



A comprehensive study of planting density and nitrogen fertilization effect on dual-purpose hemp (*Cannabis sativa* L.) cultivation

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ABSTRACT

Harvesting hemp (*Cannabis sativa* L.) for both stems and seeds is now a common practice in Europe while crop management strategies for dual-purpose hemp cultivation have not been properly addressed so far. In the present study, the effects of planting density and nitrogen fertilization on hemp stem and seed yields were tested with the cultivars Futura 75 and/or Bialobrzeskie in eight contrasting environments (Italy in 2013; Italy and Latvia in 2014; Italy (two sites), Latvia, the Czech Republic, and France in 2015). Stem yield ranged between 1.3 and 22.3 Mg ha⁻¹. The effects of planting density and nitrogen fertilization on stem yield did not interact significantly with each other, or with cultivar and harvest time. Increasing planting density from 30 to 120 plants m⁻² and increasing nitrogen fertilization rate from 0 to 60 kg N ha⁻¹ increased stem yield by 29% and 32%, respectively. Further increase in planting density and nitrogen fertilization did not result in a significant increase in stem yield. Seed yield ranged from 0.3 to 2.1 Mg ha⁻¹. The seed yield was not affected significantly by planting density between 30 and 240 plants m⁻². Although the seed yield showed an increasing trend with increasing nitrogen fertilization, the effects of nitrogen fertilization on seed yield were not statistically significant.

To grow hemp as a dual-purpose crop it is recommended to plant 90–150 plants m⁻² across all tested environments. Nitrogen fertilization rate at 60 kg N ha⁻¹ was generally sufficient in the tested environments whereas further optimization of nitrogen fertilization requires accurate assessment of plant nitrogen status. To facilitate assessing plant nutritional status, a critical nitrogen dilution curve was determined for hemp and a practical method to determine nitrogen nutritional status was discussed.

1. Introduction

Hemp (*Cannabis sativa* L.) is resurging as an ideal multipurpose crop worldwide (Amaducci et al., 2015; Aubin et al., 2016; Faux et al., 2013; Bertoli et al., 2010). For the first time, it was cultivated in Europe on more than 33,000 ha in 2016 mainly as a dual-purpose crop where stems and seeds were harvested simultaneously (Carus 2017). However, hemp was traditionally a fibre crop and most past research focused on this purpose (Westerhuis et al., 2009; Amaducci et al., 2008a, 2002a; Struik et al., 2000; Van der Werf et al., 1996). Very limited information is available on growing dual-purpose hemp (Amaducci et al., 2015). In the frame of the EC funded project Multihemp (www.multihemp.eu),

extensive experiments have been carried with the aim of providing novel information to support dual-purpose hemp cultivation in Europe. Aspects related to cultivar choice for dual-purpose hemp cultivation have been recently published by Tang et al. (2016). The present study focuses on the effect of the two main agronomic practices affecting the performance of dual-purpose hemp: planting density and nitrogen fertilization.

The effects of planting density and nitrogen fertilization on both stem and seed yields have not been properly addressed so far. Previous researches indicate that planting density has little effect on stem yield, but plants grown at high density are shorter and thinner than those grown at low density (Amaducci et al., 2002b; Struik et al., 2000).

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Slender stems are desirable for fibre hemp production because they produce more long fibre (Westerhuis et al., 2009) and require less energy for their mechanical processing (Khan et al., 2010). Thus, a high planting density is generally used, ranging from 90 plants m^{-2} to 350 plants m^{-2} (Martinov et al., 1996; Starcevic, 1996), to achieve required fibre quantity and quality. On the other hand, a low planting density, ranging from 30 plants m^{-2} to 75 plants m^{-2} , is recommended for producing hemp seeds (Amaducci and Gusovius, 2010 and references therein). Optimal planting density has not been researched for growing hemp as a dual-purpose crop.

The effect of nitrogen fertilization on stem yield varies in literature. For relatively low fertility conditions, Amaducci et al. (2002b) reported that stem yield increased by 20 kg $N\ ha^{-1}$. Finnan and Burke (2013) reported a very high stem yield increase with increasing fertilization from 0 up to 120 kg $N\ ha^{-1}$ (as high as 60 kg $N\ ha^{-1}$). In contrast, the yield response of hemp to nitrogen fertilization was found negligible when soil fertility was high (Prade et al., 2011; Struik et al., 2000). Few studies have been conducted considering the response of seed yield to nitrogen fertilization. Aubin et al. (2015) and Marija et al. (2011) reported that both stem and seed yields were positively related to nitrogen fertilization. Vera et al. (2010, 2004) reported that hemp seed yield increased progressively with increasing nitrogen availability until a high fertilization rate, ranging from 99 kg $N\ ha^{-1}$ to 198 kg $N\ ha^{-1}$ depending on growing conditions. Given the wide range of the results regarding stem and seed yields in response to nitrogen fertilization and the large variation of soil nitrogen availability, it is a challenge for farmers to optimize fertilization rate and to maximize economic return.

Nitrogen fertilization affects crop yield mainly through its effect on plant nitrogen status (Sadras and Lemaire, 2014). When nitrogen supply is deficient, aboveground biomass yield (W) increases with increasing nitrogen uptake until a critical nitrogen concentration ($N_{critical}$) has been reached; further increasing nitrogen uptake has little impact on increasing W (Lemaire and Meynard, 1997). In general, the $N_{critical}$ decreases exponentially with increasing W during plant growth, which is called the $N_{critical}$ dilution curve (Greenwood et al., 1991, 1990). Although the $N_{critical}$ dilution curve varies among species, it remains fairly consistent at different environmental growth conditions (Lemaire and Gastal, 2009). Therefore, the $N_{critical}$ dilution curve has been used to determine the nitrogen status for many crops, including rice (*Oryza sativa* L.; Ata-Ul-Karim et al., 2013; Sheehy et al., 1998), maize (*Zea mays* L.; Ziadi et al., 2010), oilseed rape (*Brassica napus* L.; Colnenne et al., 1998) and linseed (*Linum usitatissimum* L.; Flénet et al., 2006). Estimating a $N_{critical}$ dilution curve for hemp would be useful to optimize its nitrogen fertilization.

The objective of this study was to assess the effects of planting density and nitrogen fertilization across a wide range of environments to support dual-purpose hemp cultivation in Europe. First, the effects of planting density and nitrogen fertilization on hemp stem and seed yields were investigated. Second, the characteristics of hemp's nitrogen demand were analysed and a critical nitrogen dilution curve was assessed for hemp.

2. Materials and methods

2.1. Experimental locations and field layout

Field experiments were carried out at five locations in Europe: Piacenza-IT (Piacenza, Italy), Budrio-IT (Budrio, Italy), FR (La Trugalle, France), CZ (Sumperk, the Czech Republic) and LV (Vilani, Latvia) from 2013 to 2015 (Table 1). Latitude difference between the most northern (LV) and the most southern (Budrio-IT) location was 12°, which corresponds to 2 h of maximum day-length. Average temperature between May and October (during the hemp growing season) ranged from 14.9 °C (LV) to 21.8 °C (Piacenza-IT); total precipitation ranged from 212 mm (CZ) to 297 mm (FR). The most southern locations, Piacenza-IT and Budrio-IT, were hot and dry in the summer while the other three

locations were cool and humid (Supplementary Fig. 1).

Planting density was varied from 30 plants m^{-2} to 240 plants m^{-2} ; nitrogen fertilization range was from 0 to 120 kg $N\ ha^{-1}$. The planting density $\times$ nitrogen fertilization interaction was tested with the cultivar Futura 75 in Piacenza-IT in 2013. The planting density $\times$ cultivar and nitrogen fertilization $\times$ cultivar interactions were tested with the cultivars Futura 75 and Bialobrzeskie in Piacenza-IT and LV in 2014. As the effects of planting density and nitrogen fertilization on stem and seed yields did not interact with each other in 2013 and did not interact with cultivar in 2014 (see Results section), the effects of planting density and nitrogen fertilization were tested separately in 2015 for the cultivar Futura 75 at all five locations (Piacenza-IT, Budrio-IT, FR, CZ and LV).

The main factors (i.e., planting density, nitrogen fertilization and cultivar) were tested in a randomized complete block design with four replicates. Single plot size was 42 m^2 when only two harvests were scheduled, or 60 m^2 when multiple harvests were scheduled for a detailed growth analysis. Sowings were carried out as soon as the soil was accessible and average daily temperature rose above 8–10 °C. Sowing dates spanned from the earliest on 7 April 2014 in Piacenza-IT to the latest on 14 May 2015 in Budrio-IT (Supplementary Table 1). Seeds were drilled at 3–4 cm depth using experimental-plot sowing machines. The distance between rows varied between 15 cm and 25 cm, depending on the sowing machine used at each location. Seed rates were calculated based on the target density considering weight of 1000 seeds and results of seed germination tests. In Piacenza-IT and Budrio-IT, densities of 30 plants m^{-2} and 60 plants m^{-2} were obtained by sowing seeds in excess (90 plants m^{-2}) and hand-thinning to target density after emergence. Hand-thinning was conducted carefully so that any impact (e.g. increase of soil compaction due to worker's footprint) on plant growth would be minimized. Nitrogen fertilizer was distributed at sowing or immediately after emergence. Irrigation was only applied in Piacenza-IT; in total 120 mm, 60 mm and 155 mm of water was provided with a travelling sprinkler in 2013, 2014 and 2015, respectively.

2.2. Assessing crop development and yields

Seedling emergence was monitored in the plots with 120 plants m^{-2} by counting the emerged plants in one row over a length of 1 m or in two rows over a length of 50 cm, every two days from the appearance of the first plant until full emergence. Date of emergence was set when 50% of final seedlings had emerged. Flowering was monitored on 20 representative plants that were selected and labelled before flowering. A single plant was considered at the full flowering stage when its top appeared as a compact (i.e., with very short internodes) inflorescence with visible stigmata. The full flowering stage of a plot was set when 50% of the monitored plants had reached full flowering.

In each plot, harvest was carried out at least twice: at full flowering (H1) and at seed maturity (H2). At each harvest, all plants in an area of 4 m^2 were cut just above the soil level. Fresh weight of all harvested plants was assessed immediately and the number of plants in the first harvested 1 m^2 was counted. Among the plants of this first 1 m^2 , 20 representative plants were sampled. A subsample of 10 plants was dried at 75 °C until constant weight to assess dry matter content. On the remaining 10 plants, stem diameter (at 10 cm from stem base), plant height and proportion of stem, leaf and seed (H2) in the above ground biomass (after oven drying) were assessed. W (aboveground biomass yield) was calculated as the product of fresh weight and dry matter content. The yields of stem (W_{stem}), leaf (W_{leaf}) and seed (W_{seed}) were estimated as the product of W and the corresponding proportions.

In addition to H1 and H2, periodic samplings were carried out on 1 m^2 area in Piacenza-IT (2014 and 2015) and in Budrio-IT (2015), in total 4–5 times. At each sampling date, all plants in an area of 1 m^2 were cut just above the soil level using pruning scissors. Plant density, plant height, stem diameter, W , W_{stem} , W_{leaf} and W_{seed} (when present) were assessed following the same procedure described for H1 and H2.

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