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SPAD index for diagnosing nitrogen status and oil yield in 'Arbequina' olive trees

Abstract – The objective of this work was to evaluate the technical feasibility of SPAD index to estimate the N status and the oil yield of olive trees 'Arbequina'. The experiment was carried out in field conditions, over five consecutive production cycles in Southern Brazil. Treatments consisted of five doses of N applied to the soil (0, 30, 60, 90, and 120 kg ha⁻¹ of N) split into two applications. In the second half of January, which is the standard leaf-sampling period, SPAD-502 chlorophyll meter readings were performed on 20 leaves per plot, together with the collection of samples for leaf-N determination. Effects of all N doses applied to the soil were observed for SPAD index and leaf-N content, showing a positive linear correlation. Olive oil yield showed a negative correlation with the SPAD index and foliar N content in the laboratory. The SPAD index can be used in January for the nutritional diagnosis of N in 'Arbequina' olive trees with readings between 88 and 130, which falls within the range of nutrient levels considered adequate to provide satisfactory plant growth and productivity for agronomic interpretation.

Index terms: *Olea europaea*, chlorophyll meter, leaf chemical analysis, nitrogen fertilization, fruit growing, plant nutrition.

Índice SPAD para diagnóstico do estado nutricional de nitrogênio e do rendimento de azeite em oliveiras 'Arbequina'

Resumo – O objetivo deste trabalho foi avaliar a viabilidade técnica do índice SPAD, para estimar o estado nutricional de N e o rendimento de azeite em oliveiras 'Arbequina'. O experimento foi realizado em condições de campo, durante cinco ciclos consecutivos de produção, no Sul do Brasil. Os tratamentos consistiram de cinco doses de N aplicadas ao solo (0, 30, 60, 90 e 120 kg ha⁻¹ de N) e divididas em duas aplicações. Na segunda quinzena de janeiro, período padrão para diagnose nutricional, realizaram-se leituras com o medidor de clorofila SPAD-502 em 20 folhas por parcela, concomitantemente à amostragem foliar, para determinação do teor de N em laboratório. Observaram-se efeitos das doses de N aplicadas ao solo sobre o índice SPAD e o teor de N foliar, que mostraram correlação linear positiva. Houve correlação negativa entre o rendimento de azeite com o índice SPAD e o teor de N foliar. O índice SPAD pode ser utilizado em janeiro para o diagnóstico nutricional de N em oliveiras 'Arbequina', com leituras entre 88 e 130, as quais se situam na faixa de teores nutricionais considerados adequados para proporcionar crescimento vegetal e produtividade satisfatória, para fins de interpretação agrônômica.

Termos para indexação: *Olea europaea*, medidor de clorofila, análise química foliar, adubação nitrogenada, fruticultura, nutrição de plantas.

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Introduction

The evaluation of leaf macro- and micronutrient (N, P, K, Ca, Mg, B, Fe, Cu, Mn, and Zn) levels in olive trees (*Olea europaea* L.) is traditionally based on nutrient contents of mature leaves sampled from the middle portion of shoots, in January, in Rio Grande do Sul and Santa Catarina states, Brazil (CQFS – RS/SC, 2016). For the sampling, leaves should be positioned horizontally and exposed to sunlight. The sampling time (January) is when leaf nutrient levels exhibit greater stability, making it ideal for nutritional diagnosis purposes. Based on this practice, the olive fertilization program used by growers relies on leaf chemical analysis, to evaluate potential fertilization requirements (CQFS – RS/SC, 2016; Tiecher et al., 2022).

Among the macronutrients demanded in larger amounts by trees, nitrogen (N) warrants particular attention in the fertilization management. Several studies on the N application on olive cultivation, have highlighted its pivotal role in ensuring adequate tree performance and achieving satisfactory yield levels. An increased vegetative growth and oil production with N supply were observed by Haberman et al. (2019); however, excess N fertilization can heighten the tree sensitivity to frost and diseases and negatively impact the productivity, quality, and oil yield (Zipori et al., 2020). Therefore, the amount of N supplied to olive tree must adhere to precise technical recommendations, to avoid adversely affecting production.

Leaf chemical analysis to assess N requirements has its limitations, such as the time required between leaf sample collections and data acquisition (generally more than 20 days). As a result, these leaf chemical analyses can only be used to recommend fertilization in the following vegetative cycle, that is, at the beginning of next spring (eight months later) (Benati et al., 2021). Therefore, the potential for using nondestructive methods that quickly estimate leaf N content in olive trees should be investigated, to replace costly and time-consuming leaf sampling and chemical analysis. If its effectiveness is proven, this tool can have a great practical value, as it could enable the calibration of N fertilization during the current growing season and supply quickly N to the soil in the right dose.

The SPAD (soil plant analysis diagnostic), also called a chlorophyll meter, provides immediate and nondestructive reading of leaves, being an interesting

alternative for indicating levels of certain nutrients, mainly nitrogen. The potential use of chlorophyll meter for nutritional diagnosis purposes has been evaluated in several fruit tree species, in calibration studies such as on apple (Treder et al., 2016), grape (Ates & Kaya., 2021), peach (Benati et al., 2021), apricot (Khasawneh et al., 2021), and blueberry (Pinzón-Sandoval et al., 2023). For these crops, the results show strong correlations between SPAD readings with chlorophyll content, or total N content, in leaves; however, studies on the use of chlorophyll meter to estimate N content in olive leaves are still lacking. Approximately 75% of N is allocated to compounds associated with chloroplasts and chlorophyll (Evans & Clarke, 2019). The positive relationship between the SPAD index and leaf-N content can be observed in the SPAD-502 portable meter, using the quantitative determination of green intensity in leaves by measuring the light transmission at 650 nm (Ravier et al., 2017; Terassi et al., 2023).

The objective of this work was to evaluate the technical feasibility of SPAD index for diagnosing leaf-N content, as well as to determine the relationship between the leaf-N content and oil yield in 'Arbequina' olive trees.

Materials and Methods

The study was carried out in an olive farm located in the municipality of Canguçu, in the state of Rio Grande do Sul (30°94'55"S, 52°71'16"W, at 294 m altitude), in Southern Brazil. The soil is classified as Ultisols (Soil Survey Staff, 2022), which corresponds to the Argissolo Vermelho-Amarelo, according to Santos et al. (2018). In July 2018, before setting up the trial, soil samples were collected at 0-20 cm soil depth. The soil was air-dried, sieved, and prepared for chemical analysis (Table 1) (Tedesco et al., 1995).

The climate is classified as humid subtropical (Cfa), characterized by humid temperate conditions with hot summers (Alvares et al., 2013). The average meteorological data for the region shows 18°C annual temperature, 1,509 mm annual rainfall, and 78.8% air relative humidity. The monthly precipitation and average air temperature data during this trial are shown (Figure 1) (Agritempo, 2023).

The trial field area was characterized as a native grass, prior to the planting of olive trees, as it was composed of forkgrass (*Paspalum notatum*), carpetgrass (*Axonopus*

compressus), gravatá (*Eryngium horridum*) and field clover (*Desmodium incanum*). Before planting the olive nursery trees, 15.0 Mg ha⁻¹ limestone (relative total neutralizing power = 65.0%) were applied to the soil surface. Limestone was incorporated into 0-30 cm soil depth by disc plow. Subsequently, doses of 250.0 kg ha⁻¹ P₂O₅ (triple superphosphate, 42.0% P₂O₅) and 90.0 kg ha⁻¹ K₂O (K chloride, 60.0% K₂O) were applied. Afterward, fertilizers were incorporated into the 0-20 cm soil depth. Nursery trees of 'Arbequina' olive were planted in December, 2012, at 7 x 4 m spacing (357 trees ha⁻¹), with an open vase training system and without irrigation.

Nitrogen fertilization was set up in October, 2018, and treatments consisted of N doses of 0, 30, 60,

90, and 120 kg ha⁻¹. The N source was urea (45% of N). The N doses were applied annually to the soil surface, without incorporation, over an area 2.0 m wide and 4.0 m long, centered near the planting line. The N doses were split into two applications: half at full bloom (between the second half of September and first half of October); and half at stone hardening (first half of January). All the plots received equal doses of 60 kg K₂O ha⁻¹, divided into two applications carried out together with the N fertilization. Phosphate fertilization was not necessary because the soil phosphorus content was interpreted as being of “very high” grade (92.7 mg P dm⁻³ and 160.0 g clay kg⁻¹) (CQFS-RS/SC, 2016). The experimental design was a randomized complete block, with four replicates and three plants per experimental unit; sampled plants were previously selected based on visual homogeneity, and blocks were defined for uniform height, canopy, and leaf coloration, along planting rows.

Measurements of relative chlorophyll content (SPAD index readings) were carried out on the same days as the leaf sampling for chemical analysis—always in the first half of January, a period characterized by greater nutrient stability in olive plants —, in 2019, 2020, 2021, 2022, and 2023, as recommended by CQFS-RS/SC (2016). The relative leaf chlorophyll content was determined using a portable SPAD-502 Plus chlorophyll meter (Minolta, model 2900PDL), which operates by emitting two light beams through the leaf tissue: red light (~650 nm), strongly absorbed by

Table 1. Physical and chemical soil attributes at 0–20 cm soil depth, in the Ultisols soil of Fazenda Tarumã (Canguçu, RS, Brazil), in a pre-trial establishment.

Attribute	Values
Clay (g kg ⁻¹) (densimeter method)	160.0
Organic matter (g kg ⁻¹) (carbon oxidation method)	16.0
pH in water (1:1 ratio)	6.1
Available P (mg dm ⁻³) (extracted by Mehlich-1)	92.7
Exchangeable K (mg dm ⁻³) (extracted by Mehlich-1)	115.0
Available Ca (cmol _c dm ⁻³) (extracted by KCl at 1 mol L ⁻¹)	3.5
Available Mg (cmol _c dm ⁻³) (extracted by KCl at 1 mol L ⁻¹)	1.0
Exchangeable Al (cmol _c dm ⁻³) (extracted by KCl at 1 mol L ⁻¹)	0.0
CEC pH _{7.0} (cmol _c dm ⁻³)	6.7

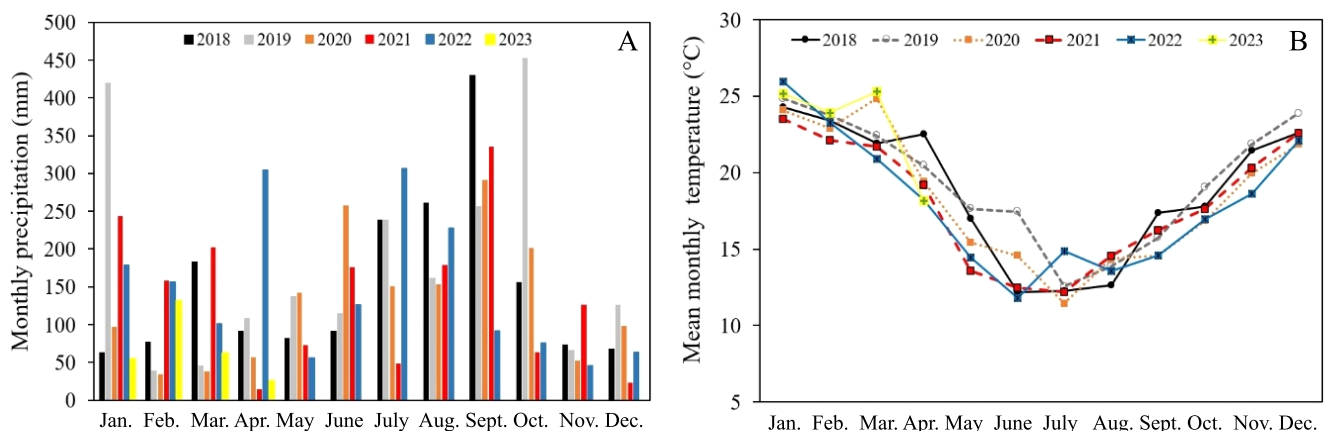


Figure 1. Monthly rainfall (A) and mean monthly air temperature (B) from January 2018 to April 2023, in the municipality of Canguçu, in the state of Rio Grande do Sul, Brazil (Agritempo, 2023).

chlorophyll; and near-infrared light (~940 nm), weakly absorbed and used as a reference. The sensor measures transmittance is performed at both wavelengths and, based on the difference of optical density, it calculates a relative index (SPAD) associated with chlorophyll content, with a precision of ± 1.0 SPAD unit within a 2 mm $\times$ 3 mm sampling area (Minolta, 1989; Benati et al., 2021).

SPAD readings were performed on the adaxial leaf surface of the median part of 20 leaves per plot, located in the middle part of the nonshaded shoots of the year. The average SPAD data were used to estimate the leaf N content.

For chemical determination of leaf N content, leaf samples (80 leaves per plot) were collected from the median portion of shoots of the year, located in the middle portion of trees. Sampled leaves were dried in an oven at 65 °C until constant mass was reached, after which they were ground. Leaf N contents were determined by the combustion method in a LECO TruSpec CHN-S Elemental Analyzer, where N was quantified (g kg⁻¹ of N) with a thermal conductivity cell.

During the olive fruit harvest (in the second half of March, when fruit were at about 30% maturity), samples of 2.0 kg of olives were collected per plot each year. Impurities and damaged fruit were handily removed. Olives were ground in a bench mill. After milling, 1.4 kg of the olive paste obtained per plot was placed in containers and malaxed for 30 min at 26°C. The olive paste was then centrifuged at 3,000 rpm for 2 min. The liquid extract was poured into a measuring cylinder, where the amount of oil was measured after 30 min of decanting (Bejaoui et al., 2017). The oil was filtered and stored in an amber container with a stopper and lid. The oil yield was calculated using the equation below:

Oil yield (%) = [(mL of oil extracted $\times$ 0.915) / mass of olive paste] $\times$ 100

The leaf-N content in 'Arbequina' olive trees –which were subjected to N rates applied to the soil surface – was estimated using the equation $y = 0.12x + 4.3933$, based on the median of each annual dataset comprising SPAD values and leaf-N content over the five years of evaluation.

The data were tested for normality by the Shapiro-Wilk test and subjected to the analysis of variance. The factors whose effects were significant by

the F-test at 5% probability were subjected to regression analysis, on order to adjust the equations. Statistical analyses were performed using the SISVAR 5.6 software (Ferreira, 2019). The relationships between the variables were obtained using the Pearson correlation coefficient, at 5% probability.

Results and Discussion

The relationship between SPAD index and leaf-N contents showed a low coefficient of determination during 2019 (R^2 0.005) and 2020 (R^2 0.163). However, in 2021 (0.709), 2022 (0.618), and 2023 (0.775), it was strong (Figure 2). The low coefficient of determination resulted in the first two years of the study could be attributed to the fertilization applied prior to the establishment of treatments, which may have masked the effect of initial N doses tested. Fruit trees, particularly those in temperate climates, have reserve structures that contribute to the supply of nutrients in subsequent cycles, during the sprouting period (Carranca et al., 2018). In a study on some N doses in peach trees, Benati et al. (2021) also obtained a low correlation between SPAD index and leaf-N content during the first year of evaluation. However, in the following vegetative cycles, fruit production and pruning depleted the nutrient reserves in control trees (not fertilized with N).

In the present work, it is highlighted that, during the 2020 production cycle, there was an alternation of olive production, possibly resulting from adverse weather conditions, such as high rainfall in October, during the flowering period (Figure 1). These conditions may have delayed the effect of treatments (N doses applied to the soil), since there was very low export of nutrients, especially N, through the olive harvest.

The results obtained from the 2021 production cycle indicate a positive relationship between the SPAD index and the increase in leaf-N contents. They also evidence the technical feasibility and increased reliability of using SPAD readings to estimate the nutritional status of N in 'Arbequina' olive trees. This condition has been also reported in experiments with N rates applied to the soil surface for pecan (Hardin et al., 2012), apple trees (Treder et al., 2016) and peach trees (Benati et al., 2021).

Leaf-N content and SPAD index increased linearly with the increase of N doses applied to the soil surface

(Figure 3). These results reflect what is reported in the literature, that 50% to 75% of the N present in the leaf is part of the chlorophyll molecule (Ravier et al., 2017;

Taiz et al., 2017; Dunn et al., 2018). Therefore, the SPAD index is well correlated with the leaf-N content increase.

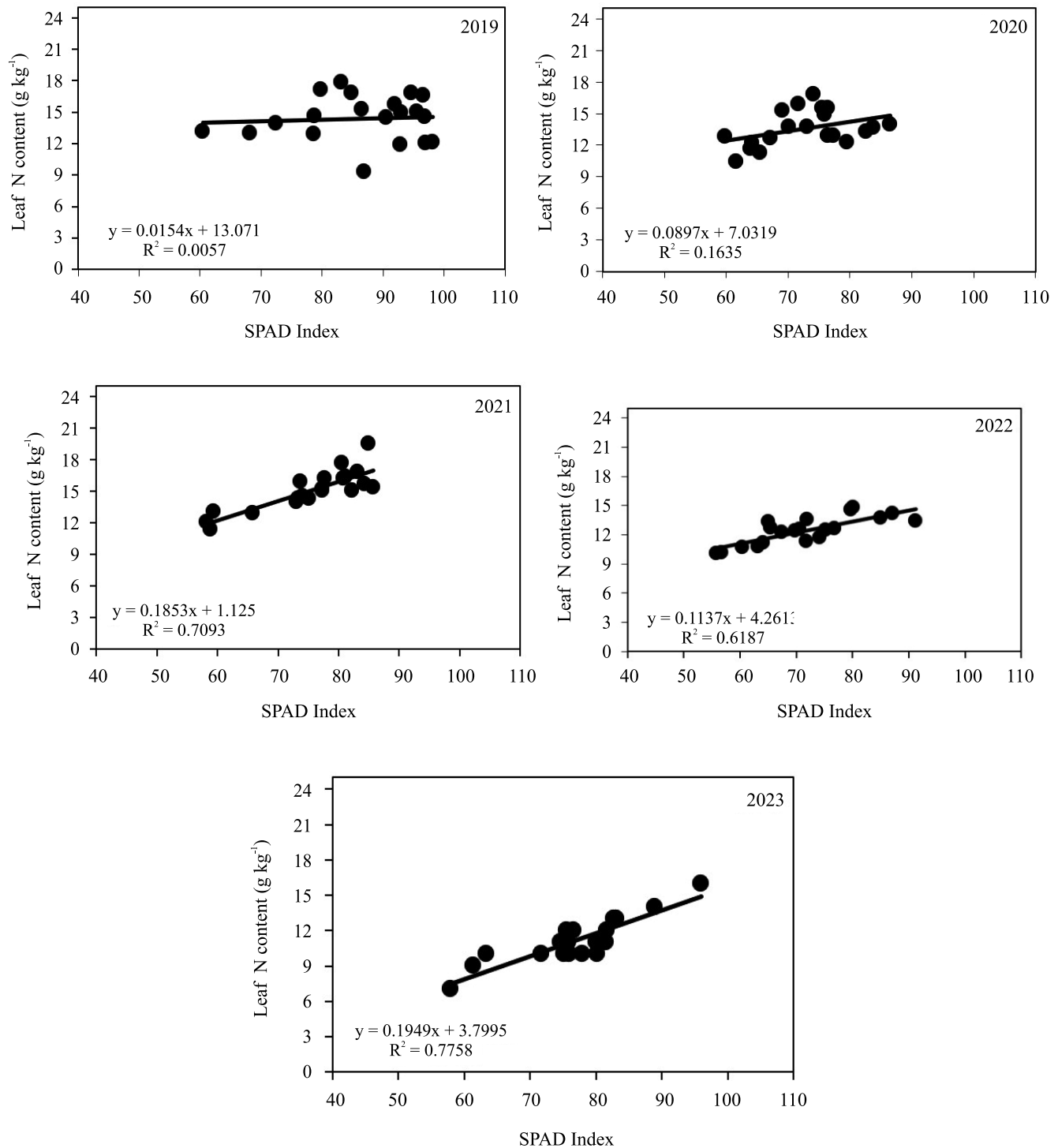


Figure 2. Relationship between leaf-N content and SPAD readings in 'Arbequina' olive (*Olea europaea*), in the years 2019, 2020, 2021, 2022, and 2023 of evaluation, at 5% probability.

The SPAD can help improve nitrogen fertilization program in olive trees because data are instantaneous and nondestructive, eliminating the need to collect leaf samples, send them to the laboratory for chemical analysis and interpret results. According to the recommendations for the states of Rio Grande do Sul

and Santa Catarina (CQFS-RS/SC, 2016), adequate leaf-N levels for olive trees range from 15 to 20 g kg⁻¹, which correspond to SPAD values between 88 and 130 (Table 2). SPAD values greater than 130 indicate an excess leaf-N content, therefore it is not necessary to apply nitrogen. However, for SPAD values lower than

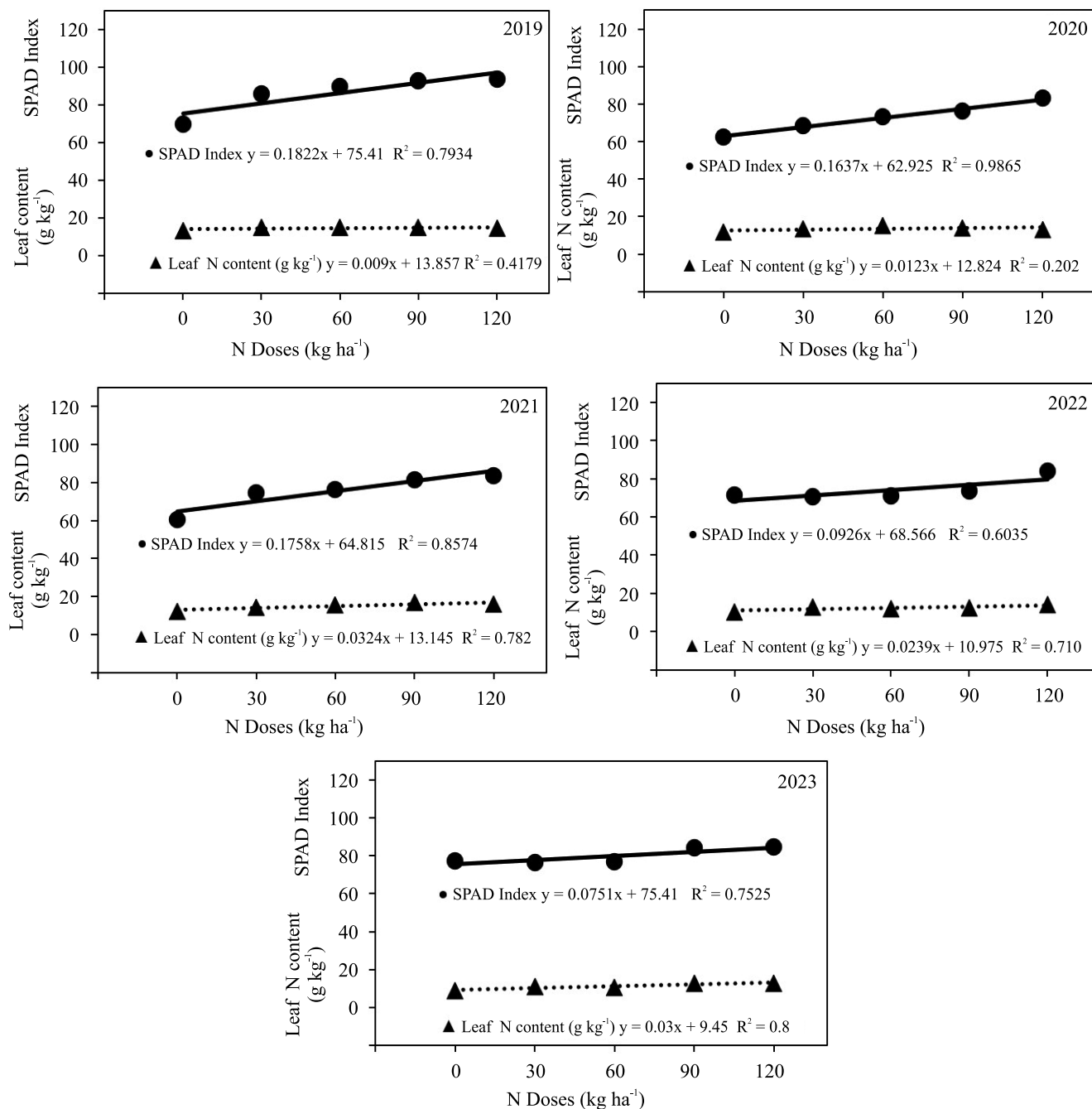


Figure 3. Estimation of leaf-N content and SPAD index in 'Arbequina' olive (*Olea europaea*), as a function of nitrogen doses applied to the soil surface, during the years 2019, 2020, 2021, 2022 and 2023, at 5% probability.

88, it is necessary to increase the N rates to meet the nutritional requirements of the crop. In a study on nitrogen fertilization in Spain, Fernández-Escobar (2016) suggests levels from 13.0 to 17.0 g kg⁻¹ as the N sufficient range for olive trees, while Erel et al. (2013), in a work conducted in Israel, propose from 16.0 to 18.0 g N kg⁻¹. Due to soil and climatic variations affecting tree nutrition, calibrating the SPAD index for temperate fruit species requires multiyear evaluations. The present study followed this approach, providing more accurate data for nutritional diagnosis across diverse edaphoclimatic conditions.

Even though, over the five years investigated, higher doses of N applied to the soil resulted in higher leaf-N contents, and the differences in slopes of lines between the years show that leaf-N concentration also depends on other practices besides soil fertilization,

such as tree pruning, climatic conditions (rainfall distribution and air temperatures) and fruit set (Zhang et al., 2020). Although the use of chlorophyll meter cannot accurately indicate the amount of N to be applied in years of low fruiting, it can help to identify the nutritional status of N in olive grove and avoid possible high and unnecessary rates of nitrogen fertilization. Studies related to the application of N in olive trees reported that this nutrient excess can lead to problems during the production cycle, such as increased susceptibility to fungal diseases (Roca et al., 2018), vulnerability to frost, delayed fruit ripening, reduced oil quality, and intensification of production alternation (Fernández-Escobar et al., 2019).

Oil yield ranged from 3.5% to 13.2%, and exhibited a reduction with increasing SPAD readings, as well as N content in the leaves (Figure 4). This adverse

Table 2. Estimation of leaf-N content in 'Arbequina' olive (*Olea europaea*) trees subjected to nitrogen doses applied to the soil surface for five years, using the equation $y = 0.12x + 4.3933$.

SPAD Index	Leaf-N content (g kg ⁻¹)	SPAD index	Leaf-N content (g kg ⁻¹)	SPAD index	Leaf-N content (g kg ⁻¹)
47	10.0	75	13.4	103	16.8
48	10.2	76	13.5	104	16.9
49	10.3	77	13.6	105	17.0
50	10.4	78	13.8	106	17.1
51	10.5	79	13.9	107	17.2
52	10.6	80	14.0	108	17.4
53	10.8	81	14.1	109	17.5
54	10.9	82	14.2	110	17.6
55	11.0	83	14.4	111	17.7
56	11.1	84	14.5	112	17.8
57	11.2	85	14.6	113	18.0
58	11.4	86	14.7	114	18.1
59	11.5	87	14.8	115	18.2
60	11.6	88	15.0	116	18.3
61	11.7	89	15.1	117	18.4
62	11.8	90	15.2	118	18.6
63	12.0	91	15.3	119	18.7
64	12.1	92	15.4	120	18.8
65	12.2	93	15.6	121	18.9
66	12.3	94	15.7	122	19.0
67	12.4	95	15.8	123	19.2
68	12.6	96	15.9	124	19.3
69	12.7	97	16.0	125	19.4
70	12.8	98	16.2	126	19.5
71	12.9	99	16.3	127	19.6
72	13.0	100	16.4	128	19.8
73	13.2	101	16.5	129	19.9
74	13.3	102	16.6	130	20.0

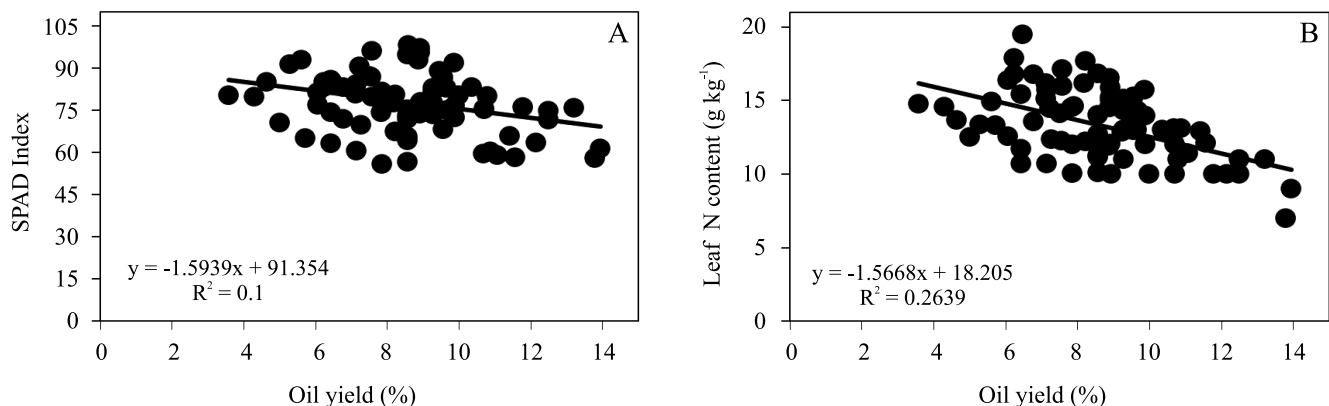


Figure 4. Relationship between SPAD readings and oil yield (A), and leaf-N content and oil yield (B) in 'Arbequina' olive (*Olea europaea*) (averages of 2019, 2021, 2022, and 2023), at 5% probability.

relationship may result from excess vigor and a low carbon/nitrogen (C/N) ratio in reproductive structures, which can also reduce ovule longevity during the flowering period (Haberman et al., 2019). The high doses of N used in the present work may have reached harmful N levels to the plants. Although the mechanism of plant reaction to excess N is unclear, it is possible that the negative effects are partly a result of excessive carbohydrate consumption by the roots for N assimilation and translocation, causing energy limitations (Haberman et al., 2019). Therefore, these results indicate that N fertilization should be carried out according to the actual needs of the olive grove, seeking a balance between plant nutrition and oil production.

Conclusions

1. In 'Arbequina' olive (*Olea europaea*) trees, the SPAD index increases with both the increase of leaf-N content and of nitrogen doses applied to the soil surface.

2. The SPAD index proved to be technically feasible for diagnosing the N status of 'Arbequina' olive trees, and it enables adjustments to nitrogen fertilization within the same production cycle.

3. SPAD readings between 88 and 130, which are obtained during the standard season for leaf sampling (January), indicate that leaf-N content in 'Arbequina' olive trees in the “normal sufficiency” grade.

4. Oil yield showed a negative correlation with increasing SPAD readings as well as foliar N content.

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