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Reaction of Selected Citrus Cultivars to Pseudocercospora Leaf and Fruit Spot Disease Under Natural Infection in Northern Uganda

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Abstract

Citrus is an important crop among many resource constrained subsistence farmers living in rural areas of Uganda. Citrus production is affected mainly by drought, declining soil fertility, pests and diseases. Among diseases, citrus leaf and fruit spot disease caused by fungus *Pseudocercospora angolensis* is currently one of the major constraints to the production of citrus in Uganda where millions of people rely on the crop for nutritional security and household income. The disease can lead to 50-100% fruit yield loss depending on environmental conditions, disease management and weather conditions. In order to identify resistance to *P. angolensis*, a study was conducted to identify variability for citrus leaf and fruit spot resistance from adapted commercial cultivars as an initial step in developing integrated disease management strategy. Six cultivars were assessed. The screening was under natural infection conditions in disease hot spots in northern Uganda in 2014a and 2014b seasons. The results showed significance difference ($p < 0.01$) for Area Under Disease Progressive Curve (AUDPC) for number of leaves with *P. angolesnis* symptoms and number of lesions. Subsequently, the study identified Kuno as resistant and Tangelo as less susceptible to Pseudocercospora leaf and fruit spot infection, and they could be recommended for citrus leaf and fruit spot disease control.

Keywords: *Pseudocercospora angolensis*, Area Under Disease Progressive Curve, leaf and fruit spot, resistance, severity

1. Introduction

Citrus is one of the most popular and widely grown fruit crops in the world (FAO, 2021). It is cultivated in more than 140 countries, with an annual world production of 143.75 million tons (FAO, 2021). Oranges account for more than half of world citrus production and are the most widely traded citrus fruit followed by tangerines, lemons and grapefruits. Africa accounts for 11% of the global output (FAO, 2021). Egypt is the leading producer of citrus in Africa, followed by South Africa, Morocco and Algeria (FAO, 2021). Citrus is an important cash crop in Uganda, where it is produced for both domestic consumption and regional markets (Uganda Investment Convention Report, 2017). The crop is grown mainly by smallholder farmers in eastern and northern parts of Uganda (Kongai et al., 2019). Commonly grown citrus cultivars include rough lemon and sweet orange including Valencia, Nova, Hamlin, Washington Navel and Kuno (Kongai, 2017).

Despite its importance, the crop is threatened by various production constraints including: declining soil fertility, socioeconomic problems (marketing, very high crop management costs, postharvest handling), pests (scales, leaf miner, mealy bugs), and diseases (gummosis, canker and leaf, citrus greening, leaf and fruit spot). In tropical Africa, particularly Sub-Saharan Africa production of citrus is seriously hampered by a fungal disease caused by *P. angolensis* (Seif & Wittle, 1984; Mohammed, 2013). The yield reduction due to this disease can reach 50-100% (Mohammed, 2013) when climatic conditions are favorable to the disease development, and a timely effective control measures are not taken (Seif, 1994). In 2010, leaf and fruit spot disease (LFSD) caused by *P. angolensis,* was identified as a new threat to the citrus especially in northern Uganda. The confirmation of the causal organism was done using DNA sequencing at CBS Fungal Biodiversity Centre, Netherlands (Pedro Crous, personal communication).

Pseudocercospora fruit and leaf spot disease is one of the major constraints for production of citrus in Sub-Saharan Africa (Pretorius, 2005; Mohammed, 2013). The disease is reported to be more serious at altitudes

over 600 m, although studies in Ghana showed that citrus in areas with altitude of less than 200 m was also severely affected (Lawson et al., 2017). All cultivatable species of citrus appear to be susceptible (CABI, 2009). Cultivated species were found to vary in susceptibility to the fungus and the effects of the disease (Sief & Hillocks, 1999). *Pseudocercospora angolensis* was reported as a disease of great economic importance, causing yield loss of 50-100% (Lawson et al., 2017; Mohammed, 2013). Similar observation was made in Uganda (Kumakech Alfred, personal communication). The devastating nature and geographic distribution of leaf and fruit spot disease makes it a major pest (Michael et al., 2017). Damage caused by *P. angolensis* primarily consists of premature abscission of young fruit and leaves, leading to direct yield loss. Additionally, development of spots on fruits leads to unmarketable fruits, thus reduction of income.

In Africa, *P. angiogenesis* was first identified in Angola and Mozambique in 1952 (De Carvalho & Mendes, 1952), from where it was spread to other parts of Africa (Seif & Hillocks, 1993). Occurrence of *P. angolensis* was reported in Eastern Africa in the 1980s (Seif & Hillocks, 1993), Ethiopia in 1990 (Derso, 1999), Guinea in 1993 (Diallo, 2001), and later in the highlands in Sierra Leone in 2010 (Harling et al., 2010). In northern Uganda, *P. angolensis* was first detected in 2010 (Kumakech, personal communication). Since then, the disease was spread to all the citrus growing districts in the northern agro-ecological zone of Uganda.

All cultivated citrus species are susceptible to *P. angolensis* (European Food Safety Authority, 2022). According to European Food Safety Authority (2022), *Citrus paradisi* (grapefruit) is more susceptible than *Citrus limon* (lemon) and *Citrus* spp. (limes). The susceptibility of *Citrus sinensis* (sweet orange) varies depending on the cultivars (Kassahun et al., 2006; Ndo et al., 2010). No reports were found of *P. angolenis* affecting non-rutaceous plant species. Citrus leaves are susceptible up to five to eight weeks after emergence. Lesions on the leaves and fruit appear two to three weeks after infection. Fruit is also susceptible to *P. angolensis*, but develops certain resistance with age (Seif & Hillocks, 1999). Long-distance spread is likely to occur through the movement of infected plant material (European Food Safety Authority, 2022).

Conventional control strategies for citrus leaf and fruit spot disease include cultural techniques such as pruning and field sanitation measures, botanical control, host plant resistance and chemical control (Manga et al., 1999; Eshetu, 1999; Kuate et al., 2006; Mohammed, 2017). Currently, use of fungicides is the main control strategy in Uganda. However, none of the chemicals is highly effective and chemical control is also harmful to the environment. Besides, Ugandan farmers are resource limited and cannot afford the fungicides, let alone the health and environmental concerns of using fungicides (Kansiime et al., 2017). No information is available in literature on citrus cultivars that are resistant to Pseudocercospora leaf and fruit spot disease in Uganda. This paper describes the reaction of selected commercial citrus cultivars to *P. angolensis* natural infection under local conditions in northern Uganda.

2. Method

2.1 Plant Materials

Six popular sweet orange cultivars viz., Washington Navel, Tangelo, Valencia, Hamlin, Nova, and Kuno were evaluated for their reaction to *P. angolensis* under natural infection conditions in northern Uganda. Budded citrus seedlings were used. Two-month-old seedlings obtained from Ngetta Zonal Agricultural Research and Development Institute was transplanted to individual buckets containing loam soil. Seedlings were grown in the screen house at a temperature of 22-28 °C under natural light conditions for one month and used for experiments when they had 3-4 new leaf flushes.

2.2 Experimental Design

Two independent experiments were established following a randomised complete block design, with four replications in a disease hot spot in an infected orchard with over 50% of the leaf area of citrus trees showing symptoms of *P. angolensis*. The experiments were established during the first and second rainy seasons in 2014, April and August, respectively.

2.3 Data Collection and Data Analysis

Weekly data on disease severity were collected for three months. Data was collected on number of leaves with lesions and number of lesions on three leaves. Severity data were used to calculate the area under disease progress curve (AUDPC) (Madden et al., 2007) prior to statistical analysis. AUDPC values were analysed by analysis of variance (ANOVA) assuming normal distribution. Data analysis was performed using GENSTAT statistical package 16th edition. The data were subjected to ANOVA, and residual plots were used to check ANOVA assumptions. Hypotheses were rejected at $P \leq 0.05$ and means compared by Tukey's test. The two-season data was pooled and mean values of results presented.

3. Results

3.1 P. angolensis Severity in Tested Citrus Cultivars

Results of disease severity are presented in Table 1. Significant differences ($P < 0.05$) in severity were recorded among citrus cultivars tested. High disease severity (AUDPC) for number of leaves with symptoms was recorded on Valencia, Hamlin, Nova and Washington Navel. Valencia had the highest severity (3.88) followed by Hamlin (2.88), Nova (2.12) and Washington Navel (2.12) at 12 weeks. On the other hand, Tangelo had a low AUDPC value (1.25) and local orange a very low value (0.21). Kuno was the only cultivar that did not have leaves with *P.angolesis* symptom; it registered an AUDPC of zero. Overall, significant differences ($P < 0.05$) in AUDPC for the average number of lesions in three leaves were also recorded among the cultivars tested. Valencia and Nova had the highest number of lesions (4.05 and 4.01, respectively), followed by Hamlin (2.73), Washington Navel (2.6) and Tangelo (1.61), local orange (0.33) and Kuno (0.0).

Table 1. Reaction of selected citrus cultivars to Pseudocercospora leaf and fruit spot natural infection in northern Uganda

Citrus cultivars	Disease severity (AUDPC values) 3 months after establishment	
	Number of leaves with lesions	Average number of lesions on 3 leaves
Hamlin	2.88ab	2.73ab
Washington Navel	2.12b	2.60ab
Tangelo	1.25bc	1.61b
Valencia	3.88a	4.05a
Nova	2.12b	4.01a
Kuno	0.00c	0.00c
Local orange	0.21c	0.33c
CV (%)	8.4	12.7

Note. [abc] Means followed by the same letter are not significantly different ($P > 0.05$).

3.2 Resistance Classification of Cultivars

The results of the reaction of selected citrus cultivars to the Pseudocercospora leaf and fruit spot natural infection is presented in Table 2. All the citrus cultivars tested exhibited susceptible reaction, except Kuno. A susceptible reaction was characterized by rapid progression of symptoms. Symptoms appeared as greenish-yellow patches, which later developed into brown leaf spots surrounded by a yellowish halo. In the susceptible cultivars, leaves were not equally susceptible. Valencia and Nova were very susceptible, followed by Washington Navel and Hamlin. Tangelo was less susceptible. Only one cultivar, Kuno exhibited a tolerant reaction characterized by absence of *P. angolensis* leaf symptoms.

Table 2. Classification of reaction of six citrus cultivars to Pseudocercospora leaf and fruit spot natural infection in northern Uganda

Cultivar	Symptom development	Reaction
Valencia	Brown leaf spots surrounded by a yellowish halo	Susceptible
Nova	Brown leaf spots surrounded by a yellowish halo	Susceptible
Hamlin	Brown leaf spots surrounded by a yellowish halo	Susceptible
Washington Navel	Brown leaf spots surrounded by a yellowish halo	Susceptible
Tangelo	Significantly fewer leaves with brown leaf spots surrounded by a yellowish halo	Less susceptible
Kuno	No leaf symptoms	Tolerant
Local orange	Very few leaves with brown leaf spot	Less susceptible

4. Discussion

Susceptibility varied among the selected citrus cultivars tested. The natural infection experiments yielded results similar to observations made in the field under conditions of northern Uganda. Valencia, Nova, Washington Navel and Hamlin were very susceptible. These findings are in agreement with the observations made in Kenya

using detached leaf assays (Seif & Hillocks, 1999). Tangelo was less susceptible, contrary to the observations made in Keya (Seif & Hillocks, 1999), where it was reported to be very susceptible. The difference in the reaction could be due to the variation in experimental conditions, natural environment versus laboratory condition. Based on the information available in the literature, Pseudocercospora leaf and fruit spot disease is reported to be associated mild temperatures between 20 and 25 °C and high rainfall of over 1,500 mm per year (Seif & Hillocks, 1999; Lawson et al., 2017). The ideal environmental conditions in the field provided a perfect condition for infection of Tangelo by *P. angolensis*. The less susceptibility of Tangelo and local oranage could therefore, be attributed to a form of resistance mechanism in the host. Further studies under controlled environments are required to validate the finding.

Results of this study further indicated that Kuno was resistant. The resistance was characterized by absence of leaf symptoms. The resistance observed could be attributed to the ability of the cultivar to overcome colonization by the pathogen. This finding provides new insights into the design of integrated disease management for Pseudocercospora leaf and fruit spot disease in Uganda. Further studies are however, needed to validate the finding and elucidate the regulatory mechanism and relevant genes involved in the resistance.

Natural infection approach under field conditions could be useful in screening for resistance of citrus to *P. angolensis*. Field evaluation is useful for germplasm characterization. If adequately standardized, this approach would facilitate comparison of results from different regions thus, permitting a greater understanding of this disease. The natural infection approach is however, not sufficient to identify components of partial resistance at various stages of Pseudocercospora leaf and fruit spot infection cycle.

Lastly, less susceptibility or resistance to *P. angolensis* as a stand-alone control method does not offer a complete solution to Pseudocercospora leaf and fruit spot disease problem in Uganda. The current study identified Kuno as the most tolerant cultivar under natural infection in the field. Kuno, however, is not popular among citrus growers in Uganda. Efforts must be made to promote and popularize the cultivar. Since the most commonly grown citrus cultivars like Valencia and Washington Navel are very susceptible under field conditions in Uganda, incorporation of chemical intervention in disease management is still necessary.

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Authors Contributions

Dr. Alfred Kumakech was responsible for study design and revising, and drafted the manuscript. Alexandrina Acipa and Allan Tekkara Obonyom were responsible for data collection. Dr. Laban F. Turyagyenda revised the manuscript. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Data Sharing Statement

No additional data are available.