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Ornamental flowers in new light: Artificial lighting shapes the microbial phyllosphere community structure of greenhouse grown sunflowers (*Helianthus annuus* L.)

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ABSTRACT

Artificial assimilation lighting is a common practice in greenhouse horticulture in the circumpolar region to compensate for natural low light conditions. To modulate plant architecture, regulate flowering of photoperiodic crops, increase plant performance per energy input and consequently profitability, light emitting diodes (LEDs) have been suggested as a powerful tool for ornamental growers in complementary or replacement of conventional lighting such as incandescent, fluorescent and high pressure sodium (HPS) lamps. As LED light differs from HPS lamps with regard to spectral output, light distribution as well as heat emission, the microclimate within the crop stand is affected. In two independent experiments conducted in fall and winter, we therefore compared the effect of two types of LED light (red 660 nm + blue 460 nm LED, 80:20 RB-LED; white LED, W-LED) with HPS lighting on ornamental sunflowers (*Helianthus annuus* cv. 'Teddy Bear'). Depending of the solar radiation (fall vs winter experiments), a same PPFD of 70–120 μmol m^{-2} s^{-1} of artificial lighting (photoperiod of 16 h) was given at the top of the plants. Plant growth performance and biomass, leaf temperature, photobiological parameters (photosynthetic activity, stomatal conductance, chlorophyll fluorescence) as well as the leaf associated microbiome, assessed using culture dependent and independent methods on apical, directly exposed to the light treatments, and basal leaves, were studied. As expected, significant differences were obtained for plant related parameters between the two repetitions of the experiment due to difference in solar radiation. Light treatments influenced plant growth performance which was lower for all parameters in sunflowers exposed to LEDs than HPS. However, no differences were found with respect to photobiological parameters. Top leaf temperature was higher in the presence of HPS than LEDs, which explained the lower plant growth performance observed under LED regimes. Colony-forming units representing culturable fungi and fluorescent pseudomonads were higher on basal leaves than on apical ones, but did not vary with respect to light treatments. On the other hand, biodiversity estimated with respect to species abundance and evenness (Shannon-H index) and species richness (Chao1) revealed different patterns for the fungal and bacterial microbiome. Regardless of the leaf position, light treatments affected fungal species abundance and evenness, which was highest on leaves exposed to HPS, but not species richness. The fungal microbiome was more diverse on apical than on basal leaves. For the bacterial microbiome, biodiversity estimates differed between the repetitions. Interactions between leaf temperature and bacterial genera were found for several of the dominant genera in the sunflower phyllosphere (*Pseudomonas*, *Staphylococcus*, *Enhydrobacter*) while other decisive bacterial and fungal genera were correlated to photobiological parameters, e.g. *Bradyrhizobium*, *Sphingomonas*, *Brevibatericum*, *Bacillus*, *Hypotrachyna*, *Aureobasidium*. The use of "new light" in greenhouse ornamentals is not only a technological change modifying plant morphology and development, but also affects the microbial ecology on plant surfaces, implying consequences on plant protection issues and biological control strategies.

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1. Introduction

Horticultural greenhouse production in circumpolar regions (>60° N latitude) is dependent of artificial assimilation lighting (Baevre and Gisleröd, 1999; Dorais, 2004; Dorais and Gosselin, 2002) which is a common tool to improve plant performance and consequently profitability of ornamental crops. In this context, light emitting diodes (LED) technology has been introduced as a measure to reduce energy consumption and energy costs per unit of product in intensive horticultural greenhouse industries as compared to high pressure sodium (HPS) lamps (Nelson and Bugbee, 2014). However, LED light differs from conventional lighting with HPS lamps with regard to spectral output, light distribution as well as heat emission (Morrow 2008). By using LEDs, the spectral composition can be matched to the photosynthetic demands of the plant (Morrow, 2008), and plant architecture and flowering of photoperiodic crops can be modulated (Massa et al., 2008). Thus, the choice of source for artificial lighting not only affects the microclimate in the crop stand but also crop physiology and morphology as reviewed by Vänninen et al. (2010) and thereby interactions with respect to the phyllosphere microbiota. Indeed, healthy leaf surfaces are colonized by bacteria, fungi, yeasts, algae and protozoa (Lindow and Leveau, 2003), amongst these also plant pathogens. Of these, bacteria are commonly described as the most dominant group (Timms-Wilson et al., 2006). A concept on interactivities between light, plant and phyllosphere microbiota is presented in Fig. 1. More specifically, light quality (spectral distribution), quantity (day light integral, light intensity) as well as diurnal changes (light period/day length, sunfleck, and diurnal artificial light modulation) affect both plants and microbiota. The light source also affects plant and microbiota indirectly (abiotic factors) through its impact on the interaction between temperature and humidity in the crop stand and on the leaf. Vice versa the prevailing light conditions within the canopy are also affected by the crop stand, as a result of plant density and canopy structure.

The phyllosphere constitutes a hostile and harsh habitat for microbial populations because they are exposed to diurnal changes and dramatic fluctuations in temperature, irradiation, relative humidity and water and nutrient availability (Lindow and Brandl, 2003; Vorholt, 2012). These environmental conditions affect the size of epiphytic microbial populations, but also their short-term and long-term community structure. Furthermore, plant species (Whipps et al., 2008; Yang et al., 2001) and plant physiological factors, such as leaf age (Ercolani, 1991; Sylla et al., 2013a; Sylla et al., 2013b), also affect the microbial community structure. In commercial greenhouse horticulture, fluctuations in environmental conditions are however less pronounced than under field conditions. Apart from control of environmental factors, such as temperature, relative humidity, and CO_2 concentration, greenhouse covering materials and shade netting alter prevailing conditions at the crop level and in the crop phyllosphere due to their impact on greenhouse light transmission, reflection, absorption and diffusion within the canopy (Diaz et al., 2006; Hemming, 2011; Stamps, 2009). Although the plant microbiome is considered as a "key determinant of plant health and productivity" (Berg et al., 2014), few studies have considered the microbial biogeography of greenhouse grown plants. To understand the impact of new lighting strategies, the microbiota associated to canopies of greenhouse crops grown under different artificial light regimes was studied. We hypothesized that (i) a change in lighting technology by using LEDs instead of HPS lamps may have a major direct and/or indirect impact on the phyllosphere microbiome of greenhouse crops and that (ii) the microbial community structure in the phyllosphere of greenhouse grown ornamental sunflower differs in response to the source of artificial lighting and its spectral band, and to the combined effect of leaf age and position.

A shift in lighting technology in greenhouses may not only address the grown crop, but also plant associated organisms of economic importance, such as plant disease occurrence, suppression and the use and efficacy of biological control agents. Therefore this technology needs to be assessed from a microbiological point of view. *Helianthus annuus* was selected because few studies were conducted in response to ornamental crops despite their high economic value, and due to its use in environmental light interception studies. The characterization of the phyllosphere microbiome exposed to different light regimes, taken on in this study, is a first step on this path.

2. Materials and methods

2.1. Plant material

Sunflowers (*Helianthus annuus* cv. 'Teddy bear') were sown on 23 August and 12 December 2013 in 35-plug trays (Vefi, Larvik, Norway) with peat-based growing medium (Hasselfors K-soil, Hasselfors AB, Örebro, Sweden). Ten days after sowing the seedling plants (20 per experimental unit) were transferred to 13 cm pots with the same growing medium, but amended with 50 g slow-release fertilizer (NPK 16-6-12, ASB Grünland Helmut Aurenz GmbH, Ludwigsburg, Germany) per 100 L of growing medium. Light treatments were started on the same day the plants were transplanted to pots.

2.2. Growing conditions

The plants were placed in a greenhouse compartment of 90 m^2 at 18 °C (set points) day/night heating temperature; ventilation by roof vents opening took place when temperature exceeded set points by 2 °C. The climate was controlled using a climate computer (Priva Intégro v.730, Priva, De Lier, The Netherlands) and climate data was logged every 5 min (Priva Office, Priva, De Lier, the Netherlands). The mean greenhouse temperature for experiment 1 was 20.3 ± 2.4 °C, and 18.3 ± 0.7 °C for experiment 2. The averaged relative humidity was 62.5 ± 14.6% and 50.0 ± 7.0% for the experiment 1 and 2, respectively. The greenhouse was not enriched with CO_2. The greenhouse compartment was lined with opaque screens, forming 3 smaller compartments (2 m x 2 m; 4 m^2). These compartments, opened in the top and the bottom, were equipped with one of the three lighting treatments: 1) white LED (W-LED; 4*90 W, Broham Invest AB, Norsjö, Sweden), 2) red/blue LED (RB-LED; 660 nm, 460 nm; 80:20; 350 W, LightGrow AB, Helsingborg, Sweden), and 3) high pressure sodium lamps (HPS 400 W, Philips, Eindhoven, The Netherlands). The spectral distributions of the different light sources are displayed in Fig. 2. The photosynthetic photon flux density (PPFD) at canopy level was adjusted to 70–120 μmol m^{-2} s^{-1} by adjustment of the distance between the light source and the top of the canopy. The distance was approx. 1 (LED) to 1.5 (HPS) m. Artificial light was given from 06:00 h to 22:00 h for a total of 16 h day^{-1}. Natural daylight (indirect through the opening at the top of the growth chambers) was let into the greenhouse for 8 h day^{-1}. The total photoperiod (natural and supplemental PPFD) was 16 h day^{-1} and the daily light integral (DLI) was 7 mol m^{-2} day^{-1}. The plants were irrigated 1–2 times per day with water via capillary uptake. The experiment was repeated twice (August–October 2013: 54 days of cultivation; December 2013–February 2014: 60 days of cultivation; BBCH stage at harvest: 55). No chemical or biological control measures were taken to control pests or diseases and no additional fertilizers were applied by foliar sprays.