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# Assessing Nitrogen level in maize (*Zea mays*) with infrared thermography

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## Abstract

Intensification of agricultural production due to the higher demand of a growing population will continue to increase pressure on natural resources. Nitrogen (N) is one of the most important nutrients for crops, due to its role in various physiological processes, such as chlorophyll synthesis. Therefore, numerous techniques aim to make nitrogen utilization more efficient, ranging from breeding programs to biological nitrogen fixation. N deficiency influences grain and plant weight, harvest index, leaf area index and crop photosynthetic rate thus, reducing the supply to the ear and, ultimately, lowering the yields.
Infrared thermography (IRT) has been widely used to observe crop water status, however, numerous researches are being developed with this instrument to detect different variables, for instance, plant disease. It is a promising method that aims to substitute direct measurements such as analyzing samples in a laboratory or individual measurements, which are time-consuming, mostly not applicable to large populations simultaneously, destructive, sometimes difficult to apply on fields, and simplify/accelerate possible responses to crop stress. To measure with IRT, the decrease of leaf temperature is directly related to stomatal closure, causing a cooling effect due to evapotranspiration. The temperature variation is captured through an infrared camera.
Previous studies identified an inverse correlation of N fertilization levels and canopy temperature in wheat (*Triticum aestivum* L.) therefore, the objective of this study was to detect nitrogen status in early stages via infrared imaging on maize (*Zea mays* variety "Amadeo"). The experiment was carried out in the greenhouse where thermal images of maize plants were taken throughout the growing stages. Four levels of N (50, 75, 100, 150%) were applied and soil and leaves samples were taken for the laboratory nitrogen analysis. Simultaneously, leaf stomatal conductance (gL) and chlorophyll content were measured to provide information on the physiological status of the plants. Temperature and humidity were also recorded.
Results present a strong correlation between treatments and chlorophyll content ($P<0.001$), however, no significant correlation was found between chlorophyll content, stomatal conductance and leaf temperature.
The relation between nitrogen fertilization and chlorophyll content is well known, as the effect of stomatal conductance and leaf temperature. Nonetheless, there is an open debate about the correlation between chlorophyll and stomatal conductance.

**Keywords:** Agriculture, nitrogen fertilization, infrared thermography
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## Introduction

According to FAO (2017), the world population is estimated to reach 9.73 billion by 2050, increasing the pressure on food production, consequently on agriculture and all natural resources. Nitrogen (N) is the most utilized nutrient for agriculture (Malhi et al., 2001), because it participates in many physiological processes, for instance, chlorophyll synthesis, and, deficiency of supply cause depletion on grain and plant weight, harvest index, leaf area index, crop photosynthetic rate, and, ultimately, reducing yields (Gallais & Hirel, 2004). Therefore, usage efficiency is crucial and numerous researches in different fields are aiming to improve N fertilization, ranging from breeding programs to biological nitrogen fixation.

To control crop nitrogen in the fields is often laborious, destructive, not applicable to large populations simultaneously and time consuming, which decreases the yield, as adjustments in the soil are not ratified instantly. Infrared thermography (IRT) is a promising non-contact approach because can rapidly capture a high number of individuals through the canopy surface. The approach has been used mainly to detect crop water status (Zia et al., 2013), however, numerous researches are being developed with this instrument to detect different variables, for instance, plant disease.

Nitrogen fertilization has a direct effect on the chlorophyll content. Ding et al. (2005) and Guo et al. (2016) used IRT and detected lower canopy temperatures for plants with higher leaf nitrogen status in wheat (*Triticum aestivum* L.), due to higher evapotranspiration. Stomatal conductance ($g_s$) is sensitive to leaf aging, health and several environmental stimuli, for example, $CO_2$ concentration, light, humidity, temperature and drought (Matsumoto et al., 2005), nonetheless, stomatal aperture is predominantly provoked by blue light and photosynthetic active radiation (PAR) (Kollist et al., 2014). Schulze et al. (1994 as cited in Guo et al., 2016) reported a close correlation between stomatal conductance and nitrogen content, leading to other studies (Roux et al., 1999 as cited in Matsumoto et al., 2005) to include leaf nitrogen content in stomatal conductance models.

A few studies are aiming to detect N levels with IRT, as cited before in the wheat experiment and, Alzaben et al., (2019) detected N levels in maize yet, with a shallow statistically significant p-value (<0.05) and a relatively low $r^2$ (maximum obtained $r^2 = 0.28$). The objective of this study was to detect nitrogen status in early stages via infrared thermography on maize (*Zea mays* variety “Amadeo”).

## Material and Methods

The experiment was carried out in a greenhouse (Fig.1) at the University of Hohenheim (Stuttgart, 48°42'41.04″N, 9°12′34.2″E). Thermal images of maize (Fig.2) plants were taken between V4 (4 fully developed leaves) until VT (tassel) 3-4 times per week, between May 23$^{rd}$ and June 30$^{th}$, 2021. Four levels of N (50, 75, 100, 150%) were applied, where each treatment had 12 pots (5L of substrate with perlite to reduce N) and two replicates per pot. Maize was fertilized in 6x to match the plants needs according to the fertilization percentage. From time to time, soil and leaves samples were taken for the laboratory nitrogen analysis. Simultaneously, leaf stomatal conductance ($g_s$) and leaf chlorophyll content were measured to provide information on the physiological status of the plants. Temperature and humidity were also recorded.

Fig. 1- Experiment setup in the greenhouse with 12 pots with two replicates per treatment (50, 75, 100 and 150%).

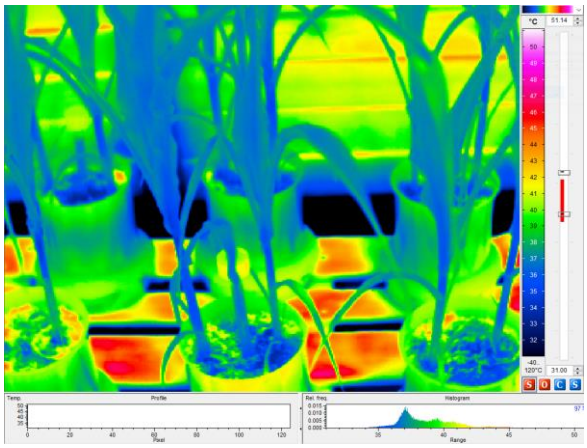

Fig. 2- Infrared picture analyzed with IRBIS3 plus

**Results and Discussion**

Results present a statistically significant difference of chlorophyll content between treatments ($P<0.001$) (Fig. 3), however, no significant correlation ($p<0.5$) was found between chlorophyll content, stomatal conductance and leaf temperature.

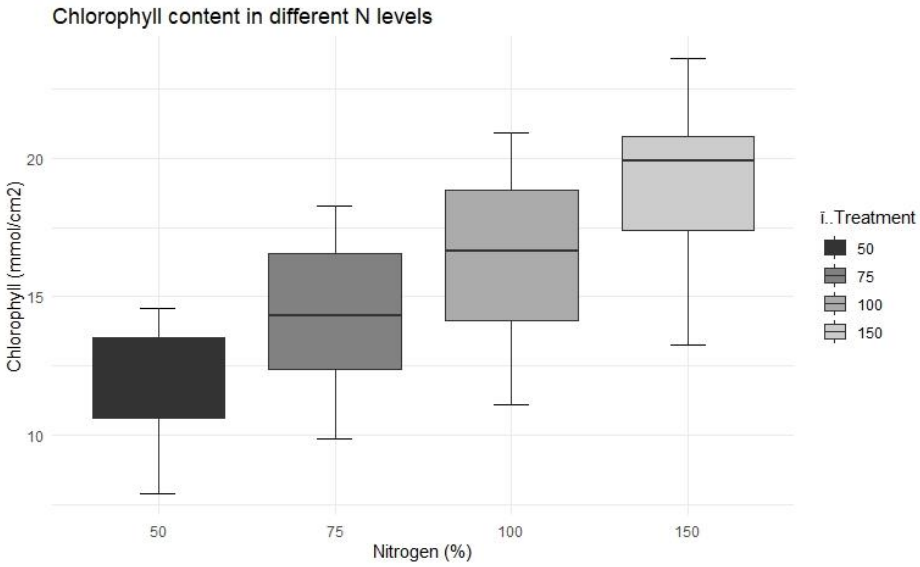


Fig. 3- Boxplot with different chlorophyll levels among the 4 treatments (50, 75, 100, 150%)

The correlation between nitrogen fertilization and chlorophyll content is well stated (Ding et al., 2005), as the effect of stomatal conductance on leaf temperature (Guo et al., 2016). Nonetheless, stomata aperture is affected by various stimuli, environmental and physiological (Matsumoto et al., 2005), obscuring the connection with chlorophyll content. Even though several authors argue that the correlation was observed by Matsumoto et al. (2005), other researchers state that stomatal conductance is largely unaffected by the chlorophyll content in the leaves or can be disrupted (von Caemmerer et al., 2004).
In this experiment, no significant correlation ($p=0.72$) was found, corroborating the arguments of von Caemmerer et al. (2004)

## Conclusions and Outlook

Further research is needed to clarify the real relation between N fertilization and leaf temperature in maize, as some authors described different canopy temperatures for different nitrogen levels, as cited before.
However, as the stomatal conductance can be affected by several factors, which could actively interfere with the results, another experiment was settled on the fields to evaluate the efficiency of the IRT in predicting nitrogen levels in the early stages of maize.

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