Effect of late Nitrogen Fertilization on nutritional status, productivity and quality of pecan (*Carya illinoinensis* Wangenh. C. Koch.)

[redacted]

Running Title: Late N fertilization of pecan

Abstract

A two-year, field study to determine the impact of late Nitrogen (N) fertilization on the nutritional status, productivity and quality of 8-year-old pecan tress was carried out. Furthermore, as potential indicator or the nutritional status of the tree, leaf greenness was measured non-destructively with a SPAD 502 Minolta on a regular basis. N was applied to the soil as ammonium sulfate (20.5% N) at increasing rates (kg ha^{-1}), namely: T1=80, T2=120, T3=160, T4 = 80+40*, T5=120+40* and T6=160+40* (*late doses). The results show that application of this macronutrient at different rates and timing (late fertilization) has an effect on nut quality and foliar nitrate (NO_3^-) content; the highest but not significant production values being reached on late N treatments. Leaf N and chlorophyll concentrations showed a positive response to seasonal fertilization during the second year of the study. The seasonal dynamics of N indicated an inverse correlation between total N and NO_3^- (σ = -45.89) and the occurrence of two critical peaks of N demand, the first one during the last week of May and the second in the last week of August, respectively.

*Additional index words***:** *Carya illinoensis*, late fertilization, Chlorophyll, SPAD measurements, total nitrogen, nitrate

N management in pecan orchards is a matter of controversy, especially regarding factors such as the type of N fertilizer, fertilizer timing, frequency of application and treatment methods (Kim et al., 2002; Wood, 2002). N supply generally increases pecan yield, but may also increase the alternate bearing tendency (Smith et al., 2004). Irregular bearing is the major problem in the production of pecan nuts (Wood, 1993 Wood et al., 2004), which may lead to depletion of N reserves in the tree, inhibiting the induction of pistillate flowers or incresase the rate of flower abortion (Acuña-Maldonado et al., 2003). An alternative to reduce the depletion of N tree reserves is to supply it when the tree need for this element is high (Rosecrance et al., 1998). The quantity of N that should applied is ruled by the size or age of the trees, availability of N in the soil and level of production expected (Lombardini, 2004). Three important periods of application have been reported: 1) during spring growth since the demand of N is high during the first phase of leaf expansion, 2) over the development of the nut, time at which N required for fruit filling (Wood, 2002), and 3) in the fall, to favor tree N accumulation and storage to increase N reserves for the following year (Lombardini, 2004). If N is applied when the tree demand is high, the efficiency of absorption, which seems to be demand-driven, is high. This would enable that treatment with low N doses may lead to a similar tree response to the one induced by high N rates applied at incorrect times over the growing season (Acuña-Maldonado et al., 2003).

Most of the N used during flowering is mobilized from the N reserves of the tree, which originated from N fertilization on the previous year (Acuña-Maldonado et al., 2003). In apple, there is a consistent response to N treatments in summer and autumn in terms of accelerating flower development, diminishing the rate of flower abortion and extending the responsiveness of the pistil, thus leading to a higher quantity of fruits for the next year (Delap, 1967; Hill-Cottingham, 1963; Hill-Cottingham and Williams, 1967; Williams, 1965). The absorption of N is related to the existing N in the tree, since the leaves serve as the primary source of N for the development of the nuts (Smith, 2002). Trees defoliate in autumn and some nutrient reserves of the leaves are translocated to the stem. Trials concerning late N applications (in September and October) in Oklahoma, showed that roots of less than 1 centimeter (cm) diameter are capable of storing N in winter, since in that region or the world the recommendation is to apply 75% of the annual N supply in the springtime and a remaining 25% in the first week of October (autumn) (Acuña-Maldonado et al., 2003).

Nitrogen fertilization in August enables good fruit filling and prevents nutrient competition between fruits and leaves, thereby allowing the tree to reach dormancy with sufficient nutrient reserves (Tarango, 2004). The roots can absorb the nutrients supplied in the autumn while the trees have foliage and the temperatures are higher than 8ºC, avoiding bud damage due to early or late frosts at the beginning and end of the growing season, respectively (Herrera, 2001).

A 7-year study carried out with pecan trees of the variety Hayes in Oklahoma, reported a 37% production increment associated with late fertilization (100 Kg ha $^{-1}$ N, applied at the beginning of October) as compared with the traditional fertilization strategy (Smith et al., 1995).

Killby (1999) investigated the effect of late fertilization on pecan trees of the variety Western Schley, for three growing seasons. Treatment with 150, 200 and 250 N units in August and September, led to a production of 2,700, 3,033 and 2,907 units per acre with a kernel percentage of 55, 59 and 56%, respectively. In this study, the production data recorded were described as extraordinarily high, and no mention was made to any negative effect associated with late fertilization (Killby, 1999). Similarly, a late fertilization (applied in August) experiment carried out with trees of the variety Castrate Fear in Alabama, led to a production of 1,850 units per acre, which more than doubled the yield of trees not subjected late fertilization (710 units per acre) (Goff, 2001). Therefore, the objective of the present work was to determine the impact of the late fertilization of N on the nutritional state, productivity and quality of pecan. The applicability of using a SPAD chlorophyll meter as non-destructive tool to estimate the level of plant pigments and plant nutrient status at three different critical phenological phases was also tested in this study.

Materials and Methods

PLANT MATERIAL AND FERTILIZER TREATMENT. This investigation was carried out for two growing seasons (2004 and 2005) in an orchard located in the region of Jiménez, which is the main pecan-producing region of the State of Chihuahua, Mexico. The selected 8-year-old, experimental trees were of the variety `Western Schley'. Fertigation treatments consisting of differential N doses applied as ammonium sulfate (20.5% N), were supplied to plants at a rate of (Kg ha^{-1}): T1=80, T2=120, T3=160, T4=80+40 *, 40 T5=120+40* and T6=160+40* (*late fertilization rates), respectively. Subsequently, T4, T5 y T6 correspond to late fertilization treatments since

all of them include a late N dose (40 kg ha^{-1}) supplied in august. According to the phenological stage of development, treatments were applied as follows: 40% of the total dose at leaf burst (on 2 and 3 April), 20% at the final stage of bud development (on 31 April and 1 May), and the remaining 40% at the time of fruit development (on 28 and 29 May). The late N treatment was applied on 15 and 20 August for the seasons 2004 and 2005, respectively.

PLANT SAMPLING. Fully expanded leaves were collected from leaflets borne in the central part of the selected branch, from April till September every 28^{th} of each month. Each sample consisted of 70 leaflets and 30 different samples were collected in each sampling date. Leaf samples were consequently taken to the Laboratory of Ground, Water and Leaf Analysis of the Faculty of Agro-technological Sciences, Universidad Autónoma de Chihuahua, Mexico. To minimize the risk of contamination, leaves were carefully washed, first in tap and then in deionized water. Thereafter, the excess water was removed and leaves were left to dry up in the shade to be further placed in paper bags and kept in the oven at 60° C for 24 h. The dried leaf tissue was subsequently ground, sieved and stored in a dry place for further use.

The parameter "productivity per hectare (ha)" was calculated as described by Westwood (1982). It is expressed as the fruit weight (kg) produced divided by the trunk section (in cm^2) and multiplied by number of trees per ha. The experimental orchard consisted of 69 trees per ha, planted at a real frame of 12 x 12 m.

As quality variables, the number of nuts per kg and the kernel percentage were assessed. The number of nuts per kg collected per experimental unit was determined as follows: a kg of nuts was weighed and the number of nuts present was subsequently counted. The kernel percentage was calculated after breaking 1 kg nuts and obtaining the kernels.

N CONCENTRATION. The concentration of total N (%) was measured by the Micro-Kjendahl method (Leyva, 2000; Bremmer and Mulvaney, 1982). Hundred mg of dry tissue were placed in a Kjendahl flask to which 0.3 g selenium and 5 mL of concentrated sulfuric acid were added. The solution was left standing for 2 h and thereafter digested until obtaining a green pistachio coloration. Once the solution was cold, 20 ml distilled water and 3 drops of the pH indicator phenolftaleine plus 40% NaOH were added until the solution turned to purple color. Then, the sample was immediately distilled and the steam was collected in a 30 mL, 4% boric acid receiver solution, adding 6 drops of bromocresol green and 6 drops of methyl red. The probe was

consequently distilled until it changed from a red to green emerald color. Thereafter the solution was titrated with 0.02N HCl (Bremmer and Mulvaney, 1982; Leyva, 2000).

Leaf nitrate concentrations (ppm) were determined by the phenol-disulfonic acid and visible UV- spectrophotometry method (Fisher and Hart, 1991). Two hundred mg of leaf tissue were weighed and 25 mL deionized water were added. The sample was shaken for 20 min until obtaining a concentrated solution, which was consequently filtered. Five mL from this concentrated solution were placed in a flask and 2 mL calcium carbonate and 1 mL hydrogen peroxide were added. The solution was digested in the oven for 12 to 15 h at 70ºC. Thereafter, the sample was taken out from the oven and was recovered by adding 3 mL phenol-disulfonic acid, 50 mL deionized water and 20 mL ammonium hydroxide (1:1) to reach a final volume of 100 mL with deionized water. The absorbance of the solution at 425 nm was determined spectrophotometrically (UV-visible) (Leyva, 2000).

LEAF CHLOROPHYLL. The concentration of Chorophyll (Chl) was estimated by two different methods: 1) with a Minolta-SPAD-502 chlorophyll meter and, 2) by Chl extraction in methanol. In the first methodology, leaf Chl was monitored non-destructively with a SPAD apparatus (Minolta 502, Osaka, Japan), on a monthly basis from April until September (every 28^{th} of each month). This technique estimates the Chl concentration from red light absorbance measurements in a column-shaped cross-section of the leaf, with a 6 mm^2 base surface**.** SPAD values were determined on fully expanded, healthy leaves of the current growing season. Sample leaves were located on the central part of the tree and measurements were taken on the blade of 3^{rd} leaflet. (Fernández et al., 2006; Krugh,et al., 1994).

Leaf total Chl (a+b) (mg L^{-1}) concentrations were quantified spectrophotometrically after extraction in methanol (Morán, 1982). Sixty mg, 5 mm diameter leaf discs were weighed and placed in glasses, adding 5 ml methanol. The probes were left standing in the dark at room temperature for more than 24 h, until full discoloration of the leaf tissue. Thereafter samples were ground and filtered and the absorbance of the resulting solution was measured at 652 nm (Fisher and Hart, 1991; Morán, 1982).

STATISTICAL ANALYSIS. Data were statistically analyzed by two-way analysis of variance (ANOVA) with the program SAS, to assess the significance of the main factors and the significance of interactions. Means were also compared using the LSD Test at $P < 0.05$ in order to find significant differences between treatments. Finally, the

levels of significance were represented as: *= P<0.05, **= P<0.01, and NS= not significant

Results and Discussion

Leaf analysis is a valuable management tool to estimate the nutritional status of N and other elements in pecan (Castellanos, 2000). In Table 1, the average values obtained for all N treatments and the level of significance between treatments are shown for the seasons 2004 and 2005. No significant response was observed on the first year, while differences between the treatments were recorded on the second experimental season. From the observed results, it is possible to remark that treatment T3, was considered optimal for an effective nutritional balance of pecan tress, which is in agreement with the findings of Wood (2002).

PRODUCTIVITY. Late fertilization positively affected nut production, as reflected in the T4 (80+40*) treatment, which led to the highest yields measured during the second experimental season. The alternating character of this culture was patent during the 2 years of study, with 2005 corresponding to a high production season. T4 represents the lowest late fertilization rate, leading to a total of 120 kg ha^{-1} of N during the growing cycle. The yield of pecan grown in the state of Chihuahua may vary between an average of 1.44 to 3.16 tons ha^{-1} (Tarango, 2004), the existing differences being due to alternate bearing. Smith (1995), found that the yield of nuts of the variety `Hayes' increased by 37% as a result of late fertilization in the beginning of October. Killby (1999) reported "extraordinarily high yields" associated with late N fertilization. Goff (2001) obtained similar positive results after fertilizing pecan trees in August. Nitrogen fertilization when trees still have foliage can promote N absorption by the root (Herrera, 2001; Acuña et al., 2003) and thus favour kernel filling (Tarango, 2004). Worley (1990) concluded that application of 1,120 kg ha^{-1} N leads to an equivalent yield to fertilizing with 224 kg ha^{-1} N. In addition, the highest dose induced a reduction of both the size of the nut and kernel percentage.

QUALITY PARAMETERS. On the first year of study, no response to the various fertilization regimes in terms of quality parameters was determined. However, on the second experimental year, the kernel percentage varied significantly between treatments. According to LSD Test, the best results were obtained with T5 and T6, the lowest kernel percentages corresponded to T2 and T3 treatments, while T1 and T4 showed similar results, T4 leading to slightly higher values (Fig. 1). All late fertilization

252 treatments (i.e. T4, T5 and T6) induced better kernel filling as compared to the effect of
253 seasonal fertilization (i.e. T1, T2 and T3). The alternate bearing cycle was obvious from
254 the quality parameters, since in the “on” year 2005 the kernel percentage was lower,
255 than for the “off” year, 2004.

256 The kernel percentage is inversely related with the number of nuts per kg (Basurto,
257 2005; Perez, et al., 2003; Ojeda et al., 2003; and Tarango, 1992). For the variety
258 `Western Schley', the average number of nuts per kilogram and the kernel percentage
259 range from 138 to 162 and from 55 to 60% , respectively (Herrera, 2004; Basurto 2005).
260 Despite the size of the nut tends to decrease with the age of the tree (Arreola, 2002), in
261 young pecans the yield per tree increases more or to the same rate as the number of nuts
262 (Tarango, 1992). High N concentrations applied the traditional way, may increase the
263 number of nuts per tree, but decrease the percentage of kernels, since the leaf area is not
264 sufficiently increased to promote maximum development of the extra amount of nuts
265 produced (Sparks, 1989) In agreement with this observation, our results show that N
266 fertilization in August led to better kernel filling, in contrast to a report in which the
267 application of N divided in summer and autumn diminished the quality of the nut
268 (Hunter and Lewis, 1942). The authors suggested that the growth stimulated by N
269 application in autumn competes with developing fruits for the carbohydrates available,
270 thereby reducing fruit quality. Nevertheless Smith, (1995) reported that N fertilization
271 in autumn did not affect the quality of the nut.

272

273 **TOTAL N.** The different N treatments applied to the root system led to differences
274 in leaf total N concentrations during the second year of study. T1 and T2 significantly
275 increased leaf total N according to test LSD (Fig. 2), whereas no significant differences
276 were determined on the rest of treatments. Leaf total N concentrations were within the
277 sufficiency range considered for the region of Chihuahua (i.e. 2.2 to 3.12 %; Meraz,
278 1999). After 16 years of study concerning pecan N fertilization, it was observed that the
279 difference in the amount of fertilizer required to increment leaf total N from 2.75 a 3.0
280 % was minimum. Maintaining total leaf N at concentration of approximately 2.75 %
281 was found to lead to higher economic returns and average yields, with no consistent
282 differences regarding kernel percentages (Worley, 1990). Trees with 2.5% total N foliar
283 concentrations were most productive after 8 years of investigation (Worley, 1990)

284 **NITRATES.** In general terms, leaf nitrate content was not affected by the applied N
285 treatments, although for the season 2005, it is noticeable that the late fertilization (T4,

T5 and T6) led to higher leaf nitrate concentrations than the seasonal fertilization treatments (T1, T2 and T3). Leaf NO_3^- and total N concentrations followed and inverse relationship (σ = -45.89). This result is in agreement with the findings of Acuña-Maldonado et al. (2003), showing that the absorbed N is inversely related to the decrease in stored N, thereby suggesting that the stored N was used until depletion and at this point N was again absorbed by the root.

Nitrogen is absorbed from the soil as NO_3^-, and then it is assimilated into amino acids (Ruiz et al., 1999), either for the root or to be transported towards the aerial plant parts via the xylem for assimilation in the leaf and storage in vacuoles (Sivansakar and Oaks, 1996). The average leaf nitrate concentrations reported for the region of Jiménez range between 728 and 983 ppm DW, with values below 371 and 1,341 ppm DW being considered as deficient and excessive, respectively (Meraz, 1999). For an average of 1,498 ppm DW nitrate, Basurto (2005) reported an inverse relationship between leaf NO_3^- concentration and root N fertilization, i.e., the highest the N input the lowest the foliar nitrate contents and the other way around. Indeed, there is no mean standard to assess leaf nitrate concentrations, since NO_3^- plays a role on the storage and assimilation of N and is, hence, chiefly linked to the phenological state of the tree. Pérez (2004) found that the dates of the highest leaf NO_3^- demand in pecan occur 36 days after leaf burst (DDB) (around 4th May), which is coincident with the time of fruit set and leaf expansion, and the second period 130 DDB (approximately, 6 August) during cell enlargement and kernel filling.

SEASONAL DYNAMICS OF LEAF N COMPOUNDS. In May, the leaf NO_3^- concentration was low and inverse to the total N content, which reached the maximum. This period corresponds to the time, of shoot growth and leaf expansion, so that the absorbed N is rather used for the synthesis of leaf structural bio-molecules of the foliage

311 and it is not stored. This fact was verified by the similar evolution of leaf chlorophyll
312 concentration, indicating that the photosynthetic apparatus is very active during the leaf
313 expansion stage. Several investigations reported the high demand of N by the tree
314 during leaf expansion and shoot growth (Acuña-Maldonado et al., 2003; Sanchéz, 2005;
315 Wood, 2002).

316 In the months of June and July, leaf NO_3^- content increased, whereas total N
317 diminished slightly. These months correspond to a period of fast fruit growth and part of
318 the assimilated N is used for fruit development while the remaining N proportion is
319 stored in the vacuole as NO_3^-. The processes of kernel filling and formation of pistillate
320 flowers during the month of August, are coincident with the drastic diminution of foliar
321 nitrate, while the level of total N decreases slightly. In this summer month,
322 photosynthesis is actively taking place with the synthesis of carbohydrates for fruit
323 formation and the initiation of flower differentiation. Tarango (2004) found that the
324 carbohydrate content of shoots decreased during the process of kernel filling. At the end
325 of September, the lowest levels of leaf total N were detected, since N the leaf is about to
326 enter the senescence phase in which active carbon assimilation is replaced by the
327 catabolism of chlorophyll and diverse bio-molecules (Azcon-Bieto, 2000). Since leaf
328 NO_3^- concentration significantly increased in this period, it may indicate that the
329 absorbed N will no longer be used for the synthesis of leaf structural bio-molecules, but
330 rather for the formation low molecular weight of amino acids, which will be stored for
331 the next growing season.

332

333 **LEAF CHLOROPHYLL CONCENTRATION**. Assessment of the leaf
334 chlorophyll status throughout the growing season was carried out destructively (Chl
335 extraction in methanol) and non-destructively (with a SPAD apparatus). The different
336 N regimes did not bring about significant differences regarding leaf Chl concentrations

337 on the first year of study. However, during the second season (2005), the T3 treatment
338 led to significantly higher leaf Chl contents (in SPAD units and μg L^{-1}) according to
339 test LSD, followed by T4 and T6, respectively.

340 A high correlation between SPAD readings and Chl concentrations was found for
341 pecan leaves (Fig. 5), with the equation: $Y= 0.2275X^2 – 18.546X + 406.47$; $R^2 =$
342 0.9382; where Y= Chl concentration (μg L^{-1}) and X=SPAD.

343 The obtained results show that after calibration, the chlorophyll meter Minolta
344 SPAD 502 is a useful and practical tool for the non-destructive estimation of leaf Chl
345 concentrations versus the use of traditional Chl extraction methods (Abadía and Abadía,
346 1993; Fernández et al., 2006). Several studies carried out with diverse plant materials
347 have shown excellent correlations between SPAD values and Chl concentrations with
348 $R^2 \leq 0.9$, e.g for tomato (Rodriguez et al., 1998); maize, (Krugh et al. 1994; Novoa and
349 Villagrán, 2000); or fruit crops such as pear or peach (Fernández et al., 2006; Álvarez-
350 Fernández et al., 2004). It must be noted that prior to the use of the SPAD apparatus to
351 assess the leaf Chl status, a calibration between the SPAD readings and leaf N
352 concentrations has to be adequately developed. The obtained data provide evidence of
353 the existing strong correlation between both parameters for pecan leaves.

354
355 **CORRELATION BETWEEN SPAD VALUES AND TOTAL N**
356 **CONCENTRATIONS.** For the leaf Chl range investigated, a strong correlation ($R^2 =$
357 0.805) was found between leaf total N concentrations and SPAD values, according to
358 the following equation: $Y = -0.0877 X + 6.6563$, with $R^2 = 0.8052$; where X=SPAD
359 units and Y= total N (%).

360 The correlation obtained for pecan leaves indicates that, as leaf greenness increases
361 total N concentrations diminish (Fig. 6). For maize leaves, chlorophyll contents are
362 positively correlated with total N concentrations and therefore, Chl estimates reflect the
363 N status of the crop (Novoa and Villagrán, 2002). A high correlation between SPAD
364 units and total N was found in tomato (Rodriguez, et al., 1998), which was consistent

under diverse husbandry and environmental conditions such as variable light intensity, temperature, relative humidity, pests, planting density, or N source (Hiderman et al., 1992, Piekielek and Fox, 1992). The ability to predict chlorophyll content on a leaf area basis from SPAD readings was demonstrated in several crops and, since leaf chlorophyll content is closely correlated with leaf N concentration, the measurement of chlorophyll provides an indirect assessment of leaf N status (Yang et al, 2003). Prior to the use of the SPAD apparatus to assess the leaf N status non-destructively and with the purpose of easily detecting possible N deficiencies, a calibration between the SPAD readings and leaf N concentrations has to be adequately carried out as reported for several crops such as maize (Krugh et al., 1994), rice (Peng et al., 1996), wheat (Spaner et al., 2005; Vidal et al., 1999; Follet et al., 1992), cocksfoot sward (Duru, 2002) fescue (Kantety et al., 1996), cotton (Wood et al., 1992) apple or grapevine (Porro et al., 2001).

CONCLUSIONS

- Late fertilization positively affected yield and quality of the pecan nut, as well as the foliar NO_3^- content.

- Maintaining adequate leaf total N concentrations was favored by seasonal fertilization, T1 and T2 being the treatments with the highest total N contents.

- Nitrate and total N foliar concentrations were found to be inversely related (σ = -45.89), which may indicate that N in the plant is first absorbed and then stored and consumed until its depletion, prior to begin to absorb it from the soil again.

- For practical purposes, two tactically important moments of peak N demand exist, namely: at the end of May and at the beginning of August.

401 **Literature Cited**
402

403 Abadia, J. and A. Abadia. 1993. Iron and plant pigments. In: Barton L.L. and B.C.
404 Hemmig (Eds.). Iron chelation in plants and soil microorganisms. Academic Press,
405 New York, pp 327-343. ISBN 0-12-079870-0.
406 Álvarez-Fernández, A., P. García-Laviña, J. Fidalgo, J. Abadía and A. Abadía. 2004.
407 Foliar fertilization to control iron chlorosis in pear (Pyrus communis L.) trees
408 Plant Soil 263, 5-15.
409 Acuña- Maldonado, L.E., M.W. Smith, N.O. Maness, B.S. Cheary and B.L. Caroll.
410 2003. Influence of nitrogen absorption, partitioning, and yield of pecan. J. Amer.
411 Soc. Hort. Sci. 128(2): 155-162.
412 Arreola, A. and M. Lagarda. 2002. Light management in pecan orchards. International
413 Pecan Symposium. Hermosillo, Sonora, Mexico. pp. 67-74
414 Azcon-Bieto, J. 2000. Plant Physiolgy. 1st Edition. McGraw-Hill Interamericana, Spain,
415 pp 235-246.
416 Basurto, S.M. 2005. Assessement of autumn nitrogen fertilization of pecan (*Carya*
417 *illinoensis* (Wangenh) K. Koch) using a spray and micro-spray system. PhD
418 dissertation. Universidad Autónoma de Chihuahua, Mexico,119 pp.
419 Bremmer, L. M. and C. S. Mulvaney. 1982. Total Nitrogen. Pp. 595-634. in: Methods
420 of soil analisis. Part 2. Chemical and microbiological properties (agronomy 9).
421 Page, R. H. Miller and D.R. Keeney (eds): 2nd edition ASA, S.S.A. Madison,
422 Wisconsin, USA.
423 Castellanos, J.Z., J.X. Uvalle-Bueno and A. Aguilar-Satelises. 2000. Manual for the
424 interpretation of soil and water analyses. INCAPA. 2nd Edition, pp 75-76
425 Delap, A.V. 1967, The effect of supplying nitrate at different seasons on the growth,
426 blossoming and nitrogen content of young apple trees in sand culture. J. Hort. Sci.
427 42:149-167
428 Duru, M. 2002. Evaluation of chlorophyll meter to assess nitrogen status of cocksfoot
429 sward. J.Plant Nutr, 25(2): 275–286
430 Fisher, H.J. and F.L. Hart. 1991. Modern food analysis methods.Ed. Acribia, Spain, pp.
431 504-549.
432 Fernández V., V. Del Río, J. Abadía and A. Abadía. 2006. Foliar iron fertilization of
433 peach (*Prunus persica* (L.) Batsch.): effects of iron compounds, surfactants and
434 other adjuvants. Plant Soil 289:239-252.
435 Follet R. H., R.F. Follet and A. D. Halverson. 1992. Use a chlorophyll meter to evaluate
436 the nitroge status of dryland winter wheat. Comm. Soil Sci. Plant Anal. 23:687-
437 697
438 Goff, B. 2001. Late Season Fertilization: Exciting New Development, Pecan South 32
439 (12): 10-11
440 Herrera, E.A. and W.C. Lindeman. 2001. Distribution de 15N labeled fertilizer applied
441 to pecan. HortScience 36(2): 308-312.
442 Hill-Cottingham, D.G. and R.R. Williams. 1967. Effect of time of application of
443 fertilizer nitrogen on the growth, flower development and fruit set of maiden apple
444 trees, var. Lord Lambourne, and the distribution of total nitrogen within the trees.
445 J. Hort. Sci. 42:319-338
446 Hiderman, J., A. Makino, Y. Kurita, T. Masa, and K. Ojiva. 1992. Changes in the leaves
447 of chlorophyll and Light-harvesting chlorophyll a/b protein of PS II in senescence.
448 Plant Cell Physiol. 53:1209-1214
449 Hunter, J.H. and R.D Lewis. 1942. Influence of fertilizer and time of its application an
450 growth, yield an quality of pecans, J. Amer. Soc. Agron. 34:175-187

Kantety R. V., E. Van Santen F. M. Woods and C. W. Wood. 1996. Chlorophyll meter predicts nitrogen status of all fescue. Plant Nutr. 19:881-889

Killby, M. V. 1999. Performance of western pecan with fall fertilization only. Extension Horticulturist University of Arizona. Southeaster Pecan Grower Association SEPGA 1999. 92nd. Annual convention. U.S.A. Proc SEPGA 92:65-67.

Kim T., H.A. Mills and H.Y. Wetzstein. 2002. Studies on the effects of nitrogen form on growth, development, and nutrient uptake in pecan. J. Plant Nutr. 25(3):497-508.

Hill-Cottingham, D.G. 1963. Effect of the time of application of fertilizer nitrogen on the grow, flowering and fruiting of maiden apple trees grown in sand culture, J. Hort. Sci. 38:242-251

Krugh B., L. Bichham and D. Miles. 1994. The solid-state chlorophyll meter, a novel instrument for rapidly and accurately determining the chlorophyll concentrations in seedling leaves. Maize genetics cooperation. News Letter 68:25-27

Leyva, V.V. 2000. Elaboración del Manual de Prácticas de Laboratorio de Análisis Químico Bromatológicos, BSc Thesis. Chemistry Faculty, Universidad Autónoma de Chihuahua, México, pp. 12-18

Lombardini, L. 2004. Aplicación nitrogenada y oportuna en nogal. Nogatec 2004. Torreón, Coah. Mexico.

Méraz, H. A., 1999. Leaf nutritional standards for pecan (*Carya ilinoensis* (Wanngeh) K. Koch) "Western Schley" based on Integrated Differential Diagnosis (IDD). MSc Thesis. Universidad Autónoma de Chihuahua. Mexico. 180 pp.

Moran, R. 1982. Formulae for Determination of Chlorophyll pigments extracted with N.N-dimethylformamide. Plant Physiol. 69:1376-1381.

Novoa, R. and A.N. Villagrán. 2002. Evaluación de un Instrumento Medidor de clorofila en la Determinación de Niveles de Nitrógeno Foliar en Maíz. Agricultura Técnica. Chile. 62(1):166-171

Ojeda D.L., Reyes A., Ramírez H., Lagarda A., Chávez F.J., Uvalle J.X., Rivero R.M. and L. Romero. 2003. Efficient pecan (*Carya illinoensis* (Wangenh) K. Koch.) nitrogen fertilization. ISBN: 8489720983

Pérez, V.A. 2003 Seasonal dynamics of leaf, rachis and soil nitrate concentrations in association with the phenology of pecan (*Carya illinoensis* (Wngenh) K. Koch) "Western Schley". MSc Thesis. Universidad Autónoma de Chihuahua. Mexico.136 pp.

Peng, S., F.V. Garcia, R.C. Laza, A.L. Sanico, R.M. Visperas, and K.G. Cassman. 1996. Increased N-use efficiency using a chlorophyll meter on high yielding irrigated rice. Field Crops Res. 47:243–252

Piekielek, W.P. and R.H. Fox. 1992. Use of a chlorophyll meter to predict nitrogen requirements for maize. Agron. J. 84: 59-65.

Porro, D., C. Dorigatti, , M. Stefanini and A. Ceschini. 2001. Use of SPAD meter in diagnosis of nutritional status in apple and grapevine. Acta Hort. 564:243-252.

Rosecrance, R.C., S.A. Weinbaum and P.H. Brown. 1998. Alternate bearing affects nitrogen, phosphorus, potassium and starch storage pools in mature pistachio trees. Ann. Bot. 82:463-470.

Rodríguez, M. M., G. G. Alcántar, S. A. Aguilar, B. J. Etchevers and R. J. Santizó. 1998. Estimation of leaf chlorophyll and nitrate concentrations in tomato with a portable chlorophyll meter. Mexico. Terra Vol. 16 No. 2.

Ruiz, J.M. 1999. Response of some metabolic processes to the application of B and Ca in tobacco plants (*Nicotiana tabacum* L.). PhD dissertation. Plant Biology Department. Universidad de Granada. Spain. Pp 93-100.

Sánchez, E., A.V. Pérez, J. M. Soto, R. M. Yáñez and F. Montes. 2005. Seasonal nitrate dynamics in relation to critical developmental stages of pecan. Memorias del XI Congreso Nacional de la Sociedad Mexicana de Ciencias Hortícolas. Mexico, pp. 193-194

Sivasankar, S. and A. Oaks 1996. Regulation of nitrate reductase during early seedling growth. Plant Physiol. 107: 1225 1231.

Smith. M.W. 2002. Pecan Autumn fertilization. Nogatec. Torreón Coahuila,. México. Pág. 5-9

Smith, M.W., B. Cheary, and B. Carroll. 1995. Time of nitrogen application and phosphorus effects on growth, yield, and fruit quality of pecan. HortScience 30(3):532-534.

Sparks, D. 1989. Pecan nutrition, Twenty-third Western Pecan conference. Proc. NMSU, pp.55-96.

Tarango, R.S.H. 1992. Fertilization and productivity of pecan, Universidad Autónoma de Chihuahua y Asociación Agrícola Local de Delicias, Productores de Nogal y nuez, ISBN 968-633-23-9. 130 pp.

Tarango, R.S.H. 2004 Manejo del nogal pecanero en base a su fenología, Folleto Técnico No. 17 del INIFAP-Delicias. Centro de investigaciones regionales norte-centro campo experimetal Delicias. Chihuahua, Mexico. 35 pp.

Vidal, I., L. Longeri and J.L. Hétier. 1999. Nitrogen uptake and chlorophyll meter measurements in spring wheat. Nutr. Cycling Agroecos. 55:1-6.

Westwood, N.H. 1982, Temperate zone pomology. Ed. Mundiprensa, Spain, pp. 247-249.

Wood, C.W., P.W. Tracy, D.W. Reeves and K.L. Edmisten. 1992. Determination of cotton nitrogen status with a hand held chlorophyll meter. J. P1ant Nutr. 15(9): 1435-1448

Wood, B.W. 2002. Late Nitrogen fertilization in pecan orchards. A review. Proceedings 36th Western Pecan Conference. pp 47-59.

Wood, B.W., P.J. Conner and R.E. Worley. 2004. Insight into alternate bearing of pecan. XXVI International Horticultural Congress: Key processes in the growth and cropping of deciduous fruit and nut tress. Acta Hort. 636:617-629

Williams, R.R. 1965. The effect of summer nitrogen aplications on the quality of apple blossom. J. Hort. Sci. 40:31-41

Worley, R.E. 1990. Long-term performance of pecan trees when nitrogen application in based on prescribed threshold concentration in leaf tissue. J. Amer. Soc. Sci. 115:745-749.

Yang, W.H, S. Peng, J. Huang, A.L. Sanico, R.J. Buresh and C. Witt. 2003. Using leaf color charts to estimate leaf nitrogen status of rice. Agronomy J. 95:212-217

551 Tables
552
553 Table 1. Influence of N treatments on: productivity, number of nuts per kg, leaf NO_3^-
554 concentration and Chl (SPAD units and mg L^{-1}), for developing pecan trees, during the
555 seasons 2004 and 2005. Data are the averages ± SE (standard error ; n = 6).
556

Treatment (kg ha^{-1} N)	Yield (tons ha^{-1})	Number of nuts per kg	$[NO_3^-]$	Leaf SPAD values	[Leaf Chl] (mg L^{-1})
			Season 2004		
T1 (80)	2.57 ± 0.30	156.6 ± 15.75	1090.1±699.95	44.22 ± 3.37	-
T2 (100)	3.14 ± 0.37	166.0 ± 5.3665	779.0 ± 232.56	42.40 ± 1.79	-
T3 (120)	3.04 ± 1.09	147.0 ± 16.38	795.1 ± 109.59	44.18 ± 0.87	-
T4 (80+40)	3.17 ± 0.25	140.2 ± 11.49	652.95 ± 895.9	43.22 ± 1.25	-
T5 (100+40)	3.36 ± 0.85	148.2 ± 13.95	652.955 ± 357	42.64 ± 1.35	-
T6 (120+40)	3.32 ± 0.29	144.2 ± 6.18	746.82± 205.1	43.72 ± 3.59	-
Level of significance	NS	NS	NS	NS	
			Season 2005		
T1 (80)	2.95 ± 0.27^{c}	140.80 ± 19.05	3659.3 ± 295.9^{a}	44.12 ± 0.83 ^{a}	20.42 ± 4.04 ^{b}
T2 (100)	3.33± 0.29^{b}	152.63 ± 8.080	3965.0 ± 489.1 ^{a}	44.48 ± 0.79 ^{b}	30.36 ± 2.78 ^{a}
T3 (120)	3.37 ± 0.21^{ab}	148.99 ± 9.570	1985.8 ± 1637 ^{b}	46.54 ± 2.38 ^{ab}	32.22 ± 3.45 ^{a}
T4 (80+40)	3.66 ± 0.31^{a}	151.26 ± 21.67	1079.3 ± 810.7 ^{bc}	45.88 ± 0.75 ^{ab}	28.04 ± 2.67 ^{a}
T5 (100+40)	2.95 ± 0.28^{bc}	140.97 ± 12.09	3337.5 ± 174.4 ^{a}	40.82 ± 2.78 ^{c}	28.90 ± 0.36 ^{a}
T6 (120+40)	3.07 ± 0.07 ^{c}	139.34 ± 9.990	553.72 ± 304.8 ^{c}	45.42 ± 2.52 ^{ab}	28.80 ± 4.40 ^{a}
Level of significance	**	NS	**	**	**

NS: Not significant
**: Significant at P<0.01

557
558
559
560
561
562
563
564
565
566
567
568
569
570
571
572
573
574
575
576
577

Figures:

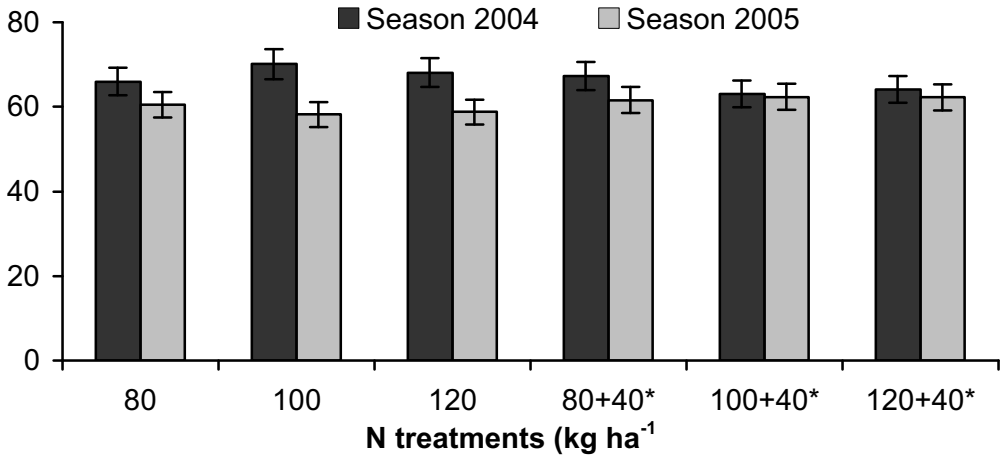


Fig. 1. Influence of N treatments on kernel percentage, for the seasons 2004 and 2005. All N treatments with an asterisk (*) correspond to late fertilization treatments.

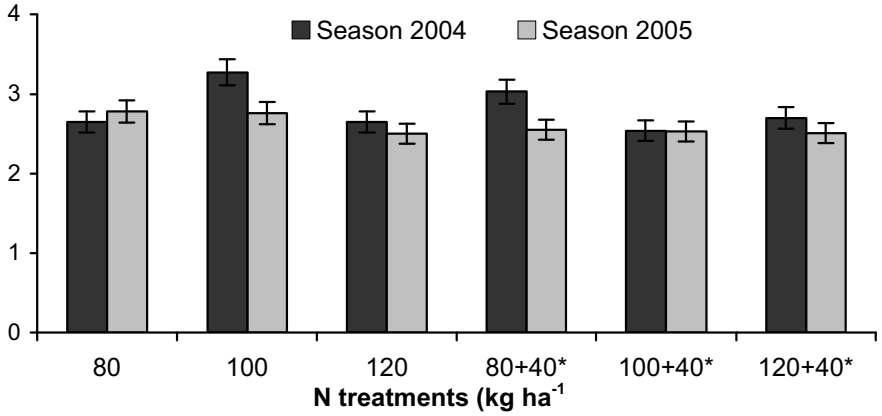


Fig. 2. Influence of the Nitrogen treatments on total N foliar concentration (%) of developing pecan trees, for the seasons 2004 and 2005. All N treatments with an asterisk (*) correspond to late fertilization treatments.

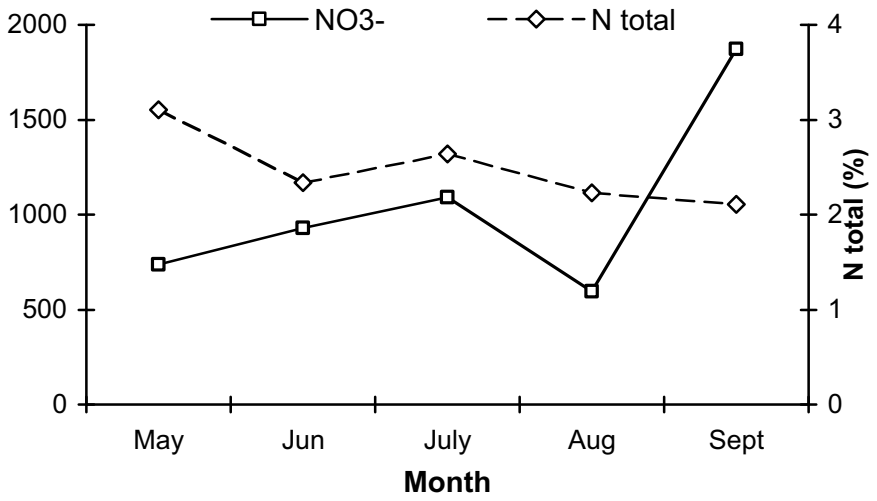
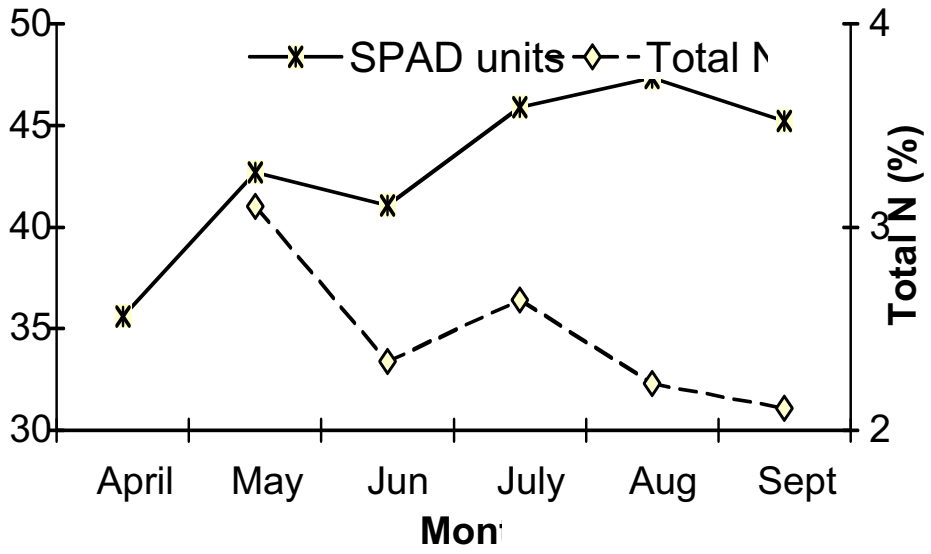


Fig. 3. Seasonal dynamics of foliar NO_3^- (ppm) and total N (%) concentrations for the treatment T1 applied to pecan. Season 2004.

671



672

673

674

675 Fig 4. Seasonal dynamics of leaf total N (%) and chlorophyll (SPAD units) contents.

676

677

678

679

680

681

682

683

684

685

686

687

688

689

690

691

692

693

694

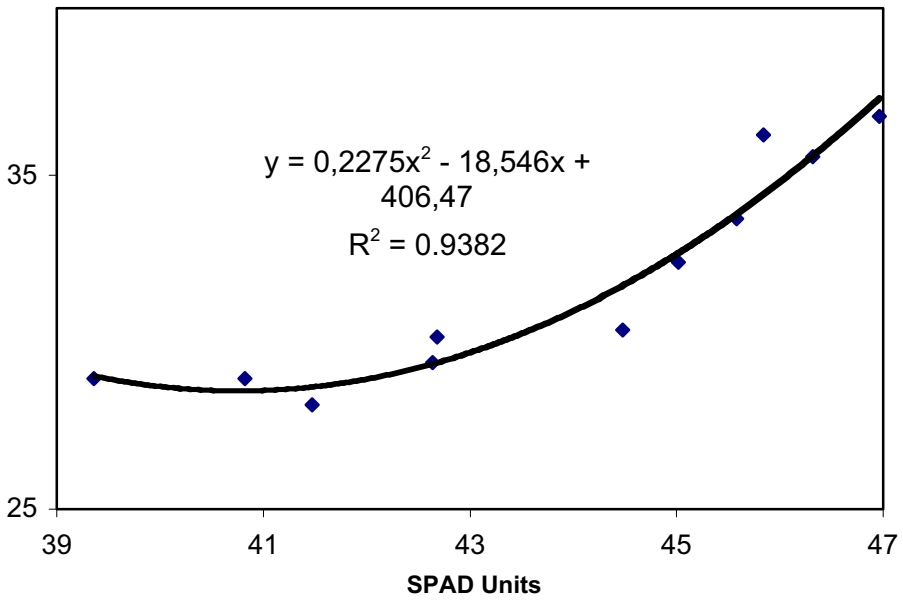
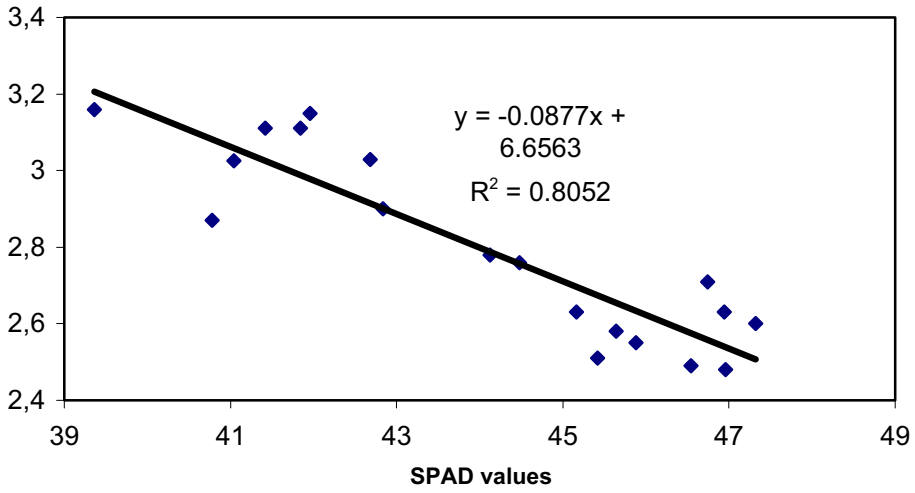


Fig. 5. Correlation between SPAD values and Chl concentration for pecan leaves of the variety `Western Schley'.

727



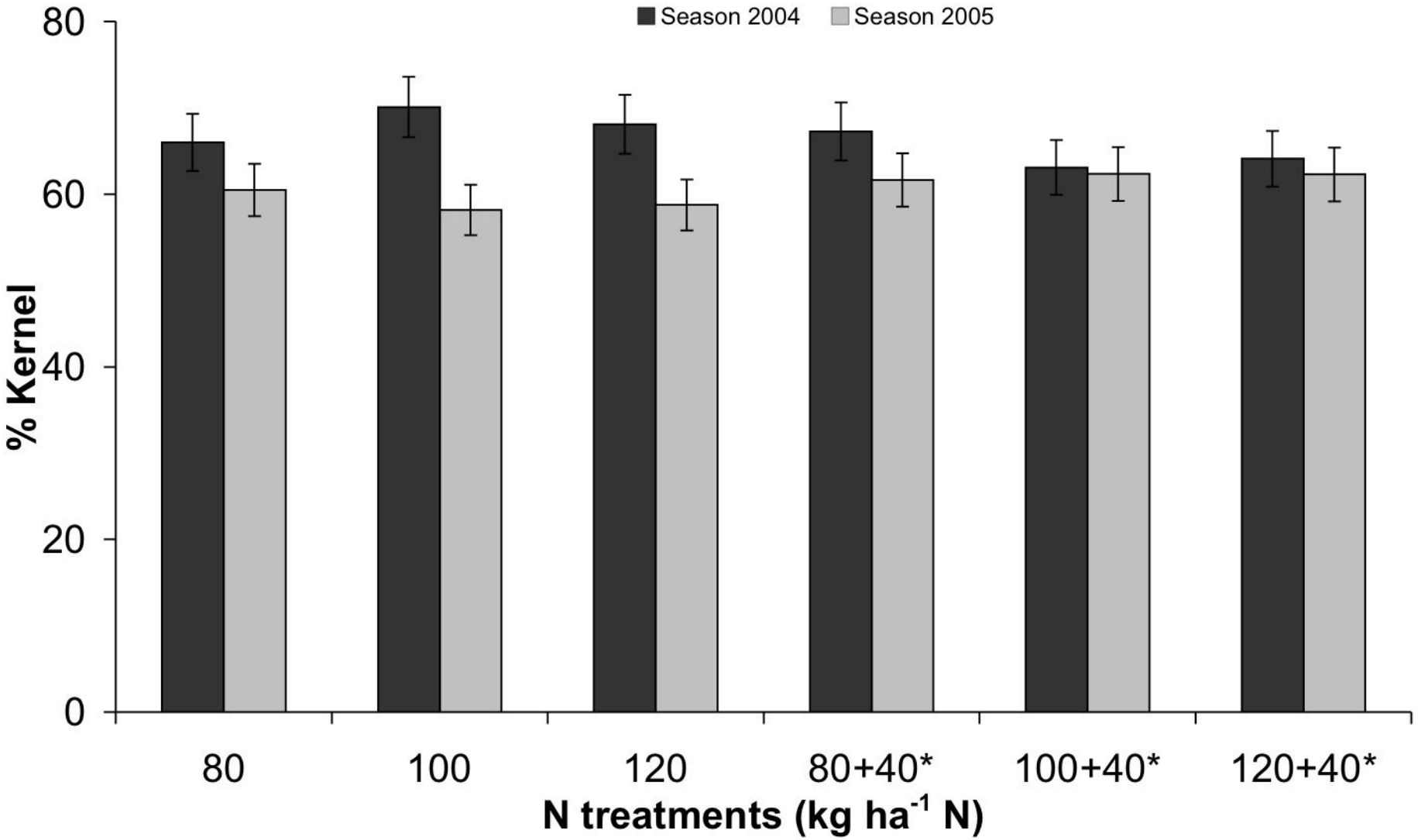
728

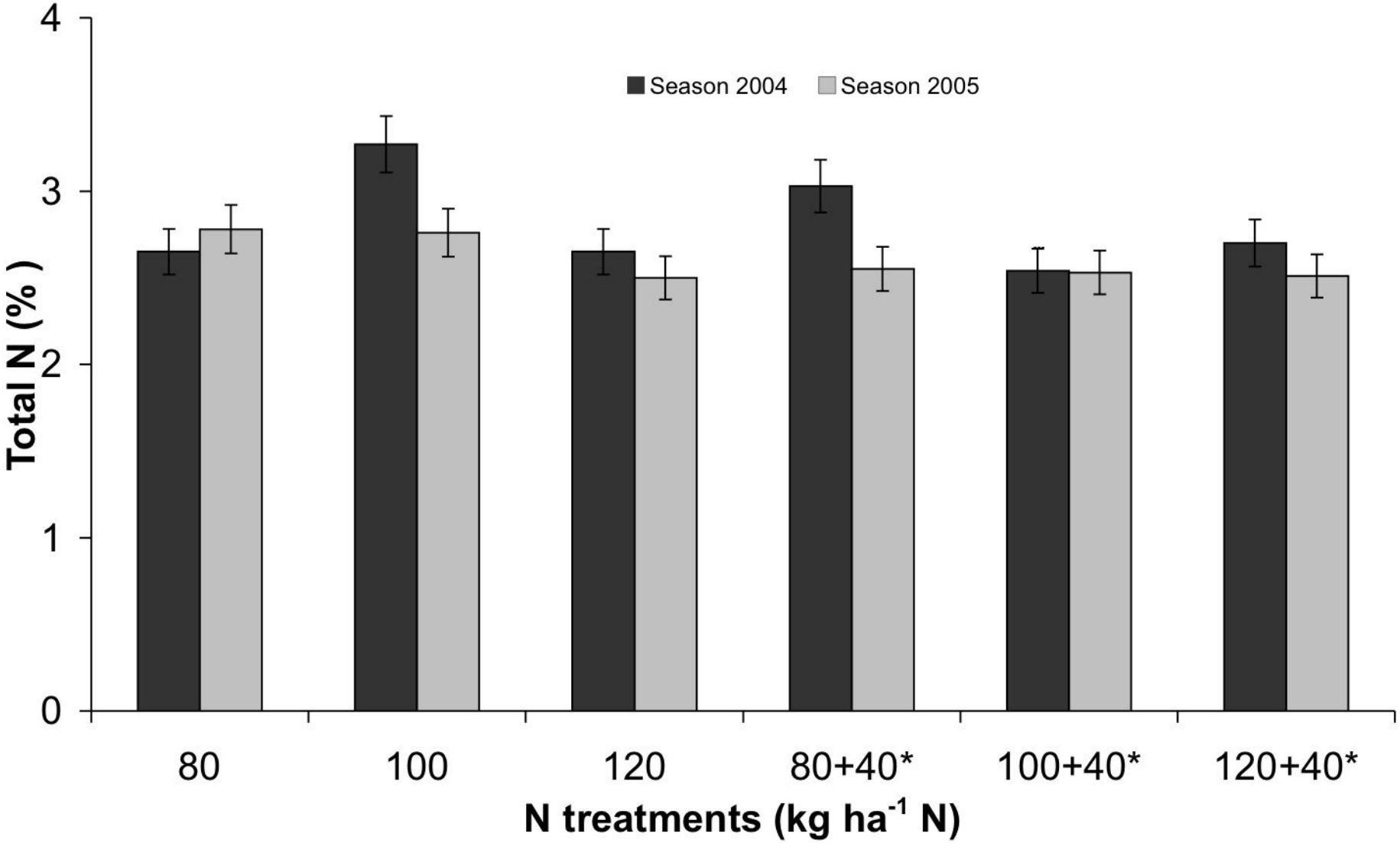
729

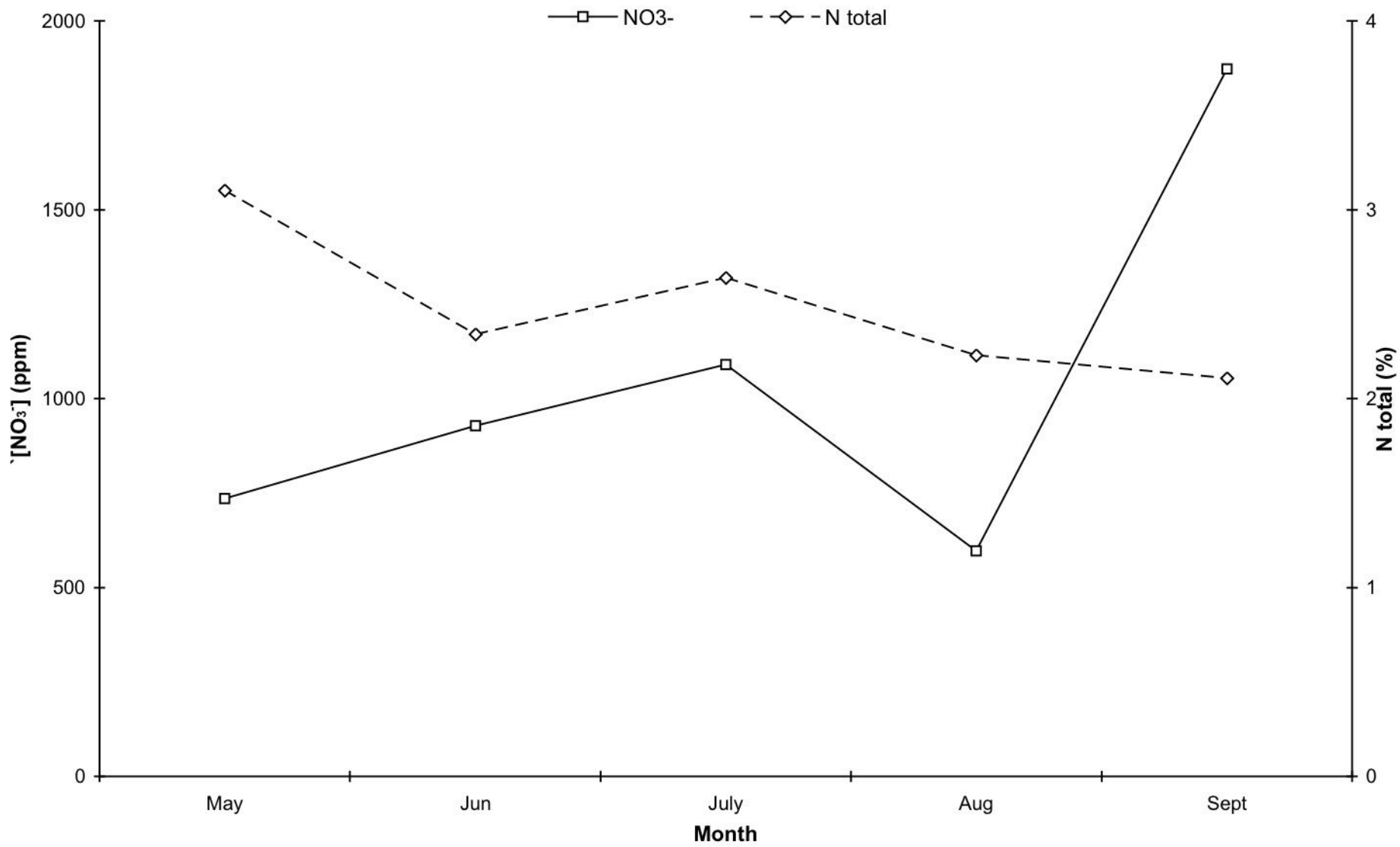
730 Fig. 6. Correlation between SPAD values and total N concentration (%) for pecan

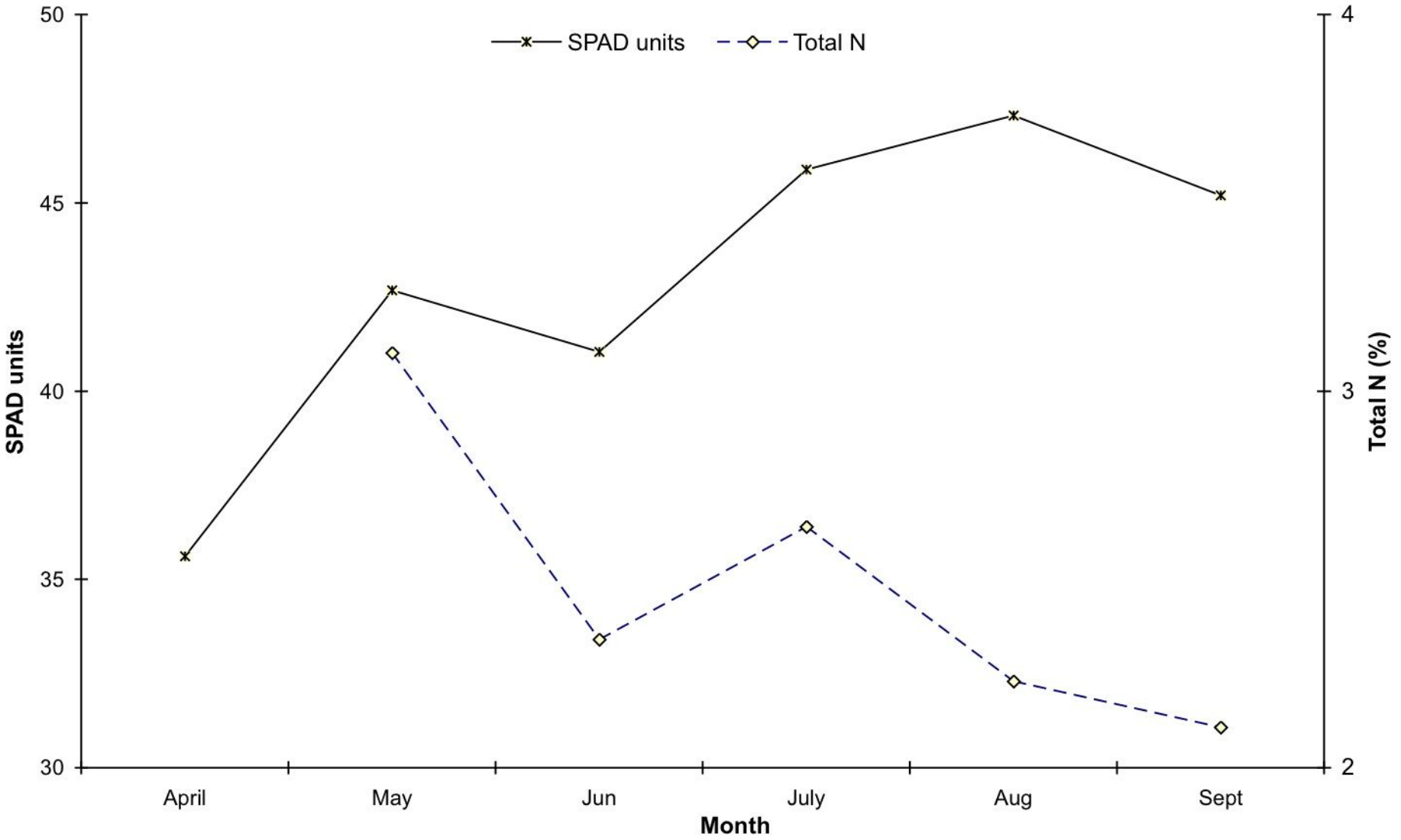
731 leaves of the variety `Western Schley'.

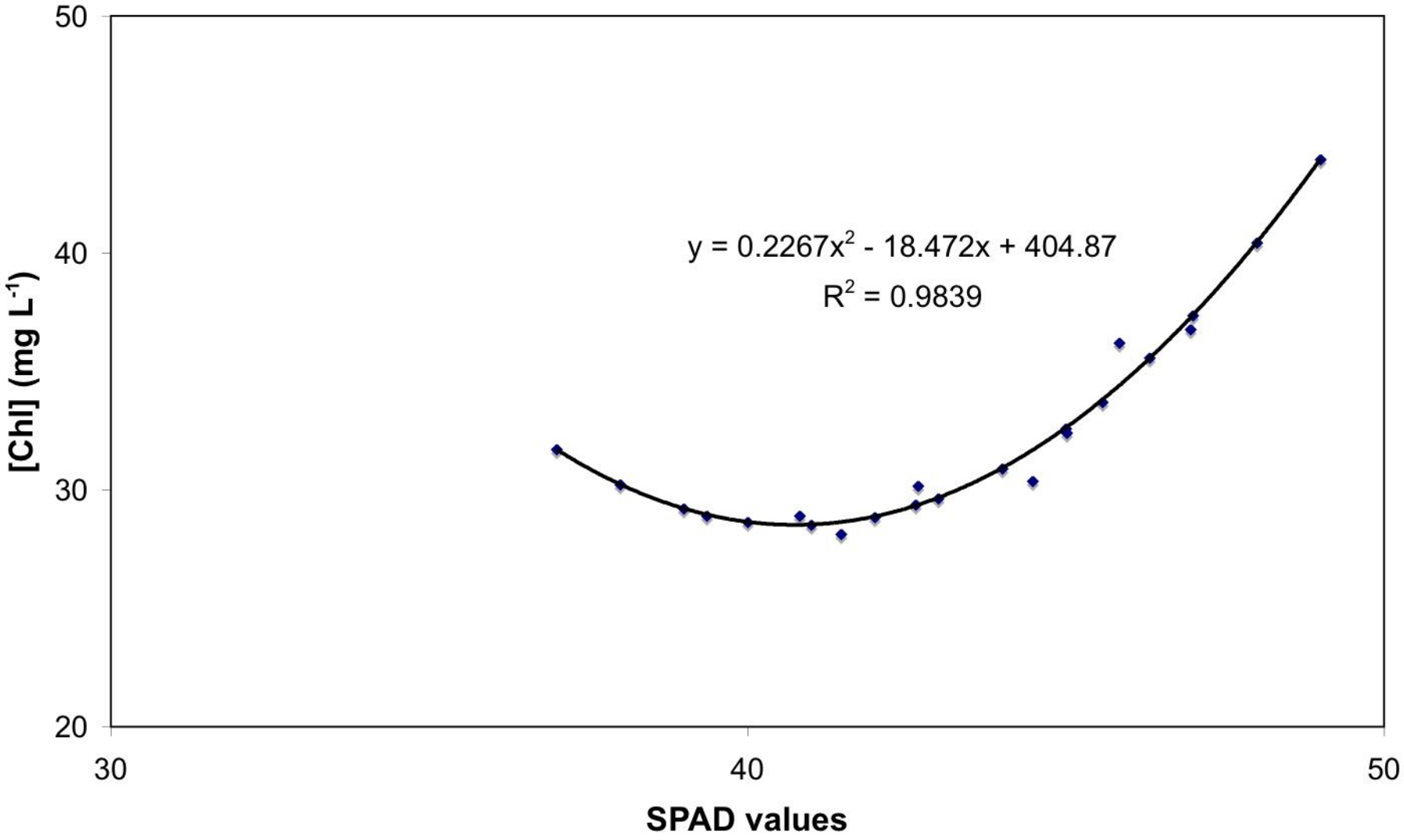
732

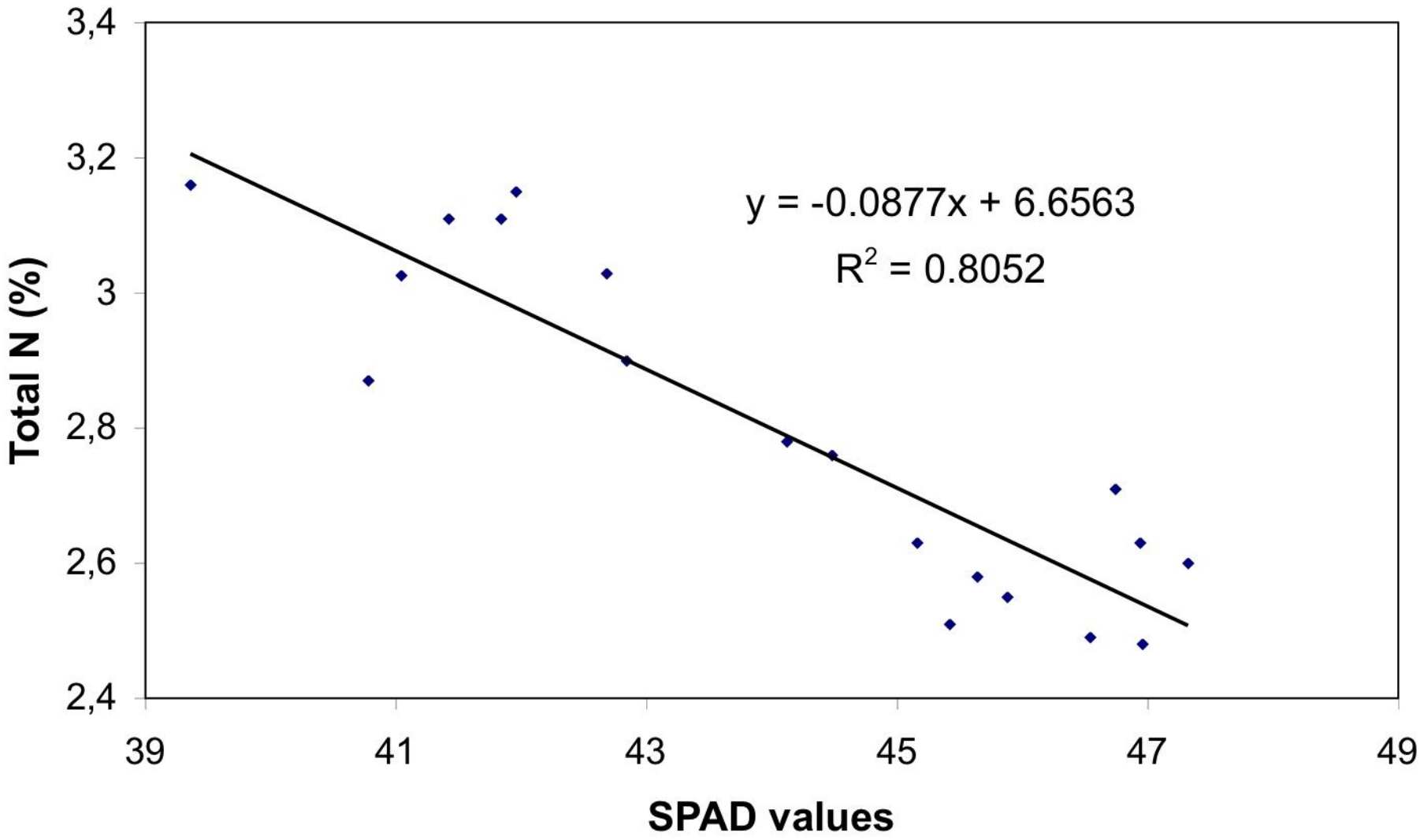

Season 2004
Season 2005
80
60
40
20
0
% Kernel
80
100
120
80+40*
100+40*
120+40*
N treatments (kg ha-1 N)


Season 2004
Season 2005
Total N (%)
0
1
2
3
4
80
100
120
80+40*
100+40*
120+40*
N treatments (kg ha-1 N)


NO3-
N total
2000
1500
1000
500
0
`[NO3] (ppm)
4
3
2
1
0
N total (%)
May
Jun
July
Aug
Sept
Month


SPAD units
Total N
50
45
40
35
30
4
3
2
SPAD units
Total N (%)
April
May
Jun
July
Aug
Sept
Month


50
40
30
20
30
40
50
[Chl] (mg L-1)
SPAD values
y = 0.2267x2 - 18.472x + 404.87
R2 = 0.9839


3,4
3,2
3
2,8
2,6
2,4
Total N (%)
y = -0.0877x + 6.6563
R2 = 0.8052
39
41
43
45
47
49
SPAD values