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Environmental Factors and Their Relationship with Nitrogen Compounds in Walnut Tree (*Carya illinoensis*)

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ABSTRACT

Total Soluble Proteins (TSP) and Total Amino Acids (TAA) help to tolerate adverse conditions in fruit trees. The objective of this research was to determine the influence of temperature and irradiation on the concentration of TSP and TAA in *Carya illinoensis* trees. Pearson's correlation and multiple regression were made using mean temperature (°C) and irradiation (MJ·m⁻²) data. The concentration of the TSP related to the temperature and irradiation in root, two correlation models were obtained, one for WN cultivar ($F = 3.969$, $df = 2.9$, $P > 0.058$, $R^2 = 0.469$) and another one for the WA cultivar ($F = 1.559$, $df = 2.9$, $P > 0.145$, $R = 0.507$) where it was observed that the varieties have the same behavior in root for TSP. However, in the stem, the WA cultivar ($R = 7.31$, $gl = 2.9$, $P < 0.013$, $R = 0.788$) presented a greater mobility of TSP. In the case of WN cultivar, a lower mobility of TSP was observed ($F = 2.407$, $gl = 2.9$, $P > 0.145$, $R = 5.90$).

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ARTICLE INFO

Received: 20 May 2025 | Revised: 5 June 2025 | Accepted: 9 June 2025 | Published Online: 9 October 2025
DOI: <https://doi.org/10.30564/re.v7i4.10106>

CITATION

García-Ramírez, J.N., Carrillo-Moreno, D.I., Valenzuela-Núñez, L.M., et al., 2025. Environmental Factors and Their Relationship with Nitrogen Compounds in Walnut Tree (*Carya illinoensis*). Research in Ecology. 7(4): 203–213. DOI: <https://doi.org/10.30564/re.v7i4.10106>

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In TAA, significant differences were observed in both cultivars: in WN cultivar, the correlation model was observed that at lower temperature and irradiation ($F = 12.988$, $gl = 2.9$, $P < 0.002$, $R = 0.862$), the concentration of TAA increases. In WA cultivar, it was found ($F = 17.481$, $gl = 2.9$, $P < 0.01$, $R = 0.892$) that lower temperature and irradiation decrease the concentration of TAA. The concentration of TAA in stem was significant in WN cultivar ($F = 6040$, $df = 2.9$, $P < 0.022$, $R = 0.757$) but not in WA cultivar ($F = 2,602$, $df = 2.9$, $P > 0.128$, $R = 0.605$). WN cultivar is the best adapted to climatic conditions in the region, due to its capacity to store an important reserve of nitrogen compounds.

Keywords: Vegetative Reserves; Environmental Factors; *Carya illinoensis*; N Cycle

1. Introduction

American pecan walnut (*Carya illinoensis* K. Koch) is a deciduous fruit tree growing in the north of Mexico and in the south of the United States, both countries are the main producers worldwide ^[1]. In Mexico, the main producing states are Chihuahua, Coahuila, Nuevo León, Durango and Sonora ^[2,3]. The pecan walnut is one of the most important fruit tree species. This species is adapted to the meteorological conditions that predominate in the north of the country where it grows at an average temperature of 25.3 to 26.7 °C. During winter it tolerates temperatures from 7.2 to 12.3 °C, these conditions are optimal for good physiological development throughout the production cycle ^[4].

Climate change is one of the most important factors in fruit development, with drastic changes in environmental factors such as temperature, irradiation, relative humidity and others ^[5]. High temperatures on fruit trees have a direct effect and may generate physiological disorders such as pollination problems, fruit setting, early fruit losses, poor quality, as well as metabolic disorders that lead to energy loss in the trees ^[6,7].

On the other hand, irradiation is an important factor in fruit trees; high irradiation levels reduce the photosynthetic rate, which causes a reduction in tree biomass, deficiency and reduction in the transport and assimilation of nutrients in plant organs ^[8]. In order to face abrupt changes in agro-meteorological conditions, trees have developed defence mechanisms involving vegetative reserves, which are defined as the elements that accumulate during a period of abundance that will be available in unfavourable times and that will help mitigate them.

A detailed study of the effects of solar radiation and temperature on fruit trees allows for better selection of varieties adapted to local climatic conditions, as well as ap-

propriate pruning management to optimize sunlight exposure and temperature. These factors influence the yield and quality of fruit trees; proper management of these factors can help optimize their growth and production. Solar radiation and temperature play a crucial role in the production of TSP and TAA, as they directly influence both photosynthesis and nutrient uptake at the root level.

Within the vegetative reserves are non-structural carbohydrates as well as nitrogenous compounds that include Total Soluble Proteins (TSP) and Total Amino Acids (TAA) and other compounds ^[9]. Amino acids are considered an important source of nitrogen; these elements have the ability to mobilize both organic and inorganic nitrogen ^[10]. In the case of TSP, these are formed from the synthesis of amino acids, one of the main functions is nitrogen storage as a reserve source, which will be available during the regrowth period ^[11,12]. These reserves will be available in unfavourable times according to the seasonality of the year and the phenology of the tree ^[10]. The objective of this work was to evaluate the impact of temperature and irradiation on the concentration of TSP and TAA in the root and stem of *C. illinoensis* on the Western (WN) and Wichita (WA) cultivars.

2. Materials and Methods

2.1. Site

The study was developed in an experimental orchard located at the Universidad Autónoma Agraria Antonio Narro Unidad Laguna in Torreón, Mexico (25° 33' 22.63" N and 103° 22' 07.77" W). Trees in the orchard are arranged in a royal frame design. The trees' average age is 40 years. Tree density is 100 trees ha⁻¹ in both Western and Wichita varieties. An annual irrigation depth (748 mm) is provided

with eight irrigations (intervals from 12 to 47 days, according to the development stage).

2.2. Sampling

Sampling was carried out in a systematic way; in order to avoid the edge effect, four trees were selected from the middle part of the orchard. Two samples of both, root and stem were extracted from each tree. The root sample was collected after digging a trench at a depth of 40 cm to locate the main root using a knife. Stem samples were taken from carrots at a height of 1.30 m. using a Pressler drill (Haglöf® Langsele, Sweden). Samples were rinsed and placed in aluminum bags; after that, they were frozen with liquid nitrogen to avoid biochemical processes in the tissues. Samples were transported to the laboratory, and stored in an ultra-freezer (Revco Value Plus® Thermo Scientific® Waltham, United States) at -70°C during seven days. Samples were then freeze-dried for one week at -40°C (Labconco Freezone Triad® Freeze Dry Systems® USA Lyophilizer). The freeze-dried samples were pulverized with a knife mill (Fritsch® Pulverisette 15®) to obtain a fine powder.

2.3. Extraction and Quantification of Total Soluble Proteins (TSP)

The concentration of TSP was determined using the Bradford methodology [13]. First, 10 mg of dry matter were weighed on an analytical balance (PW 250 Adam® Oxford, United States) and placed in 2 ml Eppendorf® microtubes (MCT-200-C ClearAxygen Scientific® Schwerte, Germany). A solution protein extraction was made (0.1 M KH_2PO_4 , 0.1 M NaHPO_4 and 13 mg PVP). Subsequently, 1 mL of this buffer was placed in the microtubes; then a steel ball was placed in each of the microtubes to shake them in a vortex (Maxi Mix II® Thermo Scientific®) during 10 min. Microtubes were centrifuged in a Spectrafuge 16M R Labnet® centrifuge at 10,000 rpm at 4°C during 15 min. After that, 500 μL were taken and placed in cuvettes with 500 μL of Quickstart® Bradford® solution, shaken and placed for 5 min at room temperature. The reading was made at an absorbance of 595 nm in a UV-Visible spectrophotometer

(Genesys 20® Thermo Scientific®), using Bovine Serum Albumine (BSA) as a standard.

2.4. Extraction and Quantification of Total Amino Acids (TAA)

The concentration of TAA was determined using the methodology of Yemm and Cocking [14]. First, 10 mg of dry matter was weighed on an analytical balance (PW 250 Adam® Oxford, United States) and placed in 2 ml Eppendorf® microtubes (MCT-200-C ClearAxygen Scientific® Schwerte, Germany). Then, 650 μL of a buffer extraction solution (ethanol/water 70/30) was added and leaved for 5 min in a cooler at 4°C . The microtubes were centrifuged in a Spectrafuge 16M R Labnet® centrifuge at 10,000 rpm at 4°C during 5 min, and the supernatant was transferred in a clean microtube (MCT-200-C ClearAxygen Scientific® Schwerte, Germany), avoiding the sediment. Subsequently, 650 μL of the extraction solution was added to the remaining sediment and the process was repeated twice. The three extractions were mixed in a new microtube. 800 μL were extracted and mixed with 200 μL of ninhydrin solution (2 g of ninhydrin in 1 L of ethanol). The microtubes were boiled during 5 min at 100°C , after that were placed at room temperature for 5 min. Reading was made at 570 nm in a UV-Visible spectrophotometer (Genesys 20® Thermo Scientific®), using leucine as a standard.

2.5. Environmental Factors

The data of mean temperature ($^{\circ}\text{C}$) and irradiation (MJ m^{-2}) were obtained from the INIFAP La Laguna Experimental Field meteorological station. Solar irradiation units and conversion factors for plants were obtained from the International System of Units and models performed at INRA (National Institute of Agricultural Research) according to Bonhomme [15].

2.6. Statistical Analysis

A Pearson correlation analysis and multiple regression were made to determine the relationship between the concentration of TSP and TA in root and stem in relation to environmental factors (temperature and irradiation). A sig-

nificance level of ($P \leq 0.05$) was considered and the analysis was performed using the SPSS® statistical software Version 18.0® Trial Version.

3. Results

3.1. Concentration of TSP in Relation to Environmental Factors in the Western (WN) Cultivar in Root and Stem

The multiple correlation between the concentrations of total soluble proteins and environmental factors in the root of the WN cultivar was not significant ($R = 0.685$, $P > 0.058$, **Figure 1**). A better fit was sought according to R^2 , but it was not significant ($F = 3.969$ df = 2.9, $P > 0.058$; R^2

= 0.469). The resulting equation was:

$$\text{TSP Concentration} = 0.706 (\text{Temperature}) - 0.086 (\text{Irradiation}) + 51.083 \quad (1)$$

In the stem of the WN cultivar the value of the multiple correlation between environmental factors and the concentration of TSP was not significant where a ($R = 0.590$, $P > 0.145$, **Figure 2**) was obtained. The R^2 was calculated to see the adjustment of the correlation, where no significant results were found ($F = 2.407$; d.f. = 2.9, $P > 0.145$; $R^2 = 0.349$). There was no violation of the assumptions of normality of the residuals. The resulting regression equation was:

$$\text{TSP Concentration} = -0.406 (\text{Temperature}) + 0.801 (\text{Irradiation}) + 60.977 \quad (2)$$

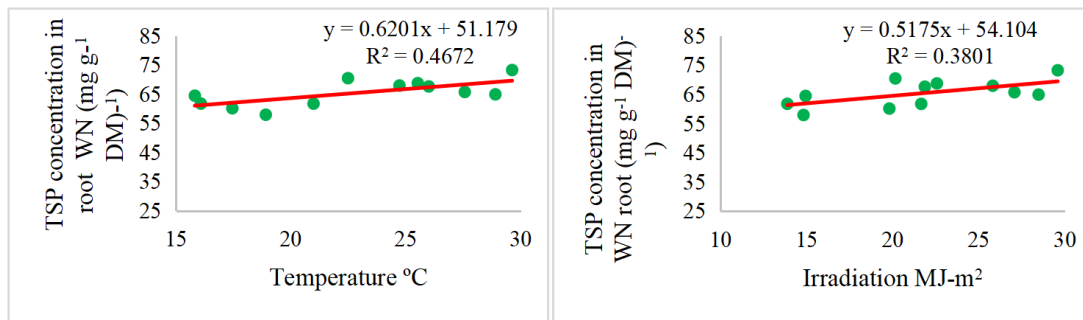


Figure 1. Relationship Between the TSP Concentration in Relation to Temperature and Irradiation on the Root of *Carya illinoensis* of the Western (WN) Cultivar. DM: Dry Matter.

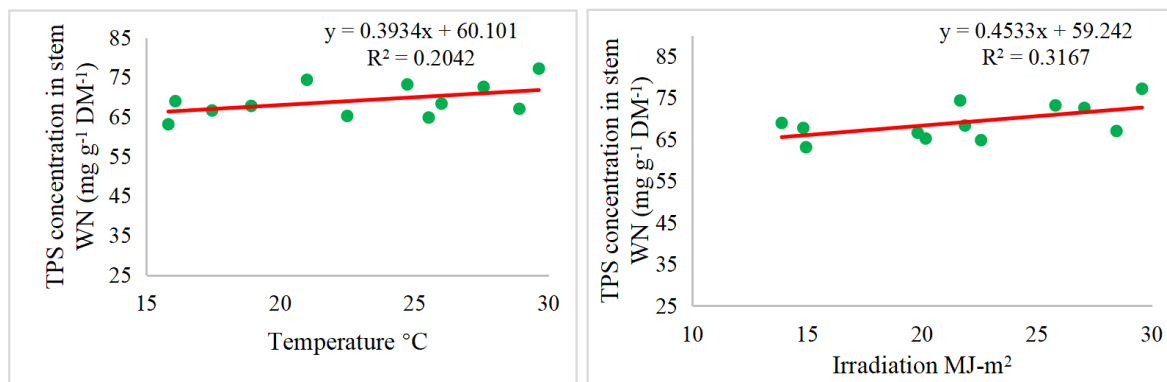


Figure 2. Relationship Between the Concentration of TSP in Relation to Temperature and Irradiation in the Stem of *Carya illinoensis* in the Western Cultivar. DM: Dry Matter.

3.2. Correlation and Regression Analysis of TSP in Relation to Environmental Factors in the Wichita (WA) Cultivar in Root and Stem

The correlation of temperature and irradiation with

the concentration of TSP in the root of the WA cultivar, no significant differences were found ($R = 0.507$, $P > 0.262$, **Figure 3**).

A better fit was sought based on R^2 where no significant differences were found ($F = 1.559$ df = 2.9, $P > 0.507$;

$R^2 = 0.257$). There was no violation of the assumptions of normality of the residuals. The regression equation was:

$$\text{TSP Concentration} = -1.566 (\text{Temperature}) + 1.446 (\text{Irradiation}) + 66.282 \quad (3)$$

In irradiation related to the content of TSP in the stem

of the WA cultivar was significant ($R = 0.788$, $P < 0.013$, **Figure 4**). Multiple linear regression analysis showed a significant relationship between TSP concentrations in the root and environmental factors ($F = 7.361$, $df = 2.9$, $P < 0.013$, $R^2 = 0.621$).

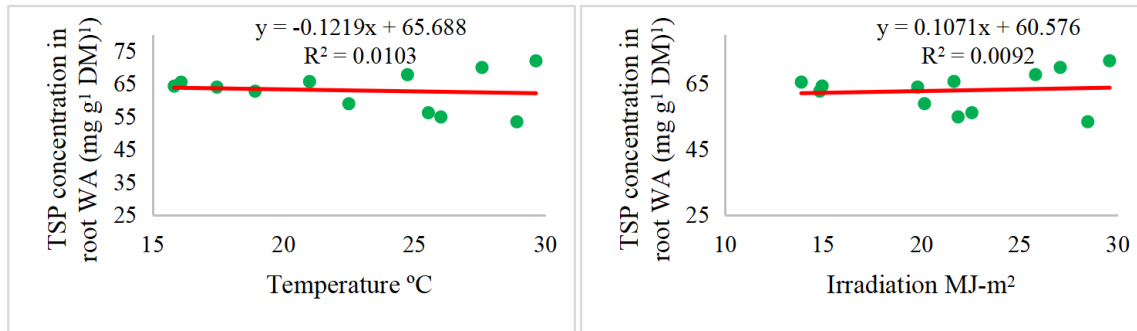


Figure 3. Relationship Between the Concentration of TSP in Relation to Temperature and Irradiation in the Root of *Carya illinoensis* Cultivar Wichita. DM: Dry Matter.

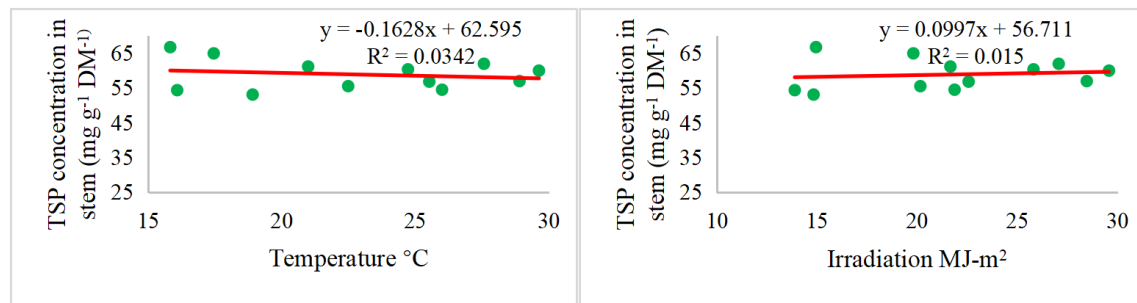


Figure 4. Relationship Between the TSP Concentration and Temperature and Irradiation in the Stem of *Carya illinoensis*, Wichita Cultivar. DM: Dry Matter.

There was no violation of the normality assumptions for the residuals. The following equation was obtained:

$$\text{TSP Concentration} = -1.790 (\text{Temperature}) + 1.630 (\text{Irradiation}) + 64.377 \quad (4)$$

The $R = 0.788$ means that 78.8% of the variability in TSP concentration must be explained by the temperature and irradiation in WA cultivar. The remaining 21.2% must be explained by other factors not included in this study (**Figure 4**).

3.3. Correlation and Regression Analysis of Total Amino Acids in Relation to Environmental Factors in the Western Cultivar in Root and Stem

The correlation of temperature and irradiation in re-

lation to the TAA concentration in the root of the WN cultivar was significant ($R = 0.862$, $P < 0.002$). Multiple linear regression analysis indicated a significant relationship between TAA concentrations in the root and environmental factors ($F = 12.988$, $df = 2.9$, $P < 0.002$, $R^2 = 0.743$).

The resulting equation was:

$$\text{TAA Concentration} = -8.712 (\text{Temperature}) + 3.967 (\text{Irradiation}) + 232.710 \quad (5)$$

The $R = 0.862$ indicates that 82.7% of the variability in the concentration of TAA can be explained by the temperature and irradiation. The remaining 18.3% must be explained by other factors not included this research (**Figure 5**).

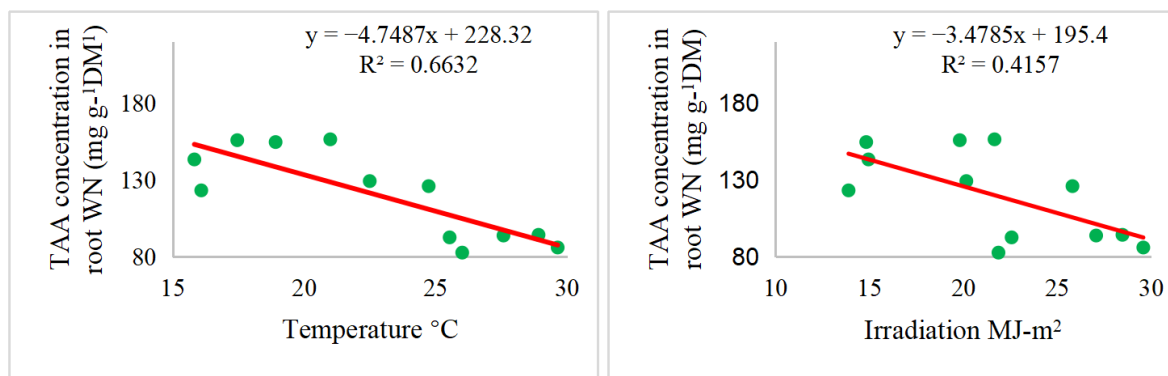


Figure 5. Relationship Between Total Amino Acid (TAA) Concentration and Temperature and Irradiation in the Root of *Carya illinoensis*, WN Cultivar. DM: Dry Matter.

The multiple correlation between environmental factors and the concentration of TAA in the stem of the WN cultivar was significant ($R = 0.757$, $P < 0.022$). Multiple linear regression analysis showed a significant

relationship between TAA concentrations in the root and environmental factors ($F = 6.040$, $df = 2.9$, $P < 0.022$, $R^2 = 0.73$) (**Figure 6**). The following equation was obtained:

$$TAA \text{ Concentration} = -2.888 (\text{Temperature}) + 3.495 (\text{Irradiation}) + 33.272 \quad (6)$$

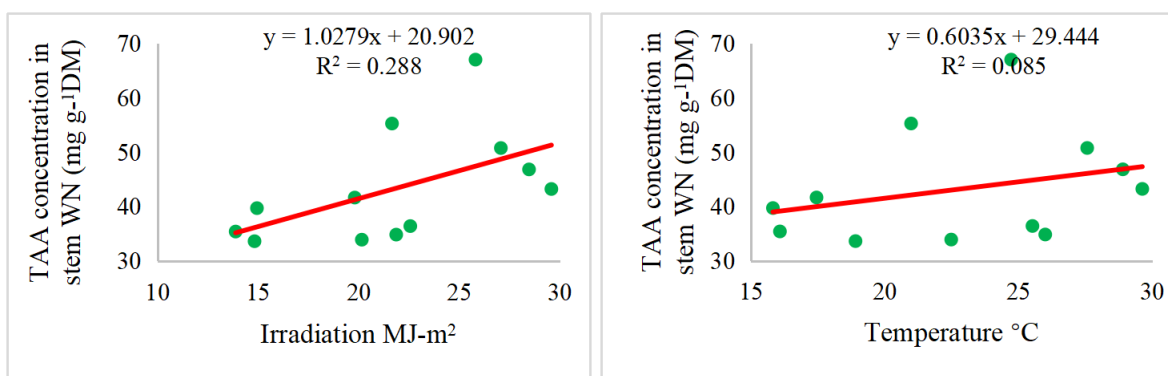


Figure 6. Relationship Between Total Amino Acid Concentration and Temperature and Irradiation in the Stem of *Carya illinoensis*, Western Variety. DM: Dry Matter.

The $R = 0.757$ indicates that 75.7% of the variability in the TAA concentration must be explained by the temperature and irradiation present in the culture. The remaining 24.3% must be explained by other factors not included in this study.

3.4. Correlation and Regression Analysis of TAA in Relation to Environmental Factors in the WA Cultivar in Root and Stem

The correlation of temperature and irradiation related to TAA concentration in the root of WA cultivar was significant ($R = 0.892$, $P < 0.001$). Multiple linear regression

indicates a significant relationship between the TAA concentrations in the root with temperature and irradiation ($F = 17.481$, $df = 2.9$, $P < 0.001$, $R^2 = 0.795$)

The following equation was obtained:

$$TAA \text{ Concentration} = 1.247 (\text{Temperature}) + 4.796 (\text{Irradiation}) + 26.798 \quad (7)$$

The $R = 0.892$ indicates that 89.2% of the variability in TAA concentration may be influenced by the temperature and irradiation in the experimental orchard. The remaining 10.8% must be explained by other factors not included in this study (**Figure 7**).

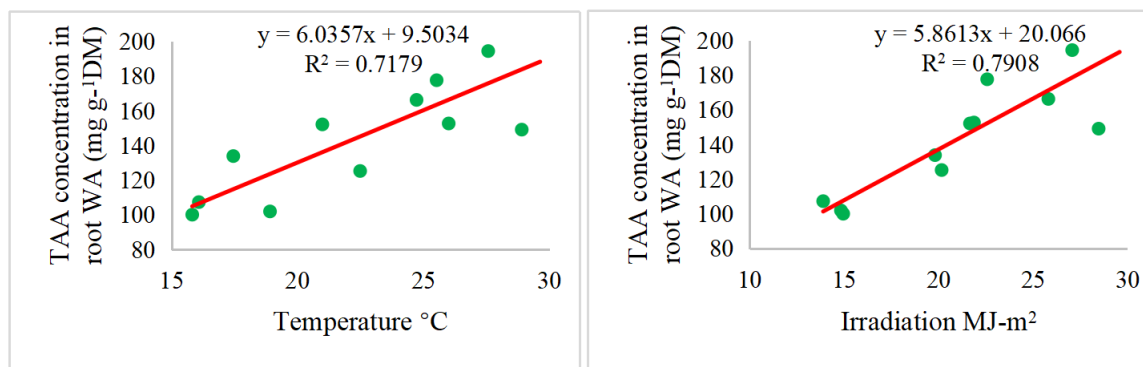


Figure 7. Relationship Between Total Amino Acid Concentration and Temperature and Irradiation in the Stem of *Carya illinoensis*, Wichita Variety. DM: Dry Matter.

The correlation of temperature and irradiation with the TAA concentration in the stem of the WA cultivar was not significant ($R = 0.605$, $P = 0.128$), so a better fit was sought

based on R^2 ($F = 2.602$, $gl = 2.9$, $P = 0.128$, $R^2 = 0.366$) where it was not significant in the relationship between environmental factors and TAA concentration (**Figure 8**).

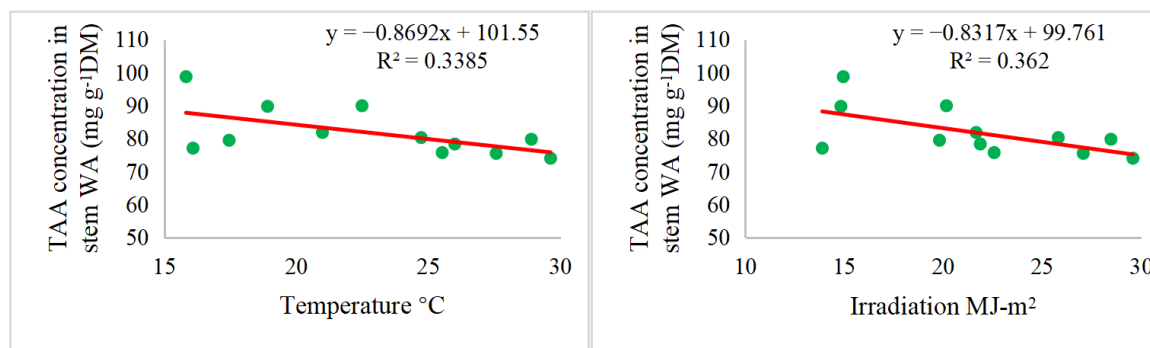


Figure 8. Relationship Between TAA Concentration and Temperature and Irradiation in the Stem of *Carya illinoensis*, Wichita Cultivar. DM: Dry Matter.

The following equation was obtained:

$$\text{TAA Concentration} = -0.265 (\text{Temperature}) - 0.605 (\text{Irradiation}) + 100 \quad (8)$$

4. Discussion

Agroclimatic conditions such as temperature and irradiation stimulate the physiological development of fruit trees, such as walnut trees [16]. These conditions stimulate the accumulation of nitrogenous compounds such as TSP and TAA. Proteins accumulate in vegetative tissues as well as in reproductive tissues [17], mainly in the root being one of the organs with the highest demand for proteins [18].

On the other hand, studies have found that citrus trees (*Poncirus trifoliata*) have a higher demand for protein when the temperature ranges from 30 to 21 °C [19]. It has been reported that (*Ficus carica* L.) trees have greater

activity in protein mobilization during April when a temperature of 29 °C is reached [20]. Eris et al. observed that in young trees of *Olea europaea* L. a higher concentration of proteins was observed when low temperatures were present [21], which does not coincide with this research. This is attributed to the fact that woody species adapted to temperate or cold climates tend to reach their maximum concentration during the winter [22].

Protein accumulation has been reported during the winter in *Populus x canadensis* trees, which show a gradual decrease during the early spring, where this decrease is in turn attributed to the increase in ambient temperature [23]. In *Quercus petraea* trees, proteins are stored during the

dormant period, after that, will decrease with the beginning of the budding stage and which represent an important source of nitrogen^[24].

The stem is considered the largest organ where its main function is the transport of nutrients from the root to the upper organs^[12], we observed that there is a close correlation between temperature and stem for protein mobilization if temperature increases, the protein mobilization arises^[25].

TAA are located in an assimilable form in tree roots^[26]. The greatest mobilization of amino acids occurs through the stem through the xylem to the upper organs and act as an important source of nitrogen reserves which will be available for the formation of new tissues^[27,28]. That is why this demand for amino acids is related to the beginning of spring and is also correlated with the increase in temperature and the demand for amino acids as seen in this research^[29,30].

Several studies have reported that nitrogen fertilization favours the availability of amino acids of up to 30% during the spring stage^[31]. On the other hand, Millard et al. reports that the greatest mobilization of amino acids in deciduous trees occurs at the sprouting stage where there is a greater demand for the formation of new tissues^[32]. This process is carried out through the xylem. This coincides with Näsholm and Ericsson who reported that in *Pinus sylvestris* trees during spring shows a greater mobilization of amino acids towards the upper organs^[33]. This agrees with Alaudinova and Mironov who reported that this mobilization of amino acids is attributed to the swelling of the buds themselves that are demanding nutrients^[34].

The concentration of TAA and TSP in relation to irradiation is one of the most important factors in fruit growing. Irradiation is the fraction of light absorbed by the trees that will be transformed into energy through photosynthesis^[35]. It has been reported that walnut seedlings show an optimal vegetative development with photosynthetically active radiation PAR of 1011 to 1181 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ^[36], in addition, it has been reported that apple trees cv Fuji tend to develop at a photosynthetically active radiation of 1107 to 1170 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ^[37]. Results in our research agree with Glenn and Yuri who reported that apple trees cv Braeburn have an optimal development at an irradiation of 700 to 1100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ^[38]. On the other hand, it has been re-

ported that in apple trees there is a close relationship between photosynthetically active irradiation with CO_2 , this will depend on the seasonality of the year as well as the irradiation assimilated by the leaves of the trees^[39].

In this research it was observed that the TSP concentration in relation to temperature and irradiation in the root of the Western and Wichita cultivars showed the same behaviour which represents that a higher the temperature and irradiation, increases the TSP concentration in the root. Otherwise, it was observed in the stem of the WA cultivar showed a greater mobility of TSP than in WN cultivar, where a lower mobility of proteins was observed.

In the case of the concentration of TAA in relation to environmental factors, significant differences were found in both varieties but in an inverse behaviour, where WN cultivar showed a higher concentration at a low temperature and irradiation the concentration of TAA in the walnut root will be higher, but as it begins to increase it will gradually decrease. In WA it was observed that at a lower both temperature and irradiation the TAA concentration in the root will be lower but as the temperature begins to increase the TAA concentrations increase too.

In the case of the TAA concentration in relation to environmental factors, significant differences were found in both cultivars showing an inverse behaviour. WN cultivar showed that at a lower temperature and irradiation the TAA concentration in the root increases, but as temperature and irradiation increases, TAA concentration gradually decreases. In WA it was found that at a lower temperature and irradiation the TAA concentration in the root will be lower, but as the temperature increases the concentrations of amino acids increases too.

On the other hand, in the TAA concentration in the stem, significant differences were observed in WN cultivar whereas in WA non-differences were observed.

An inverse behaviour was observed in cultivars where WN tends to have a lower concentration at a lower temperature and WA cultivar at a lower temperature the TAA concentration in the stem is higher.

5. Conclusions

Nitrogen compounds are a group where TSP and TAA can be found. In this study, it was found that both

elements have completely inverse behaviour depending on the cultivar.

WN is the cultivar best adapted to the climatic conditions, and we also recommend addressing more studies emphasizing environmental factors not analysed in this research, contrasting the behaviour of TSP and TAA in young trees.

It is also important to address the behaviour of nitrogen compounds in walnut trees in comparison with other regions of the country.

Author Contributions

Conceptualization, L.M.V.-N. and D.I.C.-M.; methodology, L.M.V.-N., D.I.C.-M. and V.C.-V.; software, E.A.B.-C.; validation, R.L.-S., F.A.R. and V.C.R.; formal analysis, D.I.C.-M., L.M.V.-N. and V.C.-V.; investigation, J.N.G.-R. and E.A.B.-C.; resources, D.I.C.-M. and V.C.-V.; data curation, J.N.G.-R.; writing—original draft preparation, L.M.V.-N.; writing—review and editing, E.A.B.-C., V.C.-V. and D.I.C.-M.; visualization, R.L.-S.; supervision, L.M.V.-N., D.I.C.-M. and V.C.-V.; project administration, D.I.C.-M. and V.C.-V.; funding acquisition, D.I.C.-M. All authors have read and agreed to the published version of the manuscript.

Funding

This work received no external funding.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.

Data Availability Statement

Data are unavailable due to privacy or ethical restrictions.

Acknowledgments

The authors wish to express their gratefulness to the National Council for Humanities, Science, and Technology (CONACYT) for financial supporting for José Nicolás García Ramírez's master's studies; to Dr. Ángel Lagarda Murrieta for allowing the plant sampling used for the study to be extracted from the experimental garden at the Antonio Narro Autonomous Agrarian University.

Conflicts of Interest

The authors declare no conflict of interest.

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