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Effect of Nitrogen Nutrition on the Intensity of *Cercospora* Leaf Spot of Mulberry

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ABSTRACT

Leaf spot (*Cercospora moricola*, Cooke) is a disease that negatively influences the yield of mulberry (*Morus alba* L.) plants. To determine the effect of nitrogen levels on the incidence and severity of leaf spot an experiment was carried out on mulberry plants. The nitrogen levels included 0, 100, 200 and 300 kg ha^{-1}, which were applied in two splits coinciding with the two rainy seasons. The study design was randomized complete block design (RCBD) with three replications. Determination of disease intensity involved scoring for disease intensity on a 1-5 Manandhar scale and calculation of the disease incidence were performed by expressing the number of infected leaves as a percentage of the total number of leaves. The values were translated to area under disease progress stairs (AUDPS). The means for AUDPS were subjected to analysis of variance (ANOVA) using PRO GLM in SAS and Fisher's least significant difference (LSD) used to partition the means at $p \leq 0.05$. The results showed that as the rate of nitrogen application was increased, there was a corresponding decrease in AUDPS for disease incidence and a decrease in AUDPS for disease severity. From the obtained results it can be concluded that nitrogen at an application rate of 200 kg ha^{-1} is an effective approach to suppress *Cercospora* leaf spot of mulberry and can be recommended to the farmers, where this disease is a problem for cultivation of mulberry.

Abbreviations

ANOVA: Analysis of variance, AUDPS: Area under disease progress stairs, CAN: Calcium ammonium nitrate, DI: Disease incidence, DS: Disease severity, LSD: Least significant difference, PRO GLM: Procedure for General linear models, RCBD: Randomized complete block design, SAS: Statistical analysis software

Introduction

Mulberry (*Morus alba* L.) is a plant that is grown for silkworm rearing. It is the exclusive food source for the silkworm, which during its larval life is reared for silk production (Tuikong et al., 2015). Healthy growth of silkworm depends on the quality of mulberry leaves (Sajad et al., 2014). In Kenya, the sericulture

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industry is an upcoming enterprise that is increasingly being perceived as a promising alternative source of income for rural small scale farmers (Adolkar et al., 2007). However, foliar pathogens have been a persistent problem locally causing 15 to 20% loss in leaf production. They also decrease the nutritive value of the leaves (Nderitu et al., 2012). To sustain silkworm feeding, a continuous and adequate supply of mulberry leaves with the nutrients is required. Tuikong et al. (2015) reported that one metric ton of mulberry leaves is necessary for rearing of silkworms emerging from one case of eggs, which will yield about 25 to 30 kg of cocoons of high quality. According to Ghoes et al. (2010), lack of regular and systematic studies on the leaf spot disease and epidemics has been responsible for the loss in leaf yield. The environmental factors that most affect the initiation and development of infectious plant diseases such as *Cercospora* leaf spot are temperature, humidity, and fertility that influence growth and susceptibility of the host and multiplication of the pathogen (Ghoshi et al., 2012).

Leaf spot, previously known as a major disease in cool regions, which negatively influences the mulberry leaf quality even in warmer areas (Gathumbi, 2008). The common symptoms of *Cercospora* pathogen on infected leaves are small (2-4 mm diameter) necrotic spots on the laminae and petiole of the leaves (Ray and Martin, 2012). The spots start appearing on the susceptible host at 70 d after planting and/or at 35 to 40 d after pruning (Singh and Singh, 2010). They are characterized by pale centers surrounded by darker necrotic regions. Occasionally, the spots may appear in the shape of teardrops that coalesce into rectangular shapes as the leaf matures, causing the entire leaf to turn necrotic and senescent (Ray and Martin, 2012). Leaf senescence may accelerated by the *Cercospora* disease, and the disease can rapidly spread across the laminae, causing large areas to turn brown and necrotic (Fig. 1).

Fig. 1. Mulberry leaves with *Cercospora* leaf spot symptoms (authors archive).

Under severe infections, the plant may be physiologically stressed and lose its ability to regenerate after being harvested. The *Cercospora* fungus is known to secrete cercosporin toxins that kill plant tissue (To-Anun et al., 2011). Cercosporin is a photosensitizing metabolite with host non-specific toxicity that acts during pathogenesis. Its toxic effects are the result of the production of light activated oxygen forms (Daub and Ehrenshaft, 2000). Cercosporin toxin in high concentrations incite a debilitating leaf spot disease causing the infected leaves to die back from the tip hence mulberry leaves infected with *Cercospora* become chlorotic and stressed.

Cercospora leaf spot can be controlled by spraying 0.2% Bavistin (Carbendazim 50% WP) solution on the leaves (Singh and Singh 2010). This method is untenable where the leaves are used as silkworm feed. Feeding silkworms on leaves sprayed with such chemicals has been reported to cause poisoning of the worms, poor quality cocoons, low cocoon yield and abnormal oviposition in silkworm egg production (Kuribayashi, 1986). In addition, despite their effectiveness and ease of use, chemical control has been found to cause environmental pollution, residual toxicity to silkworms and non-target organisms (Reddy et al., 2009).

Ghoshi et al. (2012) studied fertilizer application among the factors affecting mulberry growth and reported that a balanced dose application of NPK decreased *Cercospora* disease severity to 6-8%. A balanced fertilizer application in the soil resulted in lower infection by leaf pathogen in comparison with imbalanced application. Studies in Bangladesh have shown that application of nitrogen at the rate of 305 kg ha^{-1} in 4 splits enhance mulberry leaf yield (Ahmed et al., 2019). Huber et al. (2012) reported that mineral nutrients are essential for the growth and development of plants and microorganisms and are important factors in plant disease interactions. Fertilizer management practices have been reported to affect leaf quality in mulberry (Ahmed et al., 2019). Effects of application of nitrogen on the functional components of mulberry and leaf yield indicated that nitrogen increases foliar growth and also affects the functional components of the plant (Sugiyama et al., 2016). The primary hypothesis behind the effects of nitrogen on disease occurrence can lay down on this fact that plant growth at high nitrogen availability may result in increased plant susceptibility to pathogens as a result of increased foliar nitrogen concentration (Mitchell et al., 2003). However, plant response dynamics in response to nutrient availability are more complicated (Veresoglou et al., 2012). The present study aimed to determine the real effect of nitrogen application in the management of leaf spot in mulberry. Although in a vast majority of instances nitrogen fertilization increased disease severity, characteristics of different plant species and fungal pathogens can influence the disease severity following nitrogen fertilization (Veresoglou et al., 2012). Moreover, Veresoglou et al. (2012) concluded that the potential of some plant species to show reduced disease severity following nitrogen fertilization requires further investigation, as in such cases nitrogen fertilization could potentially be used as an additional means of suppressing fungal pathogens. Notwithstanding the extensive literature on the interactions between fertilization and plant growth, conclusive insights are lacking into how nutrient fertilization affects plant susceptibility to fungal pathogens (Sexton and Howlett, 2006). High Nitrogen availability may favour obligate parasites such as bio-trophic fungal plant pathogens while reducing disease severity of facultative parasites (Dordas, 2008). It has been also reported that for some obligate fungal pathogens such as rust fungi, disease severity may be responsive to nitrogen additions only when nitrogen availability limits growth (Walters and Bingham, 2007). Evidence has shown that the severity of diseases caused by bio-trophic fungi may be regulated by the type rather than the quantity of nitrogen fertilizers applied. For instance, nitrate fertilizers increase the severity of disease whereas ammonium fertilizers decrease it (Huber and Haneklaus, 2007). Nitrogen has been reported by Parthasarathy (2015) to reduce susceptibility of tomato to the powdery mildew pathogen *Odium lycopersicum* while it had no effect on susceptibility and the accompanying disease incidence to the vascular wilt pathogen *Fusarium oxyporum* f.sp. *lycopersici*. It is therefore clear that generalizing about the effects of nitrogen on plants disease is unwise.

Manipulation of nitrogen application rate upon mulberry *Cercospora* leaf spot has not been studied. The aim of the present study was to find out the effect of nitrogen levels on incidence and severity of *Cercospora* leaf spot in mulberry. The research question that this study set out to answer was whether the nitrogen fertilizer application rate is a determinant factor for the management of *Cercospora* leaf spot in mulberry.

Materials and Methods

Location of the study and source of disease

The trial was carried out at Kibos Research Center located 10 km from Lake Victoria. Temperature averages were 25 to 30 °C and 22 to 27 °C during the day and night respectively. Relative humidity varied from 60 to 69%.

One of the popularly grown varieties, Ex-Embu, sourced from the National Sericulture Research center Thika, was used in the trial. Isolates from infected leaf were identified as *Cercospora* at Maseno University Botany Lab.

Application of nitrogen fertilizers

There were 3 nitrogen fertilizer levels obtained from calcium ammonium nitrate (CAN) fertilizer at different levels including: 100, 200 and 300 kg N ha^{-1} with a non-fertilized control of 0 kg N ha^{-1}. The fertilizer was applied in two equal splits that were timed to coincide with the two rain seasons that are experienced in Kibos area at the beginning of March and September.

Determination of disease incidence and severity

Determination of disease intensity was done by separately scoring for disease severity and disease incidence. Disease severity scoring was done at monthly intervals starting at 70 d. Later on it was done at monthly intervals starting at 40 d after pruning. The scoring was done by direct estimation of severity, by assigning a severity value to individual plant leaves based on closeness of perceived severity on each of the leaves. The descriptive scale of 0-5 as used by Manandhar et al. (2016) was adopted as shown in Table 1.

Table 1. The descriptive Manandhar et al. (2016) disease severity scale

Disease severity score	Percentage of disease symptom on leaf (%)
0	0
1	1 - 10
2	11 - 25
3	26 - 50
4	51 - 75
5	Above 75

Based on the recorded mean of disease severity scores, area under disease progress stairs (AUDPS) was calculated at each scoring. The AUDPS values were then converted to area under disease progress stairs (AUDPS) in the following the method of Simko and Piepho (2012).

Disease incidence on the other hand was done by expressing the total number of leaves infected as a percentage of the total number of leaves (Kone et al., 2017). The mean disease incidence values were also converted to AUDPS.

Statistical analysis

The disease severity and disease incidence mean AUDPS values were subjected to Analysis of Variance (ANOVA) using PRO GLM in SAS (Institute, Inc.1999) and Fisher's least significant difference (LSD) used to indicate the level of difference between the means that were significantly different at $p < 0.05$.

Results

The results of disease severity AUDPS (Table 2) indicated that as the rate of nitrogen application increased, there was a corresponding decrease in AUDPS for disease severity. It was observed that application of nitrogen reduced the value for AUDPS for disease severity from 124.6 of the control treatment (0 kg N ha^{-1}) down to 41.0 for the highest rate of nitrogen application (300 kg N ha^{-1}). The results for analysis of variance for disease severity AUDPS values indicated that application of nitrogen at 100 kg ha^{-1} did not significantly ($p > 0.05$) decreased AUDPS as compared with 0 kg N ha^{-1}. However, application of nitrogen at the rate of 200 kg N ha^{-1} caused a statistically significant ($p < 0.05$) difference in the AUDPS when compared to control without nitrogen application. Application of 300 kg N ha^{-1} was not significantly different from application of 200 kg N ha^{-1}.

In the case of disease incidence, as the rate of nitrogen application was increased, there was a corresponding decrease in AUDPS for disease incidence (Table 3). It was observed that application of nitrogen reduced the value for AUDPS for disease incidence from 101.2 of the control treatment (0 kg N ha^{-1}) down to 18.2 for the highest rate of application (300 kg N ha^{-1}). The results for analysis of variance for disease severity AUDPS values indicated that application of nitrogen at 100 kg ha^{-1} was not significantly ($p > 0.05$) different from control treatment without nitrogen application (0 kg N ha^{-1}). However, application of nitrogen at the rate of 200 kg N ha^{-1} caused a significant ($p \geq 0.05$) difference in the AUDPS when compared to both control and application of nitrogen at 100 kg N ha^{-1}. Increasing the application rate to 300 kg N ha^{-1} did not cause a corresponding significant difference in AUDPS.

Comparative area under disease progress stairs for disease severity and disease incidence (Table 4) indicated that both parameters of disease intensity i.e. disease severity and disease incidence decreased with increasing nitrogen application rates.

Table 2. Results of disease progress stairs (AUDPS) for disease severity (DS) of *Cercospora* leaf spot on mulberry trees under different nitrogen levels

Treatment	Nitrogen application rate (kg ha^{-1})	AUDPS for disease severity
Control	0	124.6a
1	100	79.8ab
2	200	54.6b
3	300	41.0b
CV (%)		24
LSD		46.8

AUDPS values followed by the same letters are not significantly different at $p \leq 0.05$

Table 3. Results of disease progress stairs (AUDPS) for disease incidence (DI) of *Cercospora* leaf spot in mulberry trees under different nitrogen levels

Treatment	Nitrogen application rate (kg ha^{-1})	AUDPS for disease incidence
Control	0	101.2a
1	100	76.6a
2	200	37.8bc
3	300	18.2c
CV (%)		28
LSD		30.0

AUDPS values followed by the same letters are not significantly different at $p \leq 0.05$

Table 4. Comparative area under disease progress stairs for disease severity and disease incidence

N rate (kg ha^{-1})	Area under disease progress stairs	
	disease severity	disease incidence
0	124.6a	101.2a
100	79.8ab	76.6a
200	54.6b	37.8bc
300	41.0b	18.2c
CV (%)	24	28
LSD	46.8	30.0

Values followed by the same letters down the column are not significantly different at $p \leq 0.05$

Discussion

Decrease in AUDPS value for disease severity suggested that the percentage of *Cercospora* leaf spot infected tissue reduced with increasing nitrogen application. The more nitrogen was added the lower percentage of leaf tissue showed infection. This result was in conformity with the findings of Ghoshi et al. (2012) who reported that fertilizer application in the soil resulted in lower infection by leaf pathogen in comparison with imbalanced application. The mulberry plant and *C. moricola* fungus could be seen as a combination where disease severity decreased following nitrogen fertilization in conformity with the report of Veresoglou et al. (2012). This results therefore go against the nitrogen disease hypothesis based on the hypothesis of Mitchell et al. (2003), which stated that plant growth at high nitrogen availability may result in increased plant susceptibility to pathogens as a result of increased foliar nitrogen concentration. The decline in AUPDS conformed to the findings of Sugiyama et al. (2016) who reported that Nitrogen increases foliar growth and also affects the functional components of the plant that translates into decreased disease severity score reflected by lower AUDPS values. This results further suggested that to significantly reduce *Cercospora* disease severity, the nitrogen must be applied at a rate of 200 kg N ha^{-1} but less than 300 kg N ha^{-1}. A similar scenario was reported by Ghoshi et al. (2012) who reported that fertilizer application influenced *Cercospora moricola* occurrence and a balanced dose of NPK application decreased disease severity. A balanced fertilizer application in the soil, in this case 200 kg N ha^{-1} resulted in significant decrease in infection by *C. moricola* in comparison with imbalanced application of 100 kg N ha^{-1}. Though 300 kg N ha^{-1} decreased the disease infection as evidenced from the lower AUDPS value, it is wasteful and imbalanced since decreased infection was not statistically significant.

In the case of disease incidence, AUDPS values decreased by increasing level of nitrogen application. Furthermore, the percentage of *C. moricola* infected leaves reduced with increasing nitrogen application. The more nitrogen application, the less the number of leaves that showed infection, which is in accordance to findings of Chiang et al. (2017). The results also agreed with the findings of Parthasarathy (2015) in his study on nitrogen impact of the susceptibility of tomato to the powdery mildew pathogen (*Odium lycopersicum*). He reported that speculation on the effects of nitrogen on plants disease should not be trusted. The effect of nitrogen on plant susceptibility should be considered on case by case basis.

The results for disease intensity conformed to the findings of Daub and Ehrenshaft (2000) who reported that cercosporin, a toxin that acts during pathogenesis as a result of the production of light-activated oxygen forms, does not effectively form when the light is reduced by thick foliage. Hence the lower AUDPS values with application of nitrogen due to diminishing toxin levels is inclusive.

Conclusion

The strategy of reducing *Cercospora* leaf spot intensity in mulberry by manipulation of nitrogen in the soil is conclusive. The results of present study showed nitrogen application rate at 200 kg N ha^{-1} is a feasible approach to prevent problems related to the *Cercospora* leaf spot in this case the Kibos region of western Kenya. Therefore, nitrogen fertilization at optimum rate could potentially be used as an additional means of suppressing *C. moricola* in mulberry.

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Conflict of interest

The authors indicate no conflict of interest in this work.

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