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Source-sink relations of sunflower plants as affected by a parasite modifies carbon allocations and leaf traits

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ABSTRACT

Sunflower broomrape (*Orobanche cumana*) is a root holoparasitic plant causing major damage to sunflower (*Helianthus annuus* L.). Parasite infection initiates source-sink relations between the parasite (sink) and the host (source), allocating carbohydrates, water and nutrients to the parasite. The primary aim of the current study was to explore responses of sunflower to broomrape parasitism, specifically to examine alternations in leaf area, leaf mass per area (LMA), mesophyll structure and root hydraulic conductivity. Leaf changes revealed modifications similar to described previously in shade adapted plants, causing larger and thinner leaves. These traits were accompanied with significantly higher root hydraulics. These changes were caused by carbohydrate depletion due to source-sink relationships between the host and parasite. An Imazapic herbicide (ALS inhibitor) was used for controlling broomrape attachments and by to investigate the plasticity of the traits found. Broomrape infected plants which were treated with Imazapic had leaves similar to non-infected plants, including mesophyll structure and carbon assimilation rates. These results demonstrated source-sink effects of broomrape which cause a low-light-like acclimation behavior which is reversible.

1. Introduction

Parasitic plants rely on other vascular plants for nutrients and water that flow from the host to the parasite through the haustoria [1]. Holoparasitic plants depend fully on their hosts since they lack both functional roots and chlorophyll [2]. The *Orobanche* genus is one of the most damaging parasitic weeds worldwide, and its distribution spans from North Africa through Europe and the Mediterranean basin to China and Australia [3]. Broomrape germination, induced by root exudates strigolactones, is followed by haustorial development, attachment to the host root, and penetration to the vascular tissue, forming a physiological bridge between the vascular systems of the two organisms [4,5] The effects of the parasite's establishment upon its host are both direct and indirect and include source limitations of water and nutrients [6], resulting in stunting and yield reduction [3]. The parasite draws water and nutrients from the host plant by higher osmotic potential [7]. Sunflower broomrape (*Orobanche cumana*) is a noxious parasitic weed considered to be a specialist as it is exclusively a parasite of sunflower plants [8]. *Orobanche cumana* causes reductions in flowering head diameters [9], grain yield [10] and lower seeds oil content [11]. Breeding of resistant varieties may gain some advantages [10], however resistance was proven to brake under low temperatures [12], and more virulent biotypes of the parasite are steadily emerging overcoming the resistance [13]. In addition to yield losses, broomrape was also shown to alter anatomical and physiological traits in host plants such as delayed leaf senescence and reduced leaf mass per area (LMA; [14]), increased leaf area ratio (the ratio between leaf area per plant and plant dry mass) [15] and higher air space portion in the mesophyll layer [16]. Alterations in host photosynthesis via source-sink interactions can cause direct and indirect changes in the host plant. The parasite competes directly with the host for fixed carbon and thus serves as an extra sink for assimilates [6,17].

Producing structurally altered leaves is the primary mean for plants to respond to changes in their environment. Carbohydrate abundance is a dominant factor in leaf ontogeny and leaf traits; for example, manipulating the source to sink ratio by fruit and flower removal led to an increase in LMA [18,19]. Leaf structure [20] and nitrogen recourse distribution [21,22] are affected by environmental factors, such as light

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intensities and atmospheric CO_2 partial pressure constrain photosynthetic rates and thus influence the plant's carbohydrate balance [23]. For example, sun leaves are developed to be smaller, thicker and more efficient in high light intensities as compared to shaded leaves [20,24–27]. Higher CO_2 concentrations induced an increased leaf thickness [28–31]. In addition, various studies showed direct relations between sugar-dependent signaling and leaf ontogeny alterations, uncovering a vast and complex gene regulation of leaf development (e.g., [32–34]). Givnish [35] showed a linear relationship between leaf maximum photosynthetic rate and dark respiration rate; Therefore, when conditions are sub-optimal (i.e. low light), high-performance leaf is considered to be a waste of resources. Photosynthesis can be limited by CO_2 concentrations at the site of carboxylation, therefore CO_2 diffusion from the sub-stomatal cavities to the carboxylation site plays a major role in carbon assimilation rate (A_N; [36]). CO_2 diffusion from the sub-stomatal cavities is directly influenced by conductance through the mesophyll layer, also called internal conductance (g_i; [37]). Larger portion of air spaces in the mesophyll layer enhances g_i, and therefore facilitates net carbon assimilation rate especially under low CO_2 partial pressure [38,39]). Condensed and thick mesophyll layers reduce the intercellular air spaces and thus reduces $g_{i,}$ ([39]. Therefore, condensed mesophyll layers are negatively correlated with CO_2 diffusion, and contribute to a higher leaf dry mass and leaf area. The purpose of leaf modifications, with their strong relation to carbon budget, is to intercept more light and CO_2, leading to an increase in carbon assimilation when carbohydrate resources are limited; in an economics analogy [40], this redesigns the investment in order to harvest more of the limiting factor.

The driving force of water flow through the plant is the water potential gradient that compensates for water losses through transpiration [41]. While most of the resistance to water flow is due to stomatal aperture, the root system can represent a significant barrier, contributing up to half of the overall plant's hydraulic resistance [42,43]. Using the modified Hagen–Poisseuille's law [44], water conductance in the xylem is proportional to the summation of vessel diameter raised to the fourth power, emphasizing how important the vessel and tracheid diameters are in their influence on water conductance [45]. Various studies showed root hydraulic conductance (k) plasticity in response to environmental conditions; a reduction was observed when plants were subjected to water stress [46] and under enrich CO_2 environment [47]. Leaf traits modifications will immediately cause changes in water demand and therefore may be related to root hydraulic traits.

The primary aim of the current study was to show leaf traits modifications in response to broomrape parasitism, specifically mesophyll structure and carbon assimilation rate, and relate these traits modifications to carbon and water relations. Using Imazapic to control broomrape infection, we were able to remove the parasite stress and to examine leaf traits plasticity and reversibility.

2. Materials and methods

2.1. Greenhouse conditions and plant material

A greenhouse experiment was conducted from September 7, 2016 to November 2, 2016 at the Sede Boqer Campus of Ben-Gurion University of the Negev, Israel. The average midday photosynthetic photon flux density was approximately 900 μmol m^{-2} s^{-1}. Temperature was kept between 32 °C and 18 °C as maximum and minimum temperatures, respectively. Soil temperature was recorded hourly using a temperature data logger (HOBO data logger; Onset Computer Corporation, Bourne, MA, USA) placed at the depth of 10 cm in the pot soil [48].

Sunflower *Helianthus annuus L.* cv D. Y. 3 (*O. cumana*-susceptible; Sha'ar Ha'amakim Seeds, Sha'ar Ha'amakim, Israel) was used as the host plant. Four seeds per pot were seeded and later thinned to a single seeding per pot. Truncated cone-shaped pots of 4-L volume filled with a fine-clayey soil (montmorillonitic, thermic soil; 55% clay, 25% silt and 20% sand, 2% organic matter, pH 7.2), were used. The experiment consisted of two levels treatments: 1. *Orobanche cumana* seed presence (broomrape-infected) and the control treatment which was not infected, and 2. the effect of Imazapic (Imazapic and mock treatments); four treatment groups in total. For the broomrape-infected treatment, soil was mixed beforehand with 15 mg of *O. cumana* seeds per Kg soil, using a cement mixer [10]. Seventy-eight plants were used: 13 of each non-infected groups (treated and untreated with Imazapic, a total of 26), and 48 broomrape infected plants with same ratios as detailed above. The irrigation system automatically provided N-P-K (17-10-27) and microelement fertilizer (Poly Feed; Haifa Chemicals, Israel). The fertilizer was solubilized equivalent to 200 kg per 1000 m^3 (manufacturer recommendations) and injected into the irrigation system in a 0.5 mL/ L ratio.

2.2. Broomrape parasitism and timing of Imazapic application

The accumulative thermal time equation [48] was applied in order to predict broomrape infection, and to set the right timing of Imazapic application;

$$GDD = \sum \left[\frac{Tmax + Tmin}{2} - Tbase \right] \quad (1)$$

Whereas T base for unflower is 4 °C [49]. Minimum and maximum soil temperatures recorded were 19 and 34 °C respectively with an average minimum and maximum of 21 and 30 °C respectively. The average GDD was 21.53 °C per day. At 630 GDD (28 DAP), Imazapic was applied during the morning irrigation by adding 30 mL of Cadre (23.6% Imazapic, BASF, Germany) 2.22 microliter/liter DDW bulk solution – equivalent to 4.72 mL ha^- Imazapic in the field.

At 56 DAP, roots were gently placed onto a 2-mm mesh screen sieve, to be washed from soil. Broomrape attachments, and sunflower shoots were separately placed to dry in a 65 °C oven for 72 h, followed by dry mass measurements on a 0.01 mg accuracy scale.

2.3. Root characterization, broomrape infection and root water conductance

Root water conductance (k) was measured at 42 and 56 DAP using 21 broomrape infected and 14 non-infected plants. The stem was cut approximately 5 cm above the soil surface, quickly sealed with a water seal and connected to the high pressure flow meter device (HPFM-P-G3, Dynamax, Houston, TX) via a polyethylene flexible tube. The water flow rate into the root (F; [Kg/s]) and applied pressure (P; [MPa]) were sampled every 3 s, with the pressure steadily increased at a constant rate of 3–7 kPa s^{-1}. K was later calculated as the linear slope k = dF/dP [50].

After measuring k, the root was gently placed onto a 2-mm mesh screen sieve, to be washed from soil. Sunflower roots, broomrape attachments, and sunflower shoots were separately placed to dry in a 65 °C oven for 72 h, followed by dry mass measurements on a 0.01 mg accuracy scale.

2.4. Leaf area and leaf mass per area (LMA)

Individual leaves were placed on a flat whiteboard, next to a 20-cm^2 red colored card stock. Each leaf was photographed using a digital camera (Canon, EOS M3) at an approximately 50-cm distance, parallel to the whiteboard, thus fully capturing both the leaf and the red card stock square. The files were analyzed with Easy Leaf Area software [51]. Node numbers mentioned are relative to the first developed true leaf. In order to ensure that comparison of leaf development was done between leaves of the same age and the same node position, leaves were marked using plastic tags. Starting from 18 DAP and every few days after, young and fully expanded leaves were marked and named with respect to their node position and color. Leaf mass per area (LMA)