Transpiration and biomass production of the bioenergy crop Giant Knotweed Igniscum under various supplies of water and nutrients

Dario Mantovani¹, Maik Veste²*, Stella Gypser¹, Christian Halke¹, Laurie Koning¹, Dirk Freese¹, Stefan Lebzien³

¹ Brandenburg University of Technology Cottbus-Senftenberg, Chair of Soil Protection and Recultivation, Konrad-Wachsmann-Allee 6, D-03046 Cottbus, Germany.
² CEBrA - Centre for Energy Technology Brandenburg e.V., Friedlieb-Runge-Strasse 3, D-03046 Cottbus, Germany.
³ ENAGRA Biomasse GmbH, Auf der Grub 1, D-54472 Monzelfeld, Germany.

* Corresponding author. Tel.: +49 355 696602. E-mail: maik.veste@me.com

Abstract: Soil water availability, nutrient supply and climatic conditions are key factors for plant production. For a sustainable integration of bioenergy plants into agricultural systems, detailed studies on their water uses and growth performances are needed. The new bioenergy plant Igniscum is a cultivar of the Sakhalin Knotweed (Fallopia sachalinensis), which is characterized by a high annual biomass production. For the determination of transpiration-yield relations at the whole plant level we used wicked lysimeters at multiple irrigation levels associated with the soil water availability (25, 35, 70, 100%) and nitrogen fertilization (0, 50, 100, 150 kg N ha⁻¹). Leaf transpiration and net photosynthesis were determined with a portable minicuvette system. The maximum mean transpiration rate was 10.6 mmol m⁻² s⁻¹ for well-watered plants, while the mean net photosynthesis was 9.1 µmol m⁻² s⁻¹. The cumulative transpiration of the plants during the growing seasons varied between 49 l (drought stressed) and 141 l (well-watered) per plant. The calculated transpiration coefficient for Fallopia over all of the treatments averaged 485.6 l kg⁻¹. The transpiration-yield relation of Igniscum is comparable to rye and barley. Its growth performance making Fallopia a potentially good second generation bioenergy crop.

Keywords: Water use efficiency; Transpiration coefficient; Photosynthesis; Nitrogen; Ecophysiology; Lysimeter; Fallopia.

INTRODUCTION

Understanding the water consumption of crops under various soil water availabilities is critical for sustainable and economically feasible agricultural production under varying climatic conditions. Yield-transpiration relationships measured in a microcosm by using lysimeters under various water application regimes and nutrient content as well as soil conditions allows for an estimation of potential water requirements of crops under varying climate conditions (Ben-Gal et al., 2003; Hanks, 1974). This information is practical for use in the field. Experimental evidence that supports linear relationships between yield and potential evapotranspiration has been reported for various forage and crop biomass products and can be used for the prediction of biomass production (Ben-Gal and Shani, 2003a; Hanks and Rasmussen, 1982; Shani et al., 2007). The increased demand for bioenergy crops for biomass production and biogas (Weiland, 2010) will affect land use management systems (Gerbens-Lecuens et al., 2009) as well as local hydrological cycles (Phong et al., 2011). Therefore, before the introduction of a new species as a second generation crop for bioenergy production and for raw materials, an analysis of its water consumption is mandatory for optimal agricultural management and limited soil water availability have negative effects on crop productivity (Ings et al., 2003). Second generation bioenergy crops avoid competition with food and feed production, reduce the environmental impacts of fertilizers and herbicides, and enhance biodiversity in the landscape (Gomez et al., 2008; Havlík et al., 2011; Naik et al., 2009). Especially herbaceous perennials have great potentials to fulfill these criteria and various new species for bioenergy production have been tested, e.g., Miscanthus giganteus, Silphium perfoliatum, Sida hermaphrodita and Fallopia bohemica (Borkowska and Molas, 2012; Franzing et al., 2014; Lewandowski et al., 2000; Pude and Franken, 2001, Lewandowski and Heinz, 2003; Straviš and Kára, 2010). The Giant Knotweed, or Sakhalin Knotweed (Fallopia sachalinensis), grows natively on the Russian island of Sakhalin, in Japan and on the Korean peninsula. It is characterized by high annual biomass production (Lebzien et al., 2012; Vetter et al., 2009). The plant can reach heights of up to 3–4 m. The new cultivars Igniscum Candy and Igniscum Basic were selected and cultured from the wild form as new bioenergy crops (Veste et al., 2011) and can be effectively used in biogas plants and for combustion, respectively. First results from lab investigations and experiments in biogas plants show that with Igniscum Candy’s two possible harvests during the year and ability to be a good substitute for maize as a substrate in biogas power plants, it is a promising bioenergy crop (Seppälä et al., 2013). However, until now detailed information on its water consumption and how that affects its biomass production is still missing. The objective of this experiment was to investigate the growth, yield and transpiration responses of Igniscum Candy to various water and nitrogen supplies. The determination of the yield-transpiration relations under different under the influence of different water and nitrogen regimes will give important basic information on the growth performance under different soil water availability compared to other crops. Furthermore, the water use efficiency was investigated on the whole plant level and on the level to understand the influence of drought stress on the ecophysiological plasticity to water constrains.
MATERIAL AND METHODS

Plant material and cultivation

For the experiment we used Igniscum Candy, which is a selected cultivar of Fallopia cf. sachalinensis. One-year-old saplings of the plants obtained from Conpower Rohstoffe GmbH, Oldenburg were transplanted from small seedling pots to 7 l volume plastic pots and stored in the glasshouse at the Brandenburg University of Technology in Cottbus. The plants were maintained at temperatures above 10°C under well-watered conditions. After 60 days the saplings were cut back to facilitate homogeneous re-sprouting. Each plant was fertilized with 200 ml of Hoagland standard solution, 0.78 g calcium ammonium nitrate (NH₄NO₃) and 0.63 g superphosphate (K₂HPO₄), and then watered well. After 55 days the plants were transplanted into lysimeters where they were fertilized with 1045 ml of Hoagland standard solution, 2.58 g calcium ammonium nitrate (NH₄NO₃) and 1.59 g potassium superphosphate (K₂HPO₄), and again watered well to allow for their establishment until the start of the experiment (June 26th, 2011). After the first vegetation period the shoots were cut back and the lysimeters were stored in an area protected from frost where they could safely overwinter.

Experimental design

The experiments to determine the yield-transpiration relation were conducted by maintaining the plants for two vegetation periods under pre-defined water availability regimes and fertilization rates. All the treatments were tested in three replicates for a total of 21 lysimeters. The lysimeters where they could safely overwinter. The base connected to the drainage extension. Water was supplied from an automatic drip irrigation system controlled by a GP-1 data logger (Delta-T Devices, Cambridge, UK) when the soil moisture reached values lower than the pre-defined lower limit. To control the irrigation amount and frequency, the soil moisture was measured at a depth of 20 cm with a FDR probe (SM-200, Frequency Domain Reflectometry, Soil Moisture Sensor Delta-T Devices, Cambridge, UK). Variations in water storage were determined through calculations of the weekly water budget based on weekly measurements of the soil moisture at four depths (10, 20, 30, and 40 cm) with a portable FDR profile probe (PR2/4w-02, Delta-T Devices, Cambridge, UK). Plant transpiration was calculated from the experimental water balance following equation:

\[ T = I - (E + D + \Delta S) \]

where T is the plant transpiration, I = irrigation, E = evaporation, D = drainage and \( \Delta S \) = soil water change.

To avoid evaporation, each soil surface was covered with a garden fleece, and thus E can be set to 0.

Microclimate

Air temperature and relative air humidity were recorded directly under the roof on an hourly basis and stored in a GP-1 data logger. Daily mean values of air temperature and relative humidity were calculated for the daytime from 8:00 a.m. to 5 p.m. local time (CET). The data set obtained was used to compute the daily mean of the vapor pressure deficit (VPD). The mean of the daily temperatures recorded during the vegetation period 2011 was 23.9°C, with the daily mean temperature above 30°C on 13 days and a peak of 33.5°C occurring at the end of August (Fig. 1a). However, the atmospheric evaporative demand was higher in June, with a mean monthly VPD of 20 hPa (Fig. 1b). The VPD decreased in July, August and September with monthly means of 13.2, 13.9 and 12.9 hPa, respectively. The vegetation period 2012 was warmer compared to 2011, with a daily mean temperature of 25°C and 17 days were above 30°C. A maximum air temperature of 39.3°C occurred in the middle of August (Fig. 1b). The year 2012 was characterized by a relatively high fluctuation of the monthly VPD, with the lowest values in June (12.5 hPa) and September (13.2 hPa) and a peak in August (22.7 hPa).

Leaf gas exchange

The gas exchange of fully expanded leaves was measured with a CMS 400 minicuvette system (Heinz Walz GmbH, Effeltrich, Germany) (Midgley et al., 1997). The gas exchange measurements were carried out under ambient CO₂ concentrations (Veste and Herppich, 1995). H₂O and CO₂ fluxes were determined with an infra-red gas analyzer (BINOS 100-4P, Rosemount, Hanau, Germany) and all fluxes were expressed on the projected leaf area. Transpiration, net CO₂ exchange and leaf conductance for water vapor were calculated using the standard software DIALGAS 2.0 (Heinz Walz GmbH, Effeltrich, Germany). For the determination of the leaf area, the leaves were scanned and the surface estimated by using a digital image processing technique (GNU Image Manipulation Program 2.6, Berkeley, California, and UTHSCSA ImageTool, San Antonio, Texas). The illumination was set to a constant 1100 μmol m⁻² s⁻¹.
with an external halogen lamp to ensure light-saturation during photosynthesis. Air temperature and air humidity (measured as air to leaf water vapor pressure deficit, ALVPD) in the cuvette corresponded closely to the actual ambient conditions and were kept constant during the measurements. We selected three different temperature and humidity conditions: I) $T_{\text{cuv}} = 20^\circ\text{C}$, $rH = 74\%$, and ALVPD = 8.9 mPa Pa$^{-1}$, II) $T_{\text{cuv}} = 25^\circ\text{C}$, $rH = 53\%$ and ALVPD = 14.9 mPa Pa$^{-1}$, and III) $T_{\text{cuv}} = 30^\circ\text{C}$, $rH = 51\%$, and ALVPD = 20.8 mPa Pa$^{-1}$, representing typical summer days.

Biomass determination

Aboveground biomass was harvested in the first year of the experiment on September 16th, 2011 and on September 8th, 2012 in the second year. The fresh weight of leaves and stems were weighed separately. Plant material was oven-dried at 65$^\circ\text{C}$ until it reached a constant weight for the determination of dry weight. Yield-transpiration relations were obtained from linear regression analysis in three different ways: (I) Since the biomass production of the plant is for biogas production, which implies the use of fresh material, the yield water use efficiency (YWUE) was calculated according to the relation between the total aboveground fresh biomass and the cumulative transpiration; (II) Water use efficiency (WUE) was calculated according to the ratio between the total aboveground dry biomass evaluated at the harvest and the cumulative transpiration; (III) The transpiration coefficient (TC) was calculated according to the relation between the cumulative transpiration and the total dry matter of aboveground biomass.

Statistical analysis

The statistical analysis was performed using IBM SPSS software, version 21 (SPSS Inc., Chicago, IL, USA) and Microsoft-Excel software. A non-parametric analysis, the Mann-Whitney test (significant at level of 0.05), was performed to compare (a) cumulative transpiration, (b) yield, (c) total aboveground dry biomass, (d) YWUE (e) WUE and (f) TC at the whole plant level. Furthermore, a non-parametric Spearman’s rho analysis (significant level of 0.01** and 0.05*) and linear regression analysis were used to correlate (a) the total fresh biomass and total dry biomass with the cumulative transpiration, and (b) the total fresh biomass and total dry biomass with the fertilization amount. At the leaf level we used the Mann-Whitney test (significant at level of 0.05) to compare the (a) transpiration rate and (b) the net photosynthesis.

RESULTS AND DISCUSSION

For both water experiments in 2011 and 2012, the soil water availability reduction lead to a water use decrease (Fig. 2a). In the 2011 water experiment, the SWA 100 water use was significantly different compared to all the other treatments, being 34% and 68% higher compared to the SWA 70 and SWA 35, respectively. Significant differences in terms of cumulative transpiration were found between the water treatments during the 2012 experiment as well. The SWA 100 and SWA 70 water treatments, with water uses of 141 and 129 l, respectively, were comparable but significantly different from SWA 35 (109 l) and SWA 25 (69 l) (Fig. 2a). For the N experiments in 2011 there
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**Fig. 2.** Influence of the soil water content (nitrogen fertilization 50 kg ha\(^{-1}\)) (A–C) and nitrogen application (soil water availability 100%) (D–F) on the cumulative transpiration, fresh biomass and dry biomass of *Fallopia* plants in the 2011 and 2012 experiments.

**Fig. 3.** Influence of the soil water content (nitrogen fertilization 50 kg N ha\(^{-1}\)) (A) and nitrogen application (soil water availability 100%) (B) on the leaf area development of *Fallopia* plants in the 2011 and 2012 experiments.
CO2 ranged from 7.5 to 9.7 µmol m–2 s–1 (Fig. 4a). Raising plants (with *Solanum melongena*), while the mean net photosynthesis is 24 µmol m–2 s–1 for tomatoes and 18–26 µmol m–2 s–1 for eggplants. These measured relative high mean values for leaf transpiration net CO2 uptake and transpiration of *Fallopia* (Fig. 4), even that the differences are not significant due to the high variability between the measured leaves on different plants. Thus stomatal reaction is triggered by the soil water availability and the VPD (Gollan et al., 1985). During the days with the lowest air to leaf water pressure deficit (ALVPD), the transpiration ranged from 3.9 and 5.4 mmol m–2 s–1, while the CO2 ranged from 7.5 to 9.7 µmol m–2 s–1 (Fig. 4a). Raising the ALVPD to 14.9 mPa Pa–1, the transpiration for all the treatments increased to values of 8 and 10.6 mmol m–2 s–1, while the net CO2 exchange rates remained at the same level with mean values ranging from 7.6 to 9.1 µmol m–2 s–1. A clear response to the soil water limitation occurred when the ALVPD was 20.8 mPa Pa–1 and transpiration for the SWA 35 dropped to 6.3 mmol m–2 s–1, while the maximum for the SWA 100 was 8.6 mmol m–2 s–1 (Fig. 4c). Due to stomatal responses, the net CO2 exchange responded similarly to the water availability reduction with a minimum value for the SWA 35 of 6.1 µmol m–2 s–1 and maximum for SWA 100 of 7.9 µmol m–2 s–1. These measured relative high mean values for leaf transpiration 10.6 mmol m–2 s–1 in *Fallopia* are in line with various other crops like sunflowers (*Helianthus annuus*) and tomatoes (*Lyco-persicum esculentum*), while the mean net photosynthesis is with 9.1 µmol mmol m–2 s–1 much lower than other productive crop species with a similar transpiration. The measured net CO2 exchange rates are 10–25 µmol m–2 s–1 for sunflowers, 12–24 µmol m–2 s–1 for tomatoes and 18–26 µmol m–2 s–1 for eggplants (*Solanum melongena*) (Breckle et al., 2003). Also, *Sida hermaphrodita*, which is considered a highly productive bioenergy crop with heights up to 3–4 m, reaches net photosynthesis rates of 6 to 16 µmol m–2 s–1 in pot experiments (Veste et al., 2014), while Franzaring et al. (2014) reported values of 12–17 µmol m–2 s–1. In various experiments it could be shown that the net photosynthesis rates and the leaf conductance of annual

The consequences of the water constraints for both years in terms of aboveground biomass production are enlightened by the significant differences found between the treatments. For the watering experiment in the first vegetation period that was examined in 2011, the SWA 70 and SWA 35 biomass yields were 85 and 56% of the SWA 100 (596 g), respectively (Fig. 2b). The dry biomass followed the same trend with the SWA 70 and SWA 35 producing 94 and 50% of the SWA 100, respectively (Fig. 2c). The nitrogen fertilization amount also shaped significant differences between the treatments in terms of primary production for both years (Fig. 2e and f). The Spearman’s correlation between the aboveground fresh biomass and dry biomass with the N fertilization amount is significant at 0.528* and 731**, respectively. In 2011 the N 100 yield was the highest with 876 g, followed by a comparable amount for N 150 (734 g), while the N 50 and the N 0 had reduced yields of 596 and 594 g (Fig. 2e). In 2012 the N 150 took over the role of the best performer with a yield of 911 g. The treatments N 100 and N 50 followed with comparable productions of 827 and 830 g. The N 0 reduced in yield to 431 g. The same trend can also be recognized regarding the dry biomass production (Fig. 2f).

The leaf area is less influenced by the soil water availability compared to the total biomass (Fig 3a). The increase of biomass in the second year is due to a higher stem growth. On the other hand, the nitrogen application increases the leaf area in both years (Fig. 3b). Soil water availability influencing also the leaf transpiration net CO2 uptake and transpiration of *Fallopia* (Fig. 4), even that the differences are not significant due to the high variability between the measured leaves on different plants. Thus stomatal reaction is triggered by the soil water availability and the VPD (Gollan et al., 1985). During the days with the lowest air to leaf water pressure deficit (ALVPD), the transpiration ranged from 3.9 and 5.4 mmol m–2 s–1, while the CO2 ranged from 7.5 to 9.7 µmol m–2 s–1 (Fig. 4a). Raising the ALVPD to 14.9 mPa Pa–1, the transpiration for all the treatments increased to values of 8 and 10.6 mmol m–2 s–1, while the net CO2 exchange rates remained at the same level with mean values ranging from 7.6 to 9.1 µmol m–2 s–1. A clear response to the soil water limitation occurred when the ALVPD was 20.8 mPa Pa–1 and transpiration for the SWA 35 dropped to 6.3 mmol m–2 s–1, while the maximum for the SWA 100 was 8.6 mmol m–2 s–1 (Fig. 4c). Due to stomatal responses, the net CO2 exchange responded similarly to the water availability reduction with a minimum value for the SWA 35 of 6.1 µmol m–2 s–1 and maximum for SWA 100 of 7.9 µmol m–2 s–1. These measured relative high mean values for leaf transpiration 10.6 mmol m–2 s–1 in *Fallopia* are in line with various other crops like sunflowers (*Helianthus annuus*) and tomatoes (*Lyco-persicum esculentum*), while the mean net photosynthesis is with 9.1 µmol mmol m–2 s–1 much lower than other productive crop species with a similar transpiration. The measured net CO2 exchange rates are 10–25 µmol m–2 s–1 for sunflowers, 12–24 µmol m–2 s–1 for tomatoes and 18–26 µmol m–2 s–1 for eggplants (*Solanum melongena*) (Breckle et al., 2003). Also, *Sida hermaphrodita*, which is considered a highly productive bioenergy crop with heights up to 3–4 m, reaches net photosynthesis rates of 6 to 16 µmol m–2 s–1 in pot experiments (Veste et al., 2014), while Franzaring et al. (2014) reported values of 12–17 µmol m–2 s–1. In various experiments it could be shown that the net photosynthesis rates and the leaf conductance of annual
linear regression for all data (y = 4.93 x, R² = 0.949). (B) WUE: and water uptake variations caused by irrigation and nitrogen ferti-
Fig. 5. Fresh biomass (A) and dry matter (B) yield as functions of Fallopia transpiration evaluated for all investigated plants. Yield and water uptake variations caused by irrigation and nitrogen fertilization during the experiments in 2011 and 2012. (A) YWUE: linear regression for all data (y = 4.93 x, R² = 0.949). (B) WUE: linear regression for all data (y = 1.92 x, R² = 0.94).
These features enable the plant to start growing even during drought periods in late spring and early summer which are not uncommon in East and North Germany, when maize is just starting to grow and is sensitive to drought conditions.

CONCLUSION
Our lysimeter experiment showed that the transpiration-biomass relation of Igniscum is comparable to rye and barley in terms of dry biomass production. Contrariwise, the water use efficiency calculated for leaf transpiration and net photosynthesis is lower compared to other crop species due to the relative low CO₂ exchange rates. Despite the fact, that the calculated transpiration coefficient was higher compared to maize, which is mostly the reference for bioenergy crops, as a perennial plant IGNISCUM has various advantages under drought conditions compared to annual crops. The established deep rooting system and the rhizomes allow the plants to mobilize reserves for growth in the spring and to reach deeper soil water tables. This makes the plant more independent from the actual precipitation. These features enable the plant to start growing even during drought periods in late spring and early summer when maize is just starting to grow and is sensitive to drought conditions. These drought periods are not uncommon in eastern and northern Germany. From the viewpoint of the water use efficiency and growth performance, Fallopia has the potential to be used as a second generation bioenergy crop.

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