Crop Production

Biomass and essential oil production of hops cv Chinook in response to nitrogen fertilization[1]

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ABSTRACT

Hop (*Humulus lupulus* L.) is one of the most important raw materials of beer production. Despite being the third-largest producer and consumer of beer worldwide, Brazil imports almost all hops for production. Nitrogen is an essential macronutrient for hop development and its behavior under different doses is still unknown for Brazilian conditions. Our goal was to evaluate the dry matter and essential oil yields of the Chinook hop fertilized with five N rates during three harvests. Leaves, branches, and cones were sampled to determine their dry matter production, and cones were also analyzed for essential oil contents, extracted by hydrodistillation. Cone number per plant, cone, branch, and leaf dry matter yields, as well as total essential oil yield per plant, increased linearly as nitrogen rates were raised. The production of DM of cones obtained ranged between 245.8 (N:0) and 386.7 kg ha^{-1} (N:250 kg N ha^{-1}). Yield differences were also noted between harvest years due to weather conditions. Therefore, we can conclude that increasing N rates and climatic conditions affect N contents in hop leaves, as well as hops overall and oil yields, due to the higher cone dry matter. However, essential oil contents in cone dry matter did not change.

Keywords: *Humulus lupulus* L.; hydrodistillation; plant nutrition.

INTRODUCTION

Hop (*Humulus lupulus* L.), a dioecious species of the family Cannabaceae, is one of the raw materials used in the production processes of beers, cosmetics, and medicines (Biendl *et al.*, 2014; Solarska & Sosnowska, 2015). Although Brazil is the third-largest producer and consumer of beer in the world, the main global hop producers are the United States, Germany, the Czech Republic, and China (Cervieri Júnior *et al.*, 2014; IHGC, 2019). Brazil imports about 4,000 tons of hops per year, at a cost of over 200 million reais (Soares & Firmo, 2018). Currently, the Brazilian beer industry is expanding, mainly with the emergence of craft breweries, with 257 in 2014 and 1,383 in 2020 (Mapa, 2020).

The medicinal properties of hops may include sleep and estrogenic activity inductions (Chadwick *et al.*, 2006), dyspepsia and lack of appetite treatments (Biendl *et al.*, 2014), as well as anti-inflammatory potential (Bocquet *et al.*, 2018a) and cancer (Önder *et al.*, 2016; Ho *et al.,* 2020), tuberculosis and diabetes treatments (Bocquet *et al.*, 2018b).Active compounds important to the brewing and medicinal industries include resins, α-and β-acids, tannins, flavonoids, polyphenols, and essential oils

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(Biendl *et al.*, 2014; Nowak *et al.*, 2020). Such compounds are contained in lupulin glands of small spikes, called cones, which are female inflorescences (Biendl *et al.,* 2014). These glands store resins and essential oils (Farag & Wessjohann, 2013). Essential oils are compounds responsible for aroma and their contents may range from 0.2 to 3.0% in cone dry matter. Essential oil yield may vary with environmental conditions, growing area, cultivar used, as well as post-harvest-related factors (Biendl *et al.*, 2014).

For a good cone dry matter production with satisfactory resin and oil levels, mineral nutrients must be properly supplied. Nitrogen (N) is one of the important macronutrients for vigorous vegetative growth of crops, besides being essential during vertical and lateral plant growths, which occurs in spring (Neve, 1991; Dodds, 2017). Nitrogen recommendation for hops varies in different countries (Bavec *et al.*, 2003). However, Brazilian fertilization manuals still do not have information on N fertilization for hops. There are also few works in the literature relating cone and essential oil yields according to N rates in hops grown under Brazilian soil and climatic conditions.

Given the above, this study aimed to evaluate the hop biomass and essential oil yields under different nitrogen fertilization rates and edaphoclimatic conditions of Guarapuava, PR (Brazil).

MATERIAL AND METHODS

The experiment was installed at the Agricultural Research Foundation of the Cooperativa Agrária de Entre Rios, located in Guarapuava, PR, central-southern micro-region of Paraná State, in southern Brazil (25° 33' S; 51° 29' W, and 1,095-m altitude). According to the Köppen-Geiger classification, the local climate is classified as Cfb type, with cool summers and no defined dry season (Alvares *et al.*, 2013). The climatic data (Figure 1) during the experiment were gathered from a gauge station installed in the experimental area. The sequential water balance was calculated according to Thornthwaite & Mather (1955).

The soil in the area has a very clayey texture (650 g kg^{-1} clay, 175 g kg^{-1} sand, and 175g kg^{-1} silt in the 0-40 cm depth layer) and is classified as *Latossolo bruno alumínico* (aluminum-rich dark Oxisol). The chemical analysis results were as follows: pH $CaCl_2$ – 5.50; Al^{+3} – 0.00; H^{+}+Al^{+3} – 5.50 cmol$_c$ dm^{-3}; Ca^{+2} – 7.60 cmol$_c$ dm^{-3}; Mg^{+2} – 2.80 cmol$_c$ dm^{-3}; K^{+} - 0.52 cmol$_c$ dm^{-3}; P – 16.70 mg dm^{3}; C – 51.8 g dm^{3}; and CEC – 16.42 cmol$_c$ dm^{-3}.

Field Experiment

The experiments under field conditions were carried out during the 2018/2019, 2019/2020, and 2020/2021 crop seasons, using the cultivar 'Chinook' and nitrogen fertilization rates of 50, 100, 150, 200, and 250 kg de N ha^{-1}, plus a control (without N). Urea 46% was used as N-source and was applied by casting along 1-m wide planting rows three times: 30 kg N ha^{-1} at first-leaf formation (BBCH 1) and the remainder of each rate split into 2 equal applications at branching (BBCH 2) and main branch elongation (BBCH 3) stages, according to the phenological classification of Roßbauer *et al.* (1995).

The experimental design was randomized blocks with four replications of 8 plants spaced 1 m and rows 3 m apart. Each experimental unit had 2 plants used as borders. After sprouting, six main branches were selected and tutored by two sisal ropes fixed on wire at 5 m height.

Dry matter production determination

Cones were harvested after yellowing with petals falling to the touch, and when lupulin were dark yellow (Biendl *et al.,* 2014). The cones were samples on average 120 days after the beginning of the experiment. Cone yield, branch (main and secondary ones) and leaf dry matter, and essential oil contents were evaluated. Branch and leaf dry matters were obtained after drying in a forced-air circulation oven at 60 °C for 72 hours, until constant weight. Hop cones, in turn, were dried in the same oven, but at 35 °C for 72 hours, until constant weight (Falkenberg *et al.*, 2007). Six plants per plot were used to evaluate all variables. Harvest index (CI) was calculated as the ratio between cone dry matter and total shoot dry matter (sum of leaves, branches, and cones).

Essential oil content determination

Each treatment contained 4 repetitions of 50 g dry cones. Essential oils were extracted by hydrodistillation in a graduated Clevenger apparatus for 3 hours, containing 1,000 mL distilled water. The essential oil content was determined on a dry mass basis and expressed as a percentage (% m m^{-1}).

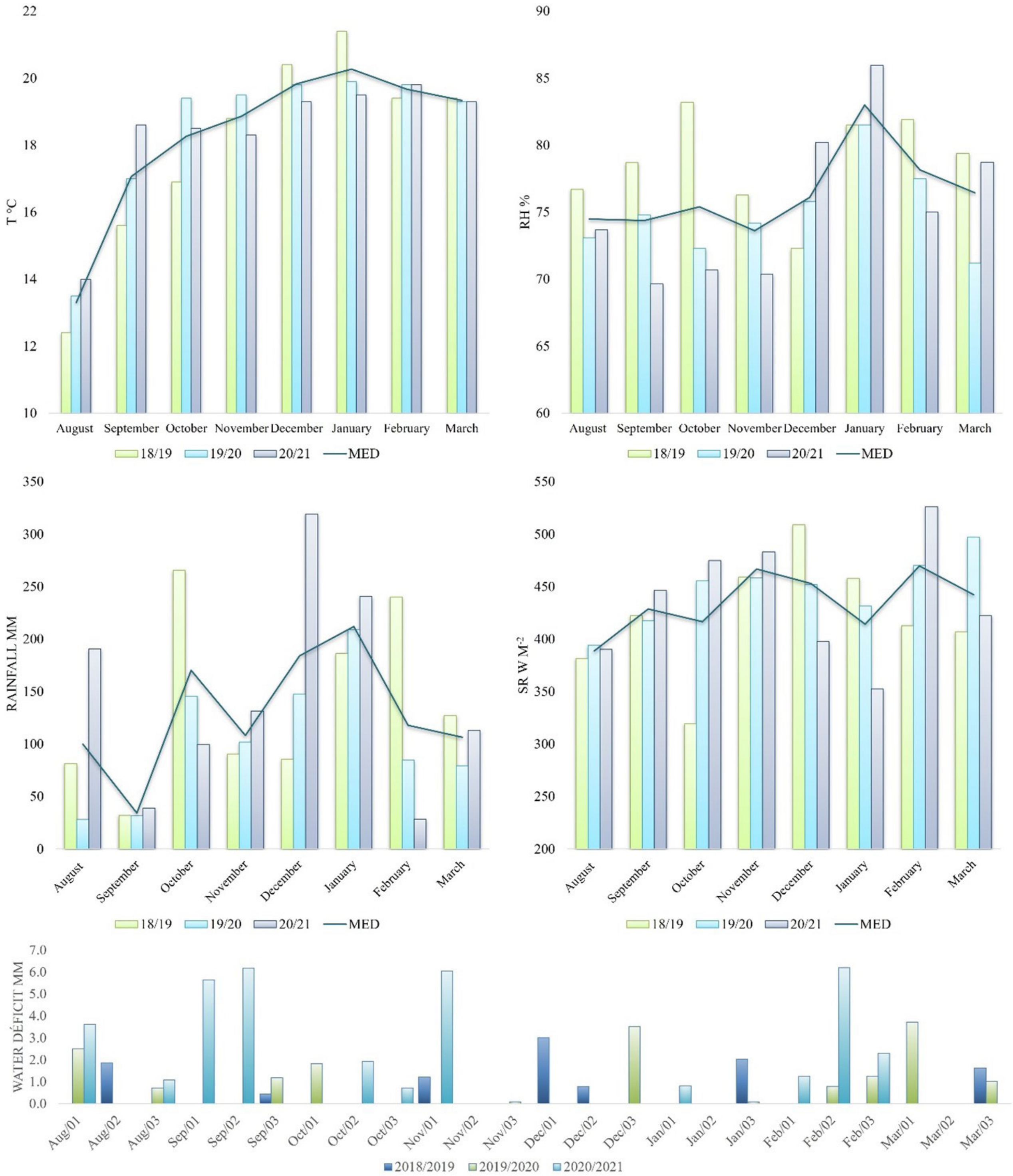

Figure 1: Monthly averages of temperature (T °C), relative humidity (RH%), solar radiation (SR w m^{-2}), and total rainfall (mm) from August to March of the 2018/2019, 2019 /2020, and 2020/2021 crop years from the FAPA gauge station, in Guarapuava-PR; 3YM - three-year mean; Source: FAPA Gauge Station, adapted by the authors (2021).

Nitrogen in hop shoot

Nitrogen content was determined by sulfuric digestion, followed by Kjehldahl determination. Dried samples (100 mg) of leaves, branches, and cones were placed into digester tubes with 1 g of a K_2S0_4 and $CuSO_4$ salt mixture at 10: 1 and 3 mL H_2SO_4 98%. Then, the tubes were slowly heated in a digester block (350 °C) until a blue-green color appears. After cooling, all tubes were filled up with water up to 50 mL. Afterwards, 10 mL NaOH (40%) was added to the digested extract and the tube was coupled to the Kjeldahl distiller. Distilled volumes were transferred to beakers containing 25 mL H_3BO_3 (2%) plus indicators (0.1% bromocresol green and 0.04% m/v methyl red). Solution coloration was corrected to wine with HCl 1 mol L^{-1} solution. After completing 45 mL distilled solution, the green-colored product was taken to titration with a 0.01 mol L^{-1} HCl until a wine color was achieved. The volume to change solution color was used to calculate N amount in the respective sample.

Statistical analysis

Statistical analysis was performed considering an arrangement time-divided plots, with 6 N levels as the main factor and 3 years the sub-factors. After analysis of variance (ANOVA), a regression analysis was performed for N levels and Scott Knott's test for the year subfactor ($p \leq 0.05$). Plant N levels were tested by ANOVA, followed by regression analysis. ANOVA conditions were verified by homoscedasticity (Bartlett, Hartley and Levene) and normality (Jarque-Berra) tests. The data were analyzed using Sisvar (Ferreira, 2019) and Speed Stat Software (Carvalho *et al.*, 2020).

RESULTS AND DISCUSSION

Temperature averages were higher between December and January in the 2018/2019 crop year (20.4 and 21.4 °C, respectively). Relative air humidity (RH%), in turn, was high in January of all crop years, especially in 2021, when it reached 83%. As for radiation, indices were below average in October 2018, December 2020, and January 2021. Average rainfall was insufficient, resulting in soil water deficit in all crops evaluated (Figure 1). All crop years showed differences for N levels; number of cones per plant; dry matter contents of branches (main and secondary), leaves, cones, and shoot; and total production of essential oil per plant. Although all variables showed differences throughout the years, there was only interaction between crop year and N level for essential oil production per plant (Table 1).

Cone yield was lower in the 2020/2021 crop year (Table 1), both in number and in dry mass per plant, with reductions of 62% and 68%, respectively, compared to the average of previous years.

Radiation availability in December 2020 and January 2021, when secondary branches and inflorescences are formed, was lower than in previous years (Figure 1). As hop is a C3 plant (Ocvirk *et al.*, 2018) and has a light saturation point between 890 and 990 W m^{-2} (Kenny, 2005), yield decrease in the 2020/ 2021 crop year was due to lower incident radiation, mainly in December and January, when plants were finishing stage 3 (elongation of main branches) at stage 4 (inflorescence emergence) and starting stage 5 (flowering), according to the scale of Roßbauer *et. al* (1995).

Average RH was excessive between December 2020 and January 2021 (80.2 and 86%, respectively). Such a high humidity increased formation of *Pseudoperonospora humuli* L. sporangia (Gent *et al.*, 2010). This fungus is the causal agent of downy mildew, which is considered the most aggressive hop diseases (Purayannur *et al.*, 2020). According to Gent & Ocamb (2009), the most severe infections occur in hops at high humidity levels in the air and in leaves, which is caused by frequent rains and relatively warm nights. This situation was observed in the experiment, especially in the third year when monthly average temperature was lower than in previous years, especially in November, December, and January (Figure 1). Thus, this resulted in lower photochemical activity and, consequently, lower rubisco carboxylation efficiency, as pointed out by Eriksen *et al.* (2020).

In the last crop (2020), rainfall was also lower in September and October than in the previous months, period in which the first sprouts appear (stage 0), leaves develop (stage 2), and lateral branches are formed (stage 3), which also contributed to lower yields in the third crop year.

Cone dry matter production in this study, whose maximum photoperiod was 13.8 hours, ranged between 245.8 and 386.7 kg ha^{-1}. These values are lower than those of the largest world producers, which are on average 1,971 kg ha^{-1} (IHGC, 2019). Hop cultivation at latitudes between 35° and 50°, mainly in the Northern Hemisphere (Ruggeri *et al.*, 2018) wherein photoperiods are longer than 15 hours, is considered ideal to increase photosynthetic rates, increasing leaf and branch formations, and thus promoting the largest number of inflorescences.

Table 1: Yield indicators and oil composition of H. lupulus L. cv. Chinook in 3 crop years under 5 N rates and control (without N) in Guarapuava-PR (Brazil). Means followed by the same letter horizontally do not differ from each other by the Scott Knott's test ($p \leq 0.05$)

RESPONSE VARIABLE	*p*-value			Crop year		
	N	Year	Inter.	2018/2019	2019/2020	2020/2021
Cone (Number per plant)	< 0.01	< 0.01	0.32	1.348.67a	1.274.23a	502.94b
Cone (mg cone^{-1})	0.73	< 0.01	0.13	96.96a	94.29a	78.83b
Main branch (g plant^{-1})	0.02	< 0.01	0.87	121.23a	130.17a	91.75b
Secondary branch (g plant^{-1})	0.02	< 0.01	0.79	144.37a	129.44a	25.19b
Branch (g plant^{-1})	< 0.01	< 0.01	0.81	265.60a	259.61a	116.94b
Leaf (g planta^{-1})	0.05	< 0.01	0.78	330.48a	256.84b	162.69c
Leaf + branch (g plant^{-1})	< 0.01	< 0.01	0.86	596.08a	516.45a	279.63b
Cone (g plant^{-1})	< 0.01	< 0.01	0.36	126.17a	120.09a	39.48b
Leaf + branch + cone (g plant^{-1})	< 0.01	< 0.01	0.79	722.25a	636.54a	319.11b
Harvest index	0.12	< 0.01	0.96	0.18a	0.19a	0.12b
Essential oil (% m.m^{-1})	0.19	< 0.01	0.08	0.31a	0.13c	0.19b
Essential oil (mg plant^{-1})	0.03	< 0.01	< 0.01	392.95a	156.79b	77.81c

*N – Nitrogen rates; Inter. – Interaction.

The yield of essential oils, per cone DM and per plant, also varied in relation to the crop seasons. The highest yields were in the first harvest (Table 1), when the highest average temperatures and highest levels of solar radiation were observed in the months of December and January (Figure 1), flowering periods and cone formation. In the first year, lower precipitation and IVR indexes were also observed in this period, especially in relation to year 3, in which the lowest oil yield per plant was observed. Intense precipitation is one of the most relevant factors in relation to the yield of essential oils (Morais, 2009), especially in the period of synthesis and storage, as they can cause the loss of water-soluble substances in the cones. Other weather conditions such as temperature also affect essential oil yield (Chrysargyris *et al.*, 2017). Thus, the combined action of these meteorological differences may be responsible for the difference in oil yield in the three years of cultivation. Other factors that might have contributed to low crop yields in the three crop years were low index and poor distribution of rainfall, which resulted in 13 ten days of water deficit in the soil, between September and December of the three years (Figure 1). Periods in which the plant is at stages 1 to 4, which include leaf development, side branch formation, main branch elongation, and inflorescence emergence.

Considering the three crop years, the increase in N rates provided a linear and positive effect on most variables (Figure 2). As average cone mass did not increase significantly, the increase in cone dry matter production may have been due to an increasing effect of N on number of cones per plant and not in cone mass and sizes.

Nitrogen fertilization increased primary and secondary branch numbers, and hence leaves per plant. As inflorescences are produced on the secondary branches (in pairs along the main branch) from buds at the intersection of leaves (upper third of the plant) with the secondary branches, we may state that the greater the number of leaves, the greater the number of inflorescences. Moreover, N contributed to the greater secondary branch production, probably due to an increase in synthesis of cytokinins, which increase cell division and act in development of lateral and axillary buds when in correct balance with auxins, also dependent on N (Kieber & Schaller, 2014).

The increase in number of cones as a function of increasing N rates also contributed to increases in total production of essential oil per plant. This was because N applications did not increase oil content per cone, which ranged between 0.17 and 0.26% (Figure 2). When comparing with the study by Kenny (1990), the production in our study was low since hop essential oil from the cultivar Chinook can vary between 0.94 and 1.73%. Essential oil production per plant increased linearly with N rates in the 2018/2019 ($Y = 238.495 + 1.236X$ - R^2 *83.32%* – $p < 0.01$) and 2019/2020 ($Y = 94.406 + 0.499X$ - R^2 *43.41%* – $p = 0.03$) crop years, but did not change in the 2020/2021 crop year.

Iskra *et al.* (2019) concluded that nitrogen fertilization can influence the quality of hop cones, changing color, percentage of acids, and essential oil contents. In our work, there was a linear increase in essential oil yield per plant with increasing N rates up to 250 kg ha^{-1}. However, oil yield increased as a function of increases in cone yield, as N did not increase oil yield per cone. This is because hop essential oils are secondary metabolism products, whose biosynthesis is not induced by different N rates.

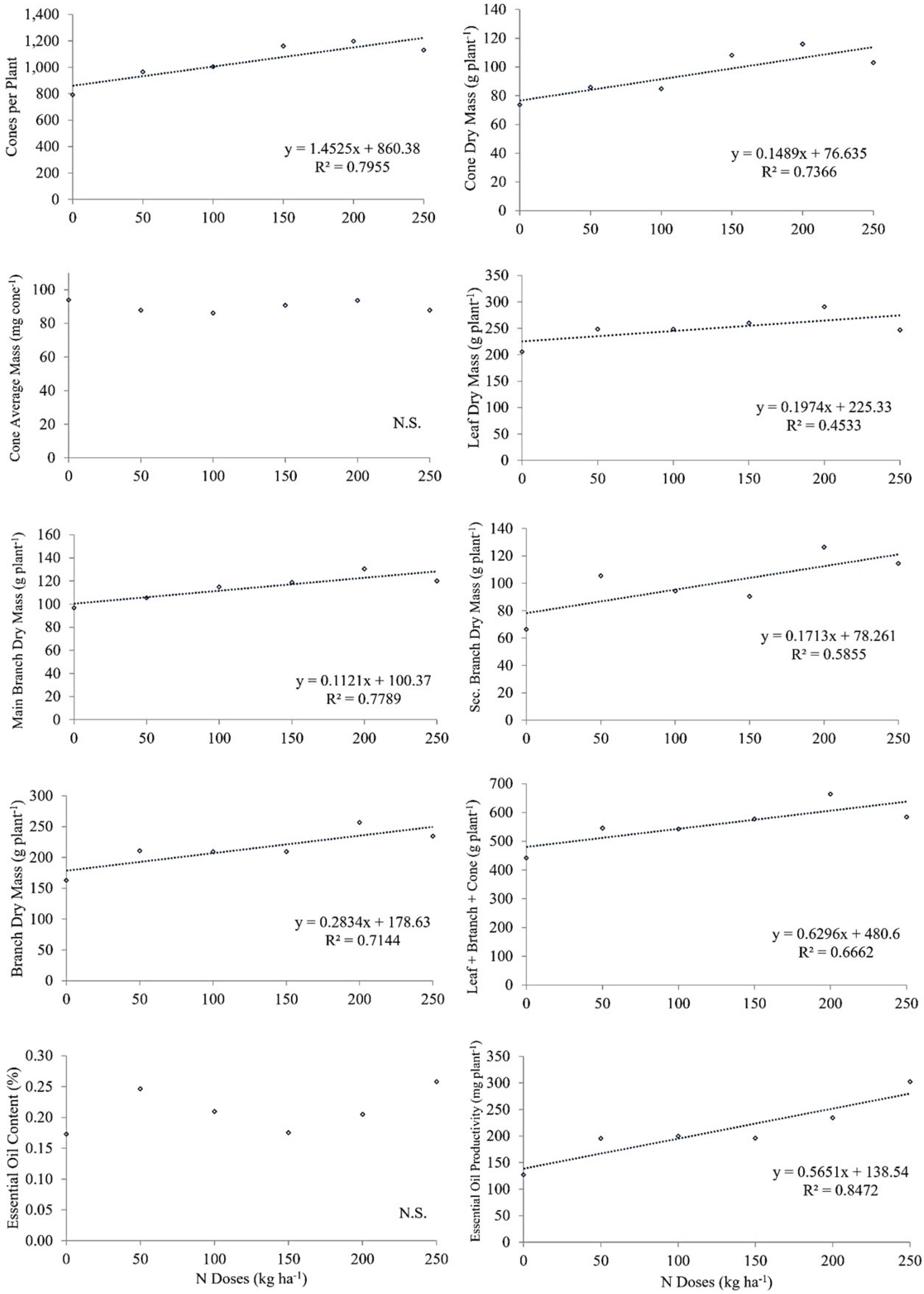

Figure 2: Yield components of H. lupulus L. cv. Chinook grown in Guarapuava-PR under different N rates. Joint analysis of the 2018/2019, 2019/2020, and 2020/2021 crop years.
*($p \leq 0.05$); NS: Non-significant ($P \leq 0.05$).

Our results demonstrate that the optimal dose for hop cone production (commercial product) is at least 250 kg N ha^{-1}. On the other hand, N application recommendations have varied considerably among the main producing countries. In the UK, they range from 180 to 220 kg ha^{-1} with soil texture (DEFRA, 2010), in Australia from 120 to 230 kg ha^{-1} (Dodds, 2017) and in the US from 120 to 170 kg. ha^{-1} in soils with moderate organic matter, reaching 230 kg ha^{-1}, and for soils with low organic matter of 250 kg ha^{-1} in Slovenia and of 270 kgha^{-1} in Germany (Bavec *et al.,* 2003).

Although the soil in the experimental area had high clay and organic matter contents, plant demands for N (≥ 250 kg ha^{-1}) were higher than the recommended values in most of the main producing countries in high latitude regions. This situation may have occurred due to the low soil moisture during applications, which occurred in the last week of September, October, and November. Low humidity can increase N losses since ammonia volatilization had been identified in the same soil in previous studies. These losses ranged from 12.5 to 25.4% of the applied N in the form of urea (Fontoura & Bayer, 2010; Viero *et al.*, 2014). Furthermore, Kolenc *et al.* (2016) reported a reduction in the activity of glutamine synthetase and alanine transferase enzymes in hops grown under water deficit, thus decreasing N assimilation.

No N deficiency symptoms were observed in plots without application (control). By contrast, in the treatment with the highest rate (250 kg N ha^{-1}), some plants showed 'angel wing' signs, which is related to nitrogen excess (Senske, 2020). However, this symptom was observed in 1 to 2 cones per plant, in less than one plant per plot. Thus, it did not affect the overall yield.

For N contents in plant shoot, a quadratic effect was observed for N rates in leaf samples ($Y = -0.00016x^2+0.0538x+30.69$), leaf and branch samples ($Y = -0.00014x^2+0.0442x+26.72$), and total shoot area (leaves, main branches, secondary branches, and cones ($Y = -0.00012x^2+0.0381x+26.58$), without differences for main branches, secondary branches, total branches, and cones (Table 2).

A maximum N accumulation efficiency was observed at the rate of 165.6 for leaves, 160.4 for leaves and branches, and 164.9 kg N ha^{-1} for total shoot dry mass. The lowest average N contents were observed in the main branches with 12.00 mg g^{-1}, while the highest concentration was in leaves, with 33.69 mg g^{-1}.

Cones had average N contents of 26.32 mg g-1. Therefore, during harvest, N contents are more concentrated in leaves and cones. As day length decreased in the autumn, a gradual leaf senescence begins and part of the N absorbed by plants is displaced to roots to form reserves for plant regrowth in the hot season (Neve, 1991).

Table 2: Nitrogen contents in shoots of H. lupulus L. plants under 5 N rates and control (without N) in Guarapuava-PR. Crop year of 2020/2021, *p ≤ 0.05; NS: Not significant P ≤ 0.05

N rate	Leaf	Secondary branch	Main branch	Branch (Main + Secondary)	Leaf+ Branch	Cone	Total
Kg ha^{-1}				N mg g^{-1}			
0	29.70	12.56	12.28	12.32	25.97	25.63	25.92
50	34.70	13.69	11.98	12.27	29.88	25.93	29.32
100	34.63	14.07	12.60	12.84	29.95	26.16	29.42
150	34.02	14.09	12.48	12.75	29.45	26.47	29.03
200	34.54	12.75	11.63	11.81	29.66	26.32	29.19
250	34.53	14.40	11.09	11.64	29.61	27.43	29.30
Average	33.69	13.59	12.00	12.27	29.09	26.32	28.70
p-value	0.02*	0.39^{NS}	0.29^{NS}	0.31^{NS}	0.02*	0.71^{NS}	0.02*
CV%	5.99	10.6	8.13	6.88	5.60	6.12	4.93
p-value RQ	0.03*	-	-	-	< 0.02*	-	0.02*
R² RQ	0.71	-	-	-	0.72	-	0.73

CONCLUSIONS

Increasing nitrogen rates linearly increase cone number per plant, cone dry matter production, leaf and branch formation, and essential oil yield in *H. lupulus* L. of the cultivar Chinook in the region of Guarapuava-PR (Brazil).

The climatic conditions (rainfall, relative humidity, radiation, and temperature) of each crop year promote changes in hop yield.

Nitrogen contents in leaves of *H. lupulus* L. cv. Chinook are affected by N rates applied.

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