.4%) bought their tomato seeds from the open market, with only a few (6.6%) who extracted seeds from selected fruits from the previous harvest. Most of the farmers interviewed (65.7%) grew tomato on the same field for three to four years, while 13.1% used the same field for tomato production for more than five years (Table 1).

Tomato farmers in the surveyed areas mainly grew four tomato varieties (Tanya, Cal J, Marglobe, and Tengeru 97). Two other varieties (Moneymaker and Roma VF) were grown to a lesser extent. All the tomato varieties grown by the farmers were susceptible to bacterial -speck and -spot diseases (Table 1). Most of the farmers preferred Tanya due to its fruit qualities such as firmness, taste, long shelf life, ability of the fruits to withstand rough post harvest handling with minimum damage and the absence of fruit cracking. Unfortunately, Tanya was also the most severely affected by the two bacterial diseases. The use of the variety correlated positively (0.27*) with high bacterial disease severity, indicating that it was highly susceptible to the two diseases.

Detection of *P. syringae* pv. tomato and *X. campestris* pv. vesicatoria in tomato seeds

The results showed that none of the tomato seed sold in the open market were infected with *P.s.* pv. *tomato* and *X.c.* pv. *vesicatoria*. Tomato seeds in the open market were marketed in hermetically sealed packets or cans, and were found to be treated with thiram.

DISCUSSION

It has been reported that host genetic resistance is the most effective strategy in managing bacterial -speck and -spot diseases (Jones and Scott, 1986; Hulbert et al., 2001). A resistance gene to *P. syringae* pv. *tomato* was discovered in a wild-type tomato species (*Lycopersicon pimpinellifolium*) (Pilovsky and Zutra, 1982). Bacterial spot resistance to Race 1 (T1) of *X. campestris* pv. *vesicatoria* has also been found in the tomato cultigen Hawaii 7998 (H7998) (Jones and Scott, 1986). The incorporation of these genes into commercially acceptable tomato genotypes is now a common practice (Gu and Martin, 1998). This has study demonstrated the need for breeding work in Tanzania to develop genetic resistance to the diseases. In undertaking this task, consideration should be given to consumer preferences, as indicated by the farmers interviewed. Tanya, the tomato variety which was preferred by most of the farmers, is also aboriginal to Tanzania, and has good adaptation to the local environment.

Although only a small number of unweeded tomato fields were observed in the study, the results evidenced the effectiveness of field sanitation as a sustainable disease management strategy. There was a positive correlation between sanitary conditions of tomato fields and the occurrence of bacterial -speck and -spot diseases (Spearman's correlation coefficients of 0.33** for sanitary condition: disease incidence and 0.38** for sanitary condition: disease severity). By dividing all the fields into two categories on the basis of sanitary conditions, it was found that the category comprising unweeded tomato fields also had high disease severity scores of 4 and above (Table 1). The two tomato fields surveyed at Mgeta (Table 1) gave a very good picture of the relationship between field sanitation and the incidence of bacterial -speck and -spot diseases. Both fields had been abandoned by their owners, who reported that they had given up on the fields as a result of their inability to manage the two bacterial diseases. The two tomato fields had disease incidences of 62 and 80% respectively, and disease severity scores of 6 each. They also turned out to be the most severely affected fields covered in the study (Table 1).

The ability of *P. syringae* pv. *tomato* and *X. campestris* pv. *vesicatoria* to survive on crop debris, volunteer pepper/tomato plants, and solanaceous weeds has been reported (Jones et al., 1986). The presence of weeds in the tomato field causes over-crowding, which leads to increased humidity in the crop micro-climate and poor air circulation. It is likely that such conditions provide a favourable environment for rapid *P. syringae* pv. *tomato* and *X. campestris* pv. *vesicatoria* multiplication and spread, leading to high disease occurrence. The findings of this study demonstrate that field sanitation is an effective and sustainable option in the management of bacterial -speck and -spot diseases of tomato.

All the farmers interviewed in this study indicated that chemical application was their first response to outbreaks of bacterial -speck and -spot diseases (Table 1). Although some of the tomato farmers did not mention the names of the chemicals they applied, those that were mentioned included Dithane® (M-45 mancozeb), Ridomyl® (Metalxyl), Cupravit® (copper oxychloride), Bravo® (Chlorothalonil), Cymbush® (cypermethrin) and Karate® (lambda-cyhalothrin). Two of the chemicals mentioned (Cymbush (cypermethrin) and Karate (lambda-cyhalothrin) are insecticides.

All the chemicals mentioned by the farmers are usually sold in the open market, and the majority of them end up in the hands of untrained farmers, leading to their indiscriminate application on tomato and other crops. Although the indiscriminate application of these chemicals has gone on for a long time, their effectiveness and long term impact on the disease management and the environment has not been assessed. As for copper-based chemicals, their use in plant disease management has ran into problems in many parts of the world due to the emergence of copper-resistant strains of many plant pathogenic bacteria (Cooksey and Koike, 1990; Ritchie and Dittapongpitch, 1991; Koller, 1998; Gore and O'Garro, 1999; Martin and Hamilton, 2004). In recent studies, it was found that 57.1% of *P. syringae* pv. *tomato* and 73.3% of *X. campestris* pv. *vesicatoria* strains collected from selected tomato-producing areas in Tanzania were resistant to 20% copper sulphate (Shenge et al., 2008c). Some reports have shown that the addition of maneb or mancozeb fungicides to the copper bactericides enhanced their efficacy (Conover and Gerhold, 1981; Marco and Stall, 1983). It is possible that some of the fungicides available in Tanzania (and their combinations) may be effective in reducing bacterial -speck and -spot diseases of tomato. It is, therefore, important to conduct further studies on the fungicides to determine combinations that have efficacy in reducing the diseases in Tanzania.

The seed-borne nature of bacterial -speck and -spot pathogens is well documented in literature (Jones et al., 1986; Sijam et al., 1991; Pohronezny et al., 1992; Jones et al., 1995). Infected seed has serious implications, because the seed-borne phase of the diseases is not only a means of pathogen survival, but also a major determinant of successful dissemination through the seed distribution system. In view of these implications, a key management strategy for the control of these diseases is to ensure the health and purity of planting material. Results of the current study indicate that tomato seeds sold in the open market in the surveyed areas were free from *P. syringae* pv. *tomato* and *X. campestris* pv. *vesicatoria* inocula. The study did not include seeds extracted from the previous crop by the farmers, as only 6.6% of the farmers were involved in the practice. Moreover, although seeds extracted from diseased fruits will most likely be contaminated by the pathogens; and thus contribute to entrenching the diseases, farmer-extracted cannot account for the initial introduction of the diseases into new areas. In spite of the finding that the tomato seeds included in this study were free from *P. syringae* pv. *tomato* and *X. campestris* pv. *vesicatoria*, it is recommended that screening of tomato seeds sold in the market should be done on a regular basis to ensure that they comply with seed health standards.

Unlike in the developed parts of the world, where vegetable production is mostly undertaken by large-scale farmers, production of vegetables in Tanzania is done by small holder, resource-poor farmers. The difficulties and constraints faced by vegetable growers in Tanzania were highlighted by Massomo et al. (2005). In that study, most of the farmers identified diseases as a major constraint to the success of their enterprise. The study by Massomo et al. (2005) also found that most of the farmers involved in vegetable production in the study area did not attend formal school beyond the secondary level. Given their educational background, training and education of farmers on disease management practices would be an important and effective way of equipping them for well-informed disease management decisions.

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