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Anatomophysiological modifications induced by solid agricultural waste (vermicompost) in lettuce seedlings (*Lactuca sativa* L.)

Modificaciones anatomofisiológicas inducidas por residuos sólidos agrícolas (vermicompuesto) en plantines de lechuga (*Lactuca sativa* L.)

Argüello JA, L Seisdedos, MC Díaz Goldfarb, EA Fabio, SB Núñez, A Ledesma

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Keywords: Sustainable management; Plant anatomy; Net Assimilation Rate; Plant growth; Assimilate partitioning; Vermicompost quality.

Resumen. El objetivo de este trabajo fue analizar el impacto del tratamiento de vermicompuesto en plántulas de lechuga en términos de modificaciones anatómicas y fisiológicas relacionadas a la partición de asimilados y crecimiento. Los resultados mostraron que el efecto de vermicompuesto en el aumento de crecimiento se explica por un aumento en la actividad del meristema fundamental de la hoja. Se observó en la hoja un mayor espesor y número de capas de clorénquima. Esto se relaciona con un incremento en la actividad fotosintética, expresado por un aumento en la Tasa de Asimilación Neta. El vermicompuesto también actuó a nivel de procambium, produciendo un aumento en el número de miembros de vasos y en el área de floema, lo que está vinculado a una mayor eficiencia en la transferencia de fotoasimilados. Este hallazgo está relacionado a un menor coeficiente de área foliar efectiva en el tratamiento con vermicompuesto, lo que indica una mayor eficiencia de producción y transferencia de fotoasimilados. La evidencia experimental presentada muestra que el vermicompuesto actuó a nivel de meristema fundamental y procambium, produciendo modificaciones anatómicas que incrementaron la biomasa y modificaron la distribución de fotoasimilados y consecuentemente el crecimiento de las plantas.

Palabras clave: Manejo sustentable; Anatomía vegetal; Tasa de Asimilación Neta; Crecimiento vegetal; Partición de Asimilados; Calidad de vermicompuesto.

Facultad de Ciencias Agropecuarias. Universidad Nacional de Córdoba, CC 509, 5000 Córdoba, Argentina.
Address Correspondence to: Juan Alberto Argüello. Facultad de Ciencias Agropecuarias. Universidad Nacional de Córdoba. CC 509. Córdoba, Argentina, *e-mail:* juanalbertoarg@gmail.com
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INTRODUCTION

As an alternative fertilization method in response to environmental concerns, the recycling of Solid Agricultural Waste has become known through the production of a biofertilizer generated by earthworms (*Eisenia foetida*). This vermicompost is traditionally used as an organic amendment. Although this is an old concept in agronomy, vermicompost is a highly useful and viable technological resource for tackling current environmental concerns. It is well-established that earthworms have beneficial physical, biological and chemical effects on soils, and many researchers have demonstrated that these effects can increase plant growth and crop yield (Edwards, 1998; Atiyeh et al., 2000; Argüello et al., 2006).

Previous studies conducted at the Laboratory of Plant Physiology (Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Argentina) showed that vermicompost stimulates lettuce seedling growth and strength (Ledesma et al., 2001). It also improves the economy of water and carbon, maximizing growth and even strengthening transplanting. In garlic, the use of vermicompost produces an earlier start of bulbification and increases the assimilate partitioning index (Argüello et al., 2006).

Vermicompost application to lettuce seedlings significantly improved growth (Atiyeh et al., 2002), increased lettuce both fresh and dry biomass weights (Ali et al., 2007), and the enhanced plant weights were independent of the nutrient content of the substrates. In other crops, this biofertilizer greatly increased foliar area and biomass (Lima & Silva, 1998). Treatments involving vermicompost generally stimulate growth.

Atiyeh et al. (2002) also mention a number of references in the literature showing that plant growth regulators, such as auxins, gibberellins and cytokinins, are produced by microorganisms. It has been suggested that the promotion of microbial activity in organic matter by earthworms may result in the production of significant quantities of plant growth regulators (Krishnamoorthy & Vajranabhiah, 1986; Tomati et al., 1983, 1988, 1990; Tomati & Galli, 1995; Edwards, 1998). Earthworm activity accelerates the humification of organic matter, and its influence in increasing microbial populations enhances the presence of auxins and gibberellin-like substances as well as humic acids (Casenave de Sanfilippo et al., 1990). Similar results were presented in biosolids by Zhang et al. (2009).

From an anatomical point of view, it has been determined that the primary growth of plants has its origin at the level of the ground meristem, procambium and protoderm (Dickinson, 2000; Evert, 2008). Mineral nutrition contributes to structural organization, since anatomical modifications are found when plants receive fertilizers, which can alter tissue thickness (Marschner, 1995). Anatomical studies in coffee to determine the effects of nutrients on anatomy have shown that modifications induced in tissues can also influence assimilate partitioning (Rosolem & Leitte, 2007). However, there is no information in the literature to date that explain how vermicompost affects the anatomy and physiology of lettuce seedlings, and impacts assimilate partitioning (Marschner, 1995).

The xylem is the tissue that transports water and minerals from the root system to the aerial portions of the plant, and the phloem translocates the products of photosynthesis from mature leaves to areas of growth and storage, including the roots. The photoassimilates move from the production zones, called sources, to metabolism or storage zones, called sinks. A mature leaf is capable of producing photosynthates in excess of its own needs (Taiz & Zeiger, 1998)

Our hypothesis is that vermicompost stimulates lettuce plantlet growth acting at the ground meristem and procambium levels of the shoot, and also optimizes physiological aspects, such as biomass increase and assimilate partitioning.

The aim of this study was to analyze the impact of vermicompost on lettuce seedlings as regards anatomo-physiological modifications related to assimilate partitioning and growth.

MATERIALS AND METHODS

Plant material. Young lettuce (*Lactuca sativa* L.) var. Criolla Verde plants were grown under greenhouse conditions at the Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Argentina. The greenhouse conditions were: temperature between 20 and 25 °C, relative humidity of approximately 70% (± 20%), and natural light. The growth medium was soil (Entic haplustoll) with and without vermicompost taken from solid waste from industry fridge (All Green Company).

Treatments were (a) soil control (C) and (b) 1 soil: 1 vermicompost (by volume) (V). The physicochemical parameters evaluated were organic carbon, phosphorus, total nitrogen, electrical conductivity and pH. The organic C content was determined by Walkley-Black (Nelson and Sommers, 1996). Total N was evaluated by Kjeldahl and extractable P by Bray and Kurtz Nº1 (Kuo, 1996). The pH values were measured in aqueous extracts 1:2.5 with a pH meter (Orion Research 901). Electrical conductivity was measured in saturated paste with a conductivity meter (DIST4 of HANNA Instrumental).

Physiological variables. Every ten days from appearance of the first pair of normal leaves, the following variables were evaluated: (1) Net Assimilation Rate (NAR, $mg/cm^2 \cdot d$) [NAR = (final total dry weight – initial total dry weight) / (final time – initial time) x {(ln (final leaf area) – ln (initial leaf area)} / (final leaf area – initial leaf area) until 65 days from seeding date] (Kvet et al., 1971; Evans, 1972; Hunt, 1982); (2) biomass in terms of total dry weight (DW, mg/plant); and (3) assimilate partitioning: Foliar Area Coefficient (FAC = Leaf area / total dry weight, cm^2/g DW), Specific Leaf Area (SLA = Leaf area / shoot dry weight, cm^2/g DW), and Harvest Index (HI = Shoot dry weight / Total dry weight) (Medina, 1977).

The experimental design was a completely randomized design with three replications and three plants per replication. Each experiment was performed at least three times. Variables were analyzed using ANOVA and measurement comparisons using Tukey tests ($p \leq 0.05$) by INFOSTAT 2010 (Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Argentina).

Anatomical variables. From sowing date and during 60 days, two weekly extractions were carried out in order to evaluate different anatomical aspects of the leaf. Of each plantlet, the second totally expanded leaf from the shoot apex was selected, and the central part of this leaf was extracted to be studied later.

Leaf structure was analyzed using freshly-collected material or material fixed in FAA. This material was cut freehand or in a rotary microtome to make semi-permanent and permanent slides for microscopic studies, carried out according to usual techniques (Johansen, 1940; D'Ambrogio, 1986).

The following variables were analyzed: number of layers and height of mesophyll; number of vessel elements of xylem and phloem area of the midrib. The phloem surface was determined at 45 days from digitalized images of the transverse sections. The Image Tool program ver.3.00 was used (Wilcox et al., 1995).

Data analysis. Variables were analyzed using ANOVA and measurement comparisons using LSD Fischer ($p < 0.05$). Discrete variables were analyzed with Poisson Regression by INFOSTAT 2010 (Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Argentina).

RESULTS

Vermicompost amendment application increased organic P content, and N. The pH of the soil and vermicompost mixture decreased slightly and electrical conductivity increased without producing salinity (Table 1).

Table 1. Substrate chemical properties.
Tabla 1. Propiedades químicas del sustrato.

Properties	Vermicompost	Soil	Soil:Vermicompost (1:1,v/v)
Organic Carbon (%)	5.75	1.9	3.96
P-Bray (ppm)	353.4	55.7	225.4
Total N %	0.46	0.10	0.317
pH	5.7	6.1	5.9
Electrical Conductivity (dS/m)	3.3	1.6	2.2

Both curves of NAR have similar dynamics, but treatment with Vermicompost (V) showed a greater NAR at day 65 (Fig. 1).

FAC and SLA (Fig. 1) were much greater with than without vermicompost in the first reading performed between sowing days 13 and 15. After 30 days, these values were rapidly reversed as an effect of the treatment, which suggests a greater transport of photoassimilates. This explains the greater HI in vermicompost treatments at the end of the cycle (Fig. 2).

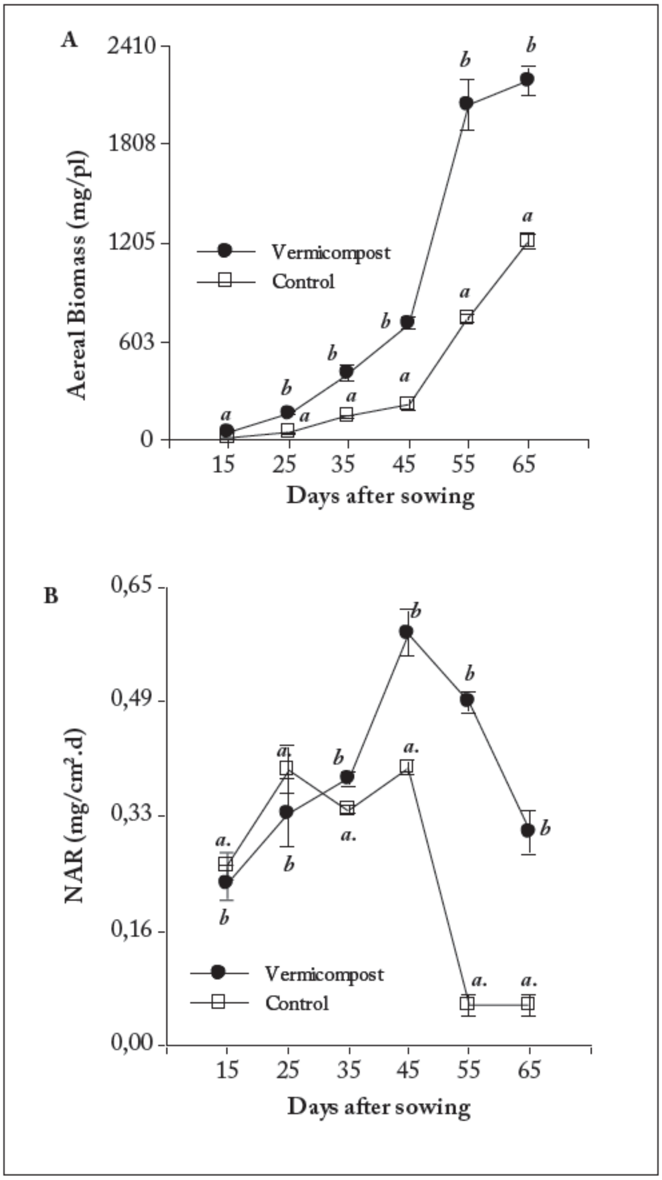

Fig. 1. Biomass (A) and Net Assimilation Rate (NAR) (B) in lettuce seedlings (*Lactuca sativa* L.) var. Criolla Verde grown on Vermicompost (V) and Control Soil (C). Different letters within a treatment date indicate statistical differences (Fisher $p \leq 0.05$).

Fig.1. Biomasa (A) y Tasa de Asimilación Neta (NAR) (B) en plántulas de lechuga (*Lactuca sativa* L.) var. Criolla Verde crecidas en Vermicompost (V) y Suelo como testigo (C). Letras diferentes entre tratamientos indican diferencias estadísticas (Fisher $p \leq 0,05$).

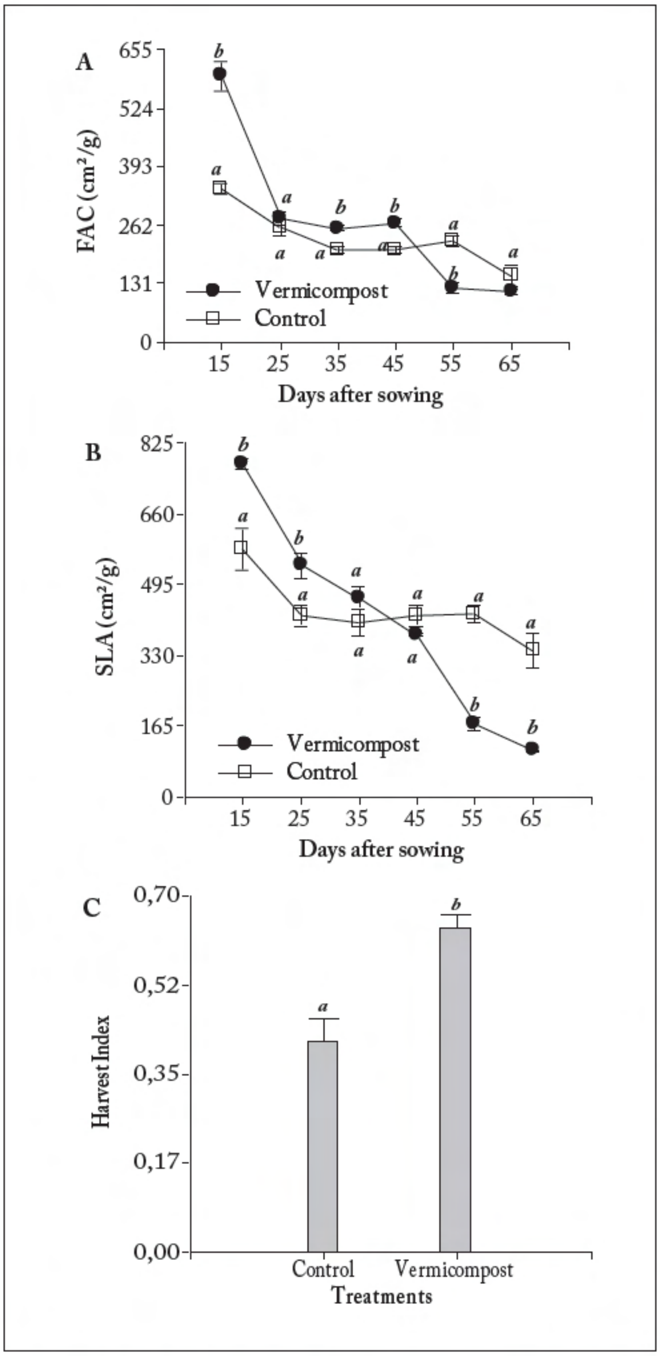

Fig. 2. Relationships between Foliar Area Coefficient (FAC) (A), Specific Leaf Area (SLA) (B) and Harvest Index (HI) (C) in lettuce seedlings (*Lactuca sativa* L.) var. Criolla Verde, produced in Vermicompost (V) and Control Soil (C). Different letters within a treatment date indicate statistical differences (Fisher p≤0.05). The vertical bars represent standard errors.

Fig. 2. Relaciones entre Coeficiente de Área Foliar (FAC) (A), Área Foliar Específica (SLA) (B) e Índice de Cosecha (HI) (C) en plántulas de lechuga (*Lactuca sativa* L.) var. Criolla Verde, producidas en Vermicompost (V) y Suelo como testigo (C). Letras diferentes entre tratamientos indican diferencias estadísticas (Fisher p≤0,05). Las barras verticales representan el error estándar.

Leaf anatomical analysis. Leaves, in transversal section, showed mesophyll layers in the control with large substomatic chambers in the abaxial epidermis (Fig. 3). Six or seven chlorenchyma layers are observed (Fig. 3: A-3; Fig. 4: A). At the midrib there are approximately thirty lignified vessel members of xylem (Fig. 3: A-1; Fig. 4: C). Near the phloem, lignified fiber elements were found, slightly differentiated in each of the three vascular bundles (Fig. 3: A-1 and A-2). In contrast, in the vermicompost treatment, the mesophyll of leaves showed a much more compact aspect and substomatic chambers on the abaxial surface, which were smaller than those in the con-

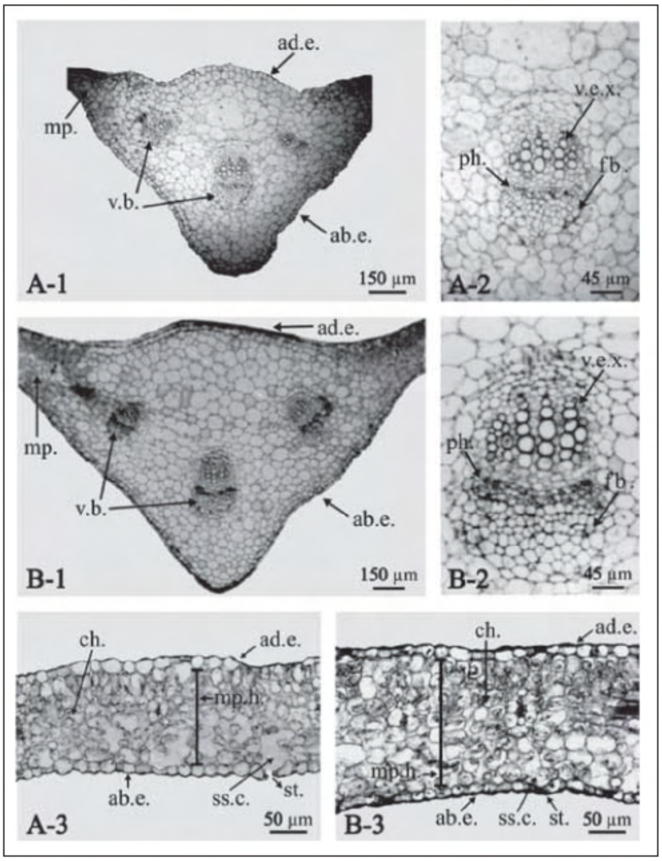

Fig. 3. Effects of vermicompost on the anatomy of *Lactuca sativa* L. leaves. Cross-section of control A-1, 2, 3; Vermicompost treatment B-1, 2, 3. Cross section of midrib A-1 and B-1; detail of vascular bundle A-2 and B-2; cross-section of mesophyll; A-3 and B-3. Abbreviations: ab.e. = abaxial epidermis; ad.e. = adaxial epidermis; ch. = chlorenchyma; fb. = fiber; mp. = mesophyll; mp.h. = mesophyll height; ph. = phloem; ss.c. = substomatal cavity; st. = stoma; v.b. = vascular bundle; v.e.x. = vessel element of xylem.

Fig. 3. Efecto de vermicompost en la anatomía de hojas de *Lactuca sativa* L. Corte transversal en testigo A-1, 2, 3; tratamiento con vermicompost B-1, 2, 3. Transcorte por nervadura central A-1 y B-1; detalle de hacecillo de conducción A-2 y B-2; transcorte por mesofilo; A-3 y B-3. Abreviaturas: ab.e. = epidermis abaxial; ad.e. = epidermis adaxial; ch. = clorénquima; fb. = fibra; mp. = mesofilo; mp.h. = altura del mesofilo; ph. = floema; ss.c. = cavidad subestomática; st. = estoma; v.b. = hacecillo vascular; v.e.x. = miembro de vaso del xilema.

trol (Fig. 3: B-3). The mesophyll showed nine to eleven chlorenchyma layers (Fig. 3: B-3; Fig. 4: A) and a greater height (Fig. 4: B). At the midrib, the number of lignified vessels of the xylem was approximately forty, showing a marked difference with the control (Fig. 3: B-1; Fig. 4: C). The fiber elements were clearly in an abaxial position with respect to the phloem (Fig. 3: B-1 and B-2). They were more lignified than in the control. The phloem area was always greater in the vermicompost than in the control treatment (Fig. 4: D).

The treatment with vermicompost increased the mesophyll layer numbers, mesophyll height, vessel element numbers of xylem and phloem area of the midrib (Fig. 4).

If vermicompost showed a growth stimulus effect, it suggests that the stimulus may be related to an increase in the meristematic activity. Ledesma et al. (2001) showed that vermicompost increases photosynthesis in terms of NAR, and this can be related to an increase in the chlorenchyma that forms the mesophyll of the foliar layer. Vermicompost might thus stimulate the activity of the ground meristem, which is finally responsible for the production of foliar chlorenchyma.

Additionally, vermicompost application can improve the pattern of assimilate partitioning (Ledesma et al., 2001). This can also be attributed to an increase in activity of the leaf procambial tissue which leads to the production of the primary vascular tissue.

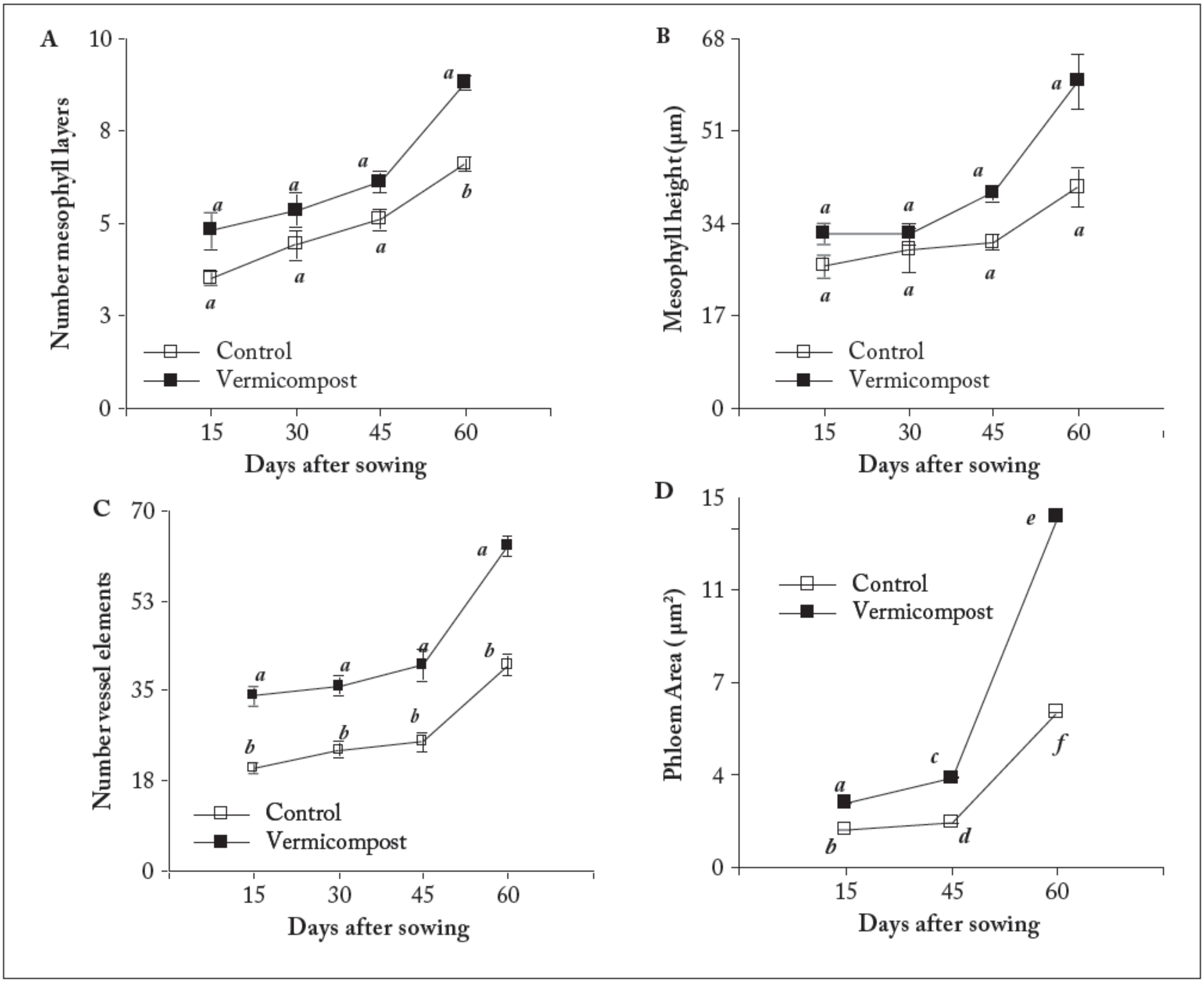

Fig. 4. Effects of vermicompost on anatomy changes in relation to numbers of mesophyll layers (A), mesophyll height (B), number of vessel elements (C) and phloem area (D) during the measurement period. Different letters show significant differences between treatments according to DGC Test ($p \leq 0.05$).

Fig 4. Efecto del vermicompost en los cambios anatómicos relacionados a número de capas del mesofilo (A), espesor del mesofilo (B), número de elementos de vasos (C) y superficie del floema (D) durante el período de evaluación. Letras diferentes muestran diferencias significativas entre tratamientos de acuerdo al test DGC ($p \leq 0,05$).

DISCUSSION

Previous studies demonstrated that lettuce seedlings treated with vermicompost showed greater vigour and growth (Ledesma et al., 2001); the experiments reported here gave similar results. One possible explanation for this is that a significant increase in aerial biomass may be generated with a consequent increase in photosynthesis (Fig. 1 A). Other authors have also found increases in biomass and foliar area in lettuce (Sganzerla, 1983; Mujahid & Gupta, 2010), in tomatoes (Atiyeh et al., 2002) and coriander (Lima & Silva, 1998). This is explained in terms of the increase in NAR (Fig. 1 B), which is related to a lower FAC in the treatment with vermicompost, indicating greater efficiency in the production of photoassimilates (Fig. 2 A). The present investigation demostrated that this increase in NAR is due to an increase of approximately 50% in the number of cell layers and in mesophyll height (Fig. 3: A-3, B-3 and Fig. 4). This increase can most probably be explained by the effect of vermicompost on the number of mesophyll layers, increasing the NAR.

In this context, it is worth asking what plants treated with vermicompost do with their greater production of photoassimilates. Previous findings indicate that vermicompost changes the assimilate partitioning pattern, prioritizing distribution to the aerial part in lettuce (Ledesma et al., 2001), and to the bulb in garlic (Argüello et al., 2006).

How then can this greater distribution be explained from the anatomical point of view? The results suggest that vermicompost acts to increase the number of vessel members (Fig. 3: B-1 and Fig. 4 C) and the phloem area (Fig. 4 D), and it also seems clear from the above discussion that vermicompost increases procambium activity. These anatomical modifications are consistent with a greater efficiency in the assimilate partitioning, which is seen in a decrease in the FAC and SLA Coefficients (Fig. 2 A-B), which in turn, accounts for the greater Harvest Index (Fig.2 C).

A deeper analysis of Figure 4 (A-B) reveals that the ground meristem activity is manifested in the vermicompost treatment by an increase (1) in the number of mesophyll layers at 30 days after sowing, and (2) of the mesophyll thickness at 45 days after sowing. On the other hand, it is important to point out the increase in the number of vessel elements (Fig. 3A and 4C) and phloem area (Fig. 3B and 4D) which occured after 15 days from sowing, as a result of the same treatment. This can be linked to anticipation in the activity of the procambium. These might be explained by the presence of growth hormones in the vermicompost, wich are absorbed in humic acids, forming complexes (Atiyeh et al., 2000, 2002; Arancon et al., 2004, 2006; Edwards et al; 2006). The hormones present constitute an external "signal" that acts on the primary meristems mentioned earlier, which are responsible for the perception of signals that stimulate growth (Argüello et al., 2008).

In conclusion, experimental evidence suggest that the vermicompost increases growth showing a greater generation and translocation of photoassimilates, which can be explained in terms of various anatomical modifications found.

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INTRODUCTION

As an alternative fertilization method in response to environmental concerns, the recycling of Solid Agricultural Waste has become known through the production of a biofertilizer generated by earthworms (*Eisenia foetida*). This vermicompost is traditionally used as an organic amendment. Although this is an old concept in agronomy, vermicompost is a highly useful and viable technological resource for tackling current environmental concerns. It is well-established that earthworms have beneficial physical, biological and chemical effects on soils, and many researchers have demonstrated that these effects can increase plant growth and crop yield (Edwards, 1998; Atiyeh et al., 2000; Argüello et al., 2006).

Previous studies conducted at the Laboratory of Plant Physiology (Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Argentina) showed that vermicompost stimulates lettuce seedling growth and strength (Ledesma et al., 2001). It also improves the economy of water and carbon, maximizing growth and even strengthening transplanting. In garlic, the use of vermicompost produces an earlier start of bulbification and increases the assimilate partitioning index (Argüello et al., 2006).

Vermicompost application to lettuce seedlings significantly improved growth (Atiyeh et al., 2002), increased lettuce both fresh and dry biomass weights (Ali et al., 2007), and the enhanced plant weights were independent of the nutrient content of the substrates. In other crops, this biofertilizer greatly increased foliar area and biomass (Lima & Silva, 1998). Treatments involving vermicompost generally stimulate growth.

Atiyeh et al. (2002) also mention a number of references in the literature showing that plant growth regulators, such as auxins, gibberellins and cytokinins, are produced by microorganisms. It has been suggested that the promotion of microbial activity in organic matter by earthworms may result in the production of significant quantities of plant growth regulators (Krishnamoorthy & Vajranabhiah, 1986; Tomati et al., 1983, 1988, 1990; Tomati & Galli, 1995; Edwards, 1998). Earthworm activity accelerates the humification of organic matter, and its influence in increasing microbial populations enhances the presence of auxins and gibberellin-like substances as well as humic acids (Casenave de Sanfilippo et al., 1990). Similar results were presented in biosolids by Zhang et al. (2009).

From an anatomical point of view, it has been determined that the primary growth of plants has its origin at the level of the ground meristem, procambium and protoderm (Dickinson, 2000; Evert, 2008). Mineral nutrition contributes to structural organization, since anatomical modifications are found when plants receive fertilizers, which can alter tissue thickness (Marschner, 1995). Anatomical studies in coffee to determine the effects of nutrients on anatomy have shown that modifications induced in tissues can also influence assimilate partitioning (Rosolem & Leitte, 2007). However, there is no information in the literature to date that explain how vermicompost affects the anatomy and physiology of lettuce seedlings, and impacts assimilate partitioning (Marschner, 1995).

The xylem is the tissue that transports water and minerals from the root system to the aerial portions of the plant, and the phloem translocates the products of photosynthesis from mature leaves to areas of growth and storage, including the roots. The photoassimilates move from the production zones, called sources, to metabolism or storage zones, called sinks. A mature leaf is capable of producing photosynthates in excess of its own needs (Taiz & Zeiger, 1998)

Our hypothesis is that vermicompost stimulates lettuce plantlet growth acting at the ground meristem and procambium levels of the shoot, and also optimizes physiological aspects, such as biomass increase and assimilate partitioning.

The aim of this study was to analyze the impact of vermicompost on lettuce seedlings as regards anatomo-physiological modifications related to assimilate partitioning and growth.

MATERIALS AND METHODS

Plant material. Young lettuce (*Lactuca sativa* L.) var. Criolla Verde plants were grown under greenhouse conditions at the Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Argentina. The greenhouse conditions were: temperature between 20 and 25 °C, relative humidity of approximately 70% (± 20%), and natural light. The growth medium was soil (Entic haplustoll) with and without vermicompost taken from solid waste from industry fridge (All Green Company).

Treatments were (a) soil control (C) and (b) 1 soil: 1 vermicompost (by volume) (V). The physicochemical parameters evaluated were organic carbon, phosphorus, total nitrogen, electrical conductivity and pH. The organic C content was determined by Walkley-Black (Nelson and Sommers, 1996). Total N was evaluated by Kjeldahl and extractable P by Bray and Kurtz Nº1 (Kuo, 1996). The pH values were measured in aqueous extracts 1:2.5 with a pH meter (Orion Research 901). Electrical conductivity was measured in saturated paste with a conductivity meter (DIST4 of HANNA Instrumental).

Physiological variables. Every ten days from appearance of the first pair of normal leaves, the following variables were evaluated: (1) Net Assimilation Rate (NAR, $mg/cm^2 \cdot d$) [NAR = (final total dry weight – initial total dry weight) / (final time – initial time) x {(ln (final leaf area) – ln (initial leaf area)} / (final leaf area – initial leaf area) until 65 days from seeding date] (Kvet et al., 1971; Evans, 1972; Hunt, 1982); (2) biomass in terms of total dry weight (DW, mg/plant); and (3) assimilate partitioning: Foliar Area Coefficient (FAC = Leaf area / total dry weight, cm^2/g DW), Specific Leaf Area (SLA = Leaf area / shoot dry weight, cm^2/g DW), and Harvest Index (HI = Shoot dry weight / Total dry weight) (Medina, 1977).

The experimental design was a completely randomized design with three replications and three plants per replication. Each experiment was performed at least three times. Variables were analyzed using ANOVA and measurement comparisons using Tukey tests ($p \leq 0.05$) by INFOSTAT 2010 (Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Argentina).

Anatomical variables. From sowing date and during 60 days, two weekly extractions were carried out in order to evaluate different anatomical aspects of the leaf. Of each plantlet, the second totally expanded leaf from the shoot apex was selected, and the central part of this leaf was extracted to be studied later.

Leaf structure was analyzed using freshly-collected material or material fixed in FAA. This material was cut freehand or in a rotary microtome to make semi-permanent and permanent slides for microscopic studies, carried out according to usual techniques (Johansen, 1940; D'Ambrogio, 1986).

The following variables were analyzed: number of layers and height of mesophyll; number of vessel elements of xylem and phloem area of the midrib. The phloem surface was determined at 45 days from digitalized images of the transverse sections. The Image Tool program ver.3.00 was used (Wilcox et al., 1995).

Data analysis. Variables were analyzed using ANOVA and measurement comparisons using LSD Fischer ($p<0.05$). Discrete variables were analyzed with Poisson Regression by INFOSTAT 2010 (Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Argentina).

RESULTS

Vermicompost amendment application increased organic P content, and N. The pH of the soil and vermicompost mixture decreased slightly and electrical conductivity increased without producing salinity (Table 1).

Table 1. Substrate chemical properties.
Tabla 1. Propiedades químicas del sustrato.

Properties	Vermicompost	Soil	Soil:Vermicompost (1:1,v/v)
Organic Carbon (%)	5.75	1.9	3.96
P-Bray (ppm)	353.4	55.7	225.4
Total N %	0.46	0.10	0.317
pH	5.7	6.1	5.9
Electrical Conductivity (dS/m)	3.3	1.6	2.2

Both curves of NAR have similar dynamics, but treatment with Vermicompost (V) showed a greater NAR at day 65 (Fig. 1).

FAC and SLA (Fig. 1) were much greater with than without vermicompost in the first reading performed between sowing days 13 and 15. After 30 days, these values were rapidly reversed as an effect of the treatment, which suggests a greater transport of photoassimilates. This explains the greater HI in vermicompost treatments at the end of the cycle (Fig. 2).

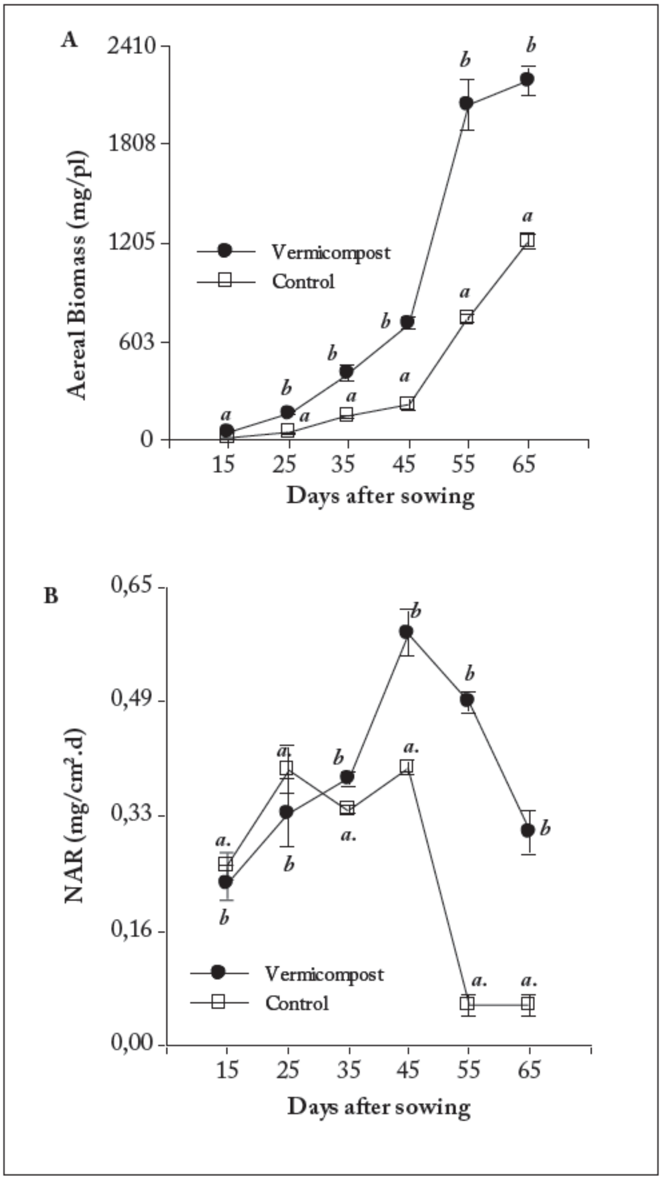

Fig. 1. Biomass (A) and Net Assimilation Rate (NAR) (B) in lettuce seedlings (*Lactuca sativa* L.) var. Criolla Verde grown on Vermicompost (V) and Control Soil (C). Different letters within a treatment date indicate statistical differences (Fisher $p \leq 0.05$).
Fig.1. Biomasa (A) y Tasa de Asimilación Neta (NAR) (B) en plántulas de lechuga (*Lactuca sativa* L.) var. Criolla Verde crecidas en Vermicompost (V) y Suelo como testigo (C). Letras diferentes entre tratamientos indican diferencias estadísticas (Fisher $p \leq 0{,}05$).

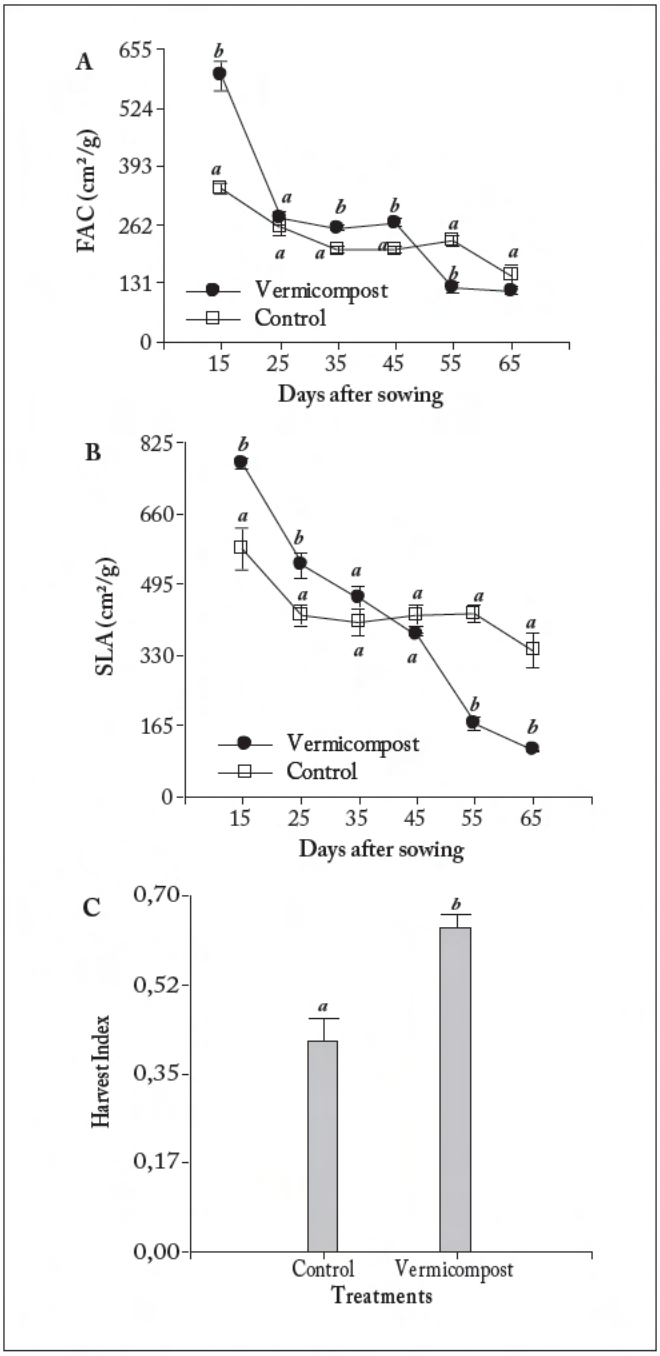

Fig. 2. Relationships between Foliar Area Coefficient (FAC) (A), Specific Leaf Area (SLA) (B) and Harvest Index (HI) (C) in lettuce seedlings (*Lactuca sativa* L.) var. Criolla Verde, produced in Vermicompost (V) and Control Soil (C). Different letters within a treatment date indicate statistical differences (Fisher $p \leq 0.05$). The vertical bars represent standard errors.

Fig. 2. Relaciones entre Coeficiente de Área Foliar (FAC) (A), Área Foliar Específica (SLA) (B) e Índice de Cosecha (HI) (C) en plántulas de lechuga (*Lactuca sativa* L.) var. Criolla Verde, producidas en Vermicompost (V) y Suelo como testigo (C). Letras diferentes entre tratamientos indican diferencias estadísticas (Fisher $p \leq 0{,}05$). Las barras verticales representan el error estándar.

Leaf anatomical analysis. Leaves, in transversal section, showed mesophyll layers in the control with large substomatic chambers in the abaxial epidermis (Fig. 3). Six or seven chlorenchyma layers are observed (Fig. 3: A-3; Fig. 4: A). At the midrib there are approximately thirty lignified vessel members of xylem (Fig. 3: A-1; Fig. 4: C). Near the phloem, lignified fiber elements were found, slightly differentiated in each of the three vascular bundles (Fig. 3: A-1 and A-2). In contrast, in the vermicompost treatment, the mesophyll of leaves showed a much more compact aspect and substomatic chambers on the abaxial surface, which were smaller than those in the con-

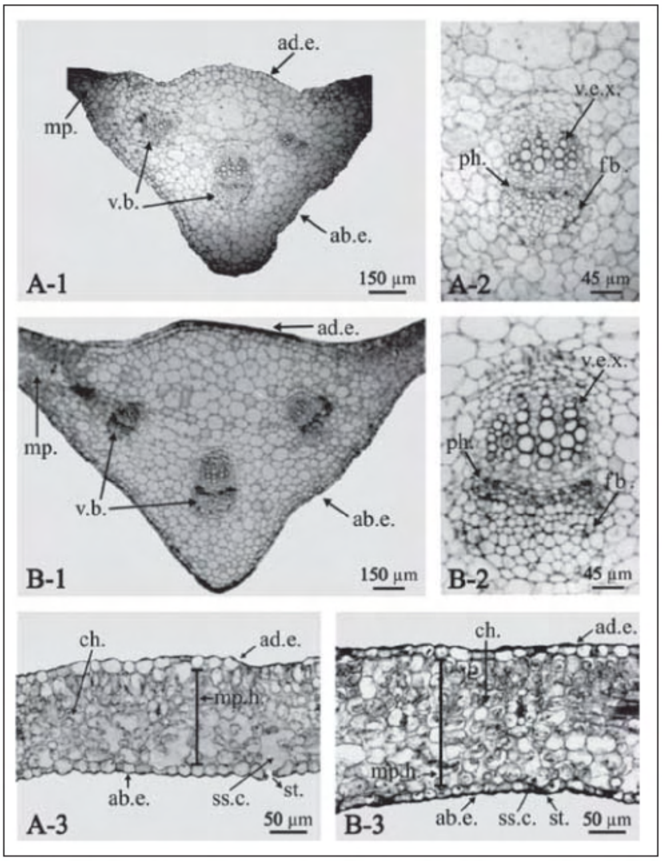

Fig. 3. Effects of vermicompost on the anatomy of *Lactuca sativa* L. leaves. Cross-section of control A-1, 2, 3; Vermicompost treatment B-1, 2, 3. Cross section of midrib A-1 and B-1; detail of vascular bundle A-2 and B-2; cross-section of mesophyll; A-3 and B-3. Abbreviations: ab.e. = abaxial epidermis; ad.e. = adaxial epidermis; ch. = chlorenchyma; fb. = fiber; mp. = mesophyll; mp.h. = mesophyll height; ph. = phloem; ss.c. = substomatal cavity; st. = stoma; v.b. = vascular bundle; v.e.x. = vessel element of xylem.

Fig. 3. Efecto de vermicompost en la anatomía de hojas de *Lactuca sativa* L. Corte transversal en testigo A-1, 2, 3; tratamiento con vermicompost B-1, 2, 3. Transcorte por nervadura central A-1 y B-1; detalle de hacecillo de conducción A-2 y B-2; transcorte por mesofilo; A-3 y B-3. Abreviaturas: ab.e. = epidermis abaxial; ad.e. = epidermis adaxial; ch. = clorénquima; fb. = fibra; mp. = mesofilo; mp.h. = altura del mesofilo; ph. = floema; ss.c. = cavidad subestomática; st. = estoma; v.b. = hacecillo vascular; v.e.x. = miembro de vaso del xilema.

trol (Fig. 3: B-3). The mesophyll showed nine to eleven chlorenchyma layers (Fig. 3: B-3; Fig. 4: A) and a greater height (Fig. 4: B). At the midrib, the number of lignified vessels of the xylem was approximately forty, showing a marked difference with the control (Fig. 3: B-1; Fig. 4: C). The fiber elements were clearly in an abaxial position with respect to the phloem (Fig. 3: B-1 and B-2). They were more lignified than in the control. The phloem area was always greater in the vermicompost than in the control treatment (Fig. 4: D).

The treatment with vermicompost increased the mesophyll layer numbers, mesophyll height, vessel element numbers of xylem and phloem area of the midrib (Fig. 4).

If vermicompost showed a growth stimulus effect, it suggests that the stimulus may be related to an increase in the meristematic activity. Ledesma et al. (2001) showed that vermicompost increases photosynthesis in terms of NAR, and this can be related to an increase in the chlorenchyma that forms the mesophyll of the foliar layer. Vermicompost might thus stimulate the activity of the ground meristem, which is finally responsible for the production of foliar chlorenchyma.

Additionally, vermicompost application can improve the pattern of assimilate partitioning (Ledesma et al., 2001). This can also be attributed to an increase in activity of the leaf procambial tissue which leads to the production of the primary vascular tissue.

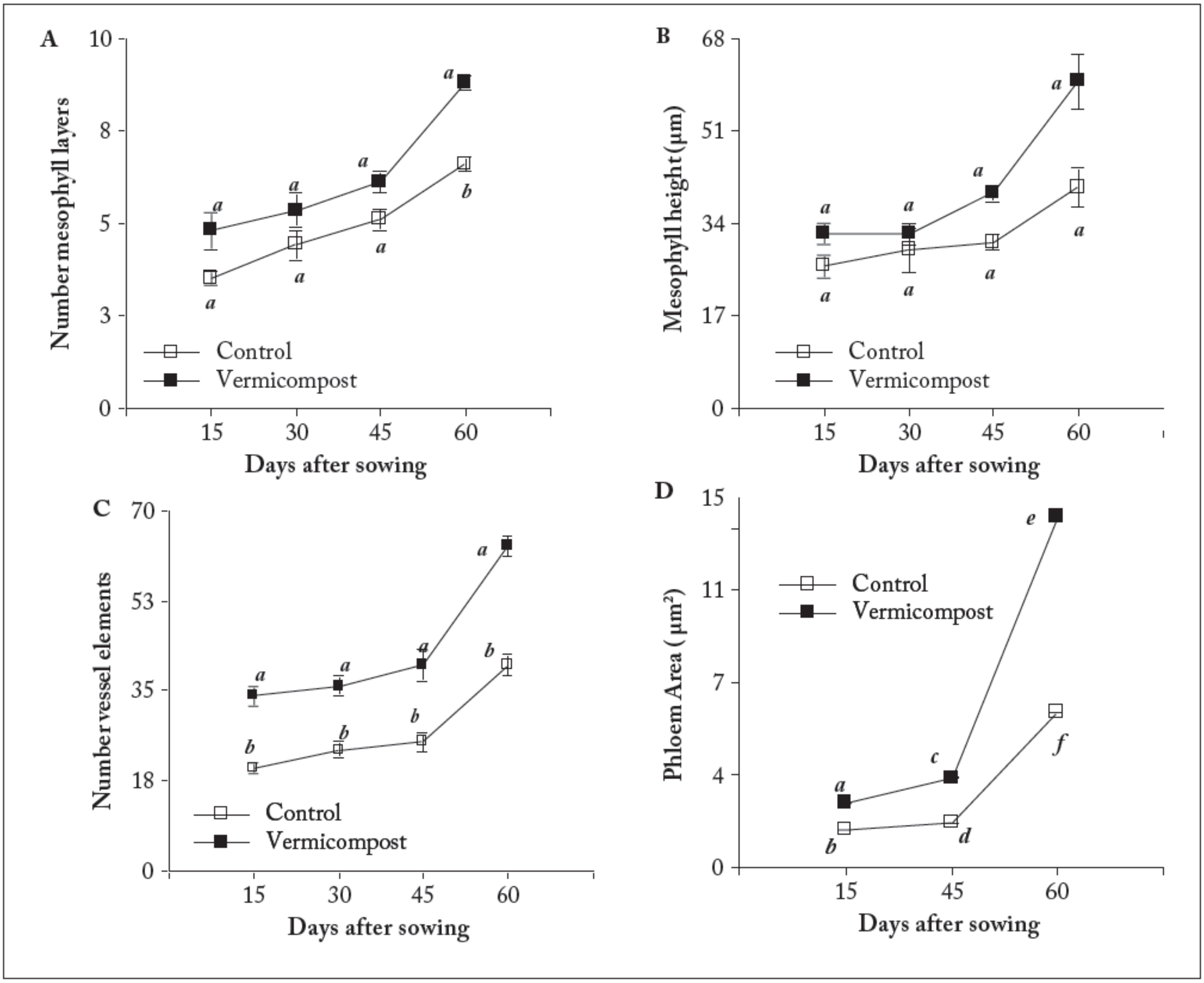

Fig. 4. Effects of vermicompost on anatomy changes in relation to numbers of mesophyll layers (A), mesophyll height (B), number of vessel elements (C) and phloem area (D) during the measurement period. Different letters show significant differences between treatments according to DGC Test ($p \leq 0.05$).

Fig 4. Efecto del vermicompost en los cambios anatómicos relacionados a número de capas del mesofilo (A), espesor del mesofilo (B), número de elementos de vasos (C) y superficie del floema (D) durante el período de evaluación. Letras diferentes muestran diferencias significativas entre tratamientos de acuerdo al test DGC ($p \leq 0,05$).

DISCUSSION

Previous studies demonstrated that lettuce seedlings treated with vermicompost showed greater vigour and growth (Ledesma et al., 2001); the experiments reported here gave similar results. One possible explanation for this is that a significant increase in aerial biomass may be generated with a consequent increase in photosynthesis (Fig. 1 A). Other authors have also found increases in biomass and foliar area in lettuce (Sganzerla, 1983; Mujahid & Gupta, 2010), in tomatoes (Atiyeh et al., 2002) and coriander (Lima & Silva, 1998). This is explained in terms of the increase in NAR (Fig. 1 B), which is related to a lower FAC in the treatment with vermicompost, indicating greater efficiency in the production of photoassimilates (Fig. 2 A). The present investigation demostrated that this increase in NAR is due to an increase of approximately 50% in the number of cell layers and in mesophyll height (Fig. 3: A-3, B-3 and Fig. 4). This increase can most probably be explained by the effect of vermicompost on the number of mesophyll layers, increasing the NAR.

In this context, it is worth asking what plants treated with vermicompost do with their greater production of photoassimilates. Previous findings indicate that vermicompost changes the assimilate partitioning pattern, prioritizing distribution to the aerial part in lettuce (Ledesma et al., 2001), and to the bulb in garlic (Argüello et al., 2006).

How then can this greater distribution be explained from the anatomical point of view? The results suggest that vermicompost acts to increase the number of vessel members (Fig. 3: B-1 and Fig. 4 C) and the phloem area (Fig. 4 D), and it also seems clear from the above discussion that vermicompost increases procambium activity. These anatomical modifications are consistent with a greater efficiency in the assimilate partitioning, which is seen in a decrease in the FAC and SLA Coefficients (Fig. 2 A-B), which in turn, accounts for the greater Harvest Index (Fig.2 C).

A deeper analysis of Figure 4 (A-B) reveals that the ground meristem activity is manifested in the vermicompost treatment by an increase (1) in the number of mesophyll layers at 30 days after sowing, and (2) of the mesophyll thickness at 45 days after sowing. On the other hand, it is important to point out the increase in the number of vessel elements (Fig. 3A and 4C) and phloem area (Fig. 3B and 4D) which occured after 15 days from sowing, as a result of the same treatment. This can be linked to anticipation in the activity of the procambium. These might be explained by the presence of growth hormones in the vermicompost, wich are absorbed in humic acids, forming complexes (Atiyeh et al., 2000, 2002; Arancon et al., 2004, 2006; Edwards et al; 2006). The hormones present constitute an external "signal" that acts on the primary meristems mentioned earlier, which are responsible for the perception of signals that stimulate growth (Argüello et al., 2008).

In conclusion, experimental evidence suggest that the vermicompost increases growth showing a greater generation and translocation of photoassimilates, which can be explained in terms of various anatomical modifications found.

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