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Title: EFFECTS OF PLANT DENSITY AND CUTTING-TYPE ON ROOTING AND GROWTH OF AN EXTENSIVE GREEN ROOF OF SEDUM SEDIFORME (JACQ.) PAU IN A MEDITERRANEAN ENVIRONMENT

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Corresponding Author: Dr. Salvatore La Bella, Ph.D

Corresponding Author's Institution: Università of Palermo

First Author: Simona Aprile, Ph.D.

Order of Authors: Simona Aprile, Ph.D.; Teresa Tuttolomondo, Ph.D.; Maria Cristina Gennaro, M.D.; Claudio Leto, M.D.; Salvatore La Bella, Ph.D; Mario Licata, Ph.D.

Abstract: Species of the Sedum genus are a popular choice for green roofs as they thrive in shallow growth layers and are resilient to extreme environmental conditions; however, they are also easy to propagate from seed, plantlings or plant parts. The use of rooted cuttings is most widespread due to good rates of establishment. In the interest of cost reduction, the direct spreading of Sedum cuttings over the roof area, especially over large areas, has attracted attention in recent years. Considering the interest on the above method and the seeming lack of agronomic experimental data available, this study aims to further knowledge in this area. Experimental tests on direct spreading of non-rooted cuttings of Sedum sediforme were carried out in order to study the effects of plant density and cutting-type both on rooting and on the growth and cover dynamics in a green roof simulated on the ground. The choice of species was based on previous studies using a number of wild Sedum taxa which showed interesting biotechnical characteristics, amongst which Sedum sediforme. The results highlight that stem-tip cuttings responded more quickly to rooting and with higher percentages compared to nodal cuttings. However, nodal cutting, when used at greater density, created more continuous plant cover in a shorter period of time.

Suggested Reviewers: Stefano Macolino PhD
Assistant Professor, Department of Agronomy, Food, Natural resources, Animals and Environment, University of Padova
stefano.macolino@unipd.it
He has continued studying extensive green roof. He has published extensively in peer reviewed journals.

Maria Papafotiou PhD
Full Professor, Department of Floriculture & Landscape Architecture,, Agricultural University of Athens

mpapaf@aua.gr
Expert in Floriculture and Turfgrass Sciences. She is a specialist on exploitation of Mediterranean flora and on creation of green roof systems. She has published extensively in peer reviewed journals

Alban Ibraliu PhD
Researcher, Agricultural University of Tirana
albanibraliu@ubt.edu
Specialist in aromatic, medicinal plants and Mediterranean plants. He has published extensively in peer reviewed journals.

Geanina Bireescu PhD
Researcher, Institute of Biological Research Cluj-Napoca
geanina.bireescu@icbiasi.ro
She is a specialist on soil and substrate. She has published extensively in peer reviewed journals.

Hamid El Kherrak PhD
Researcher, Dépt. Architecture du paysage, Institut Agronomique et Vétérinaire Hassan II
h.elkherrak@iav.ac.ma
Specialist in landascape architecture.

COVER LETTER

Dear Editorial Board of Scientia Horticulturae,

Please find enclosed the manuscript: “Effects of plant density and cutting-type on rooting and growth of an extensive green roof of *Sedum sediforme* (Jacq.) Pau in Mediterranean environment”, by Simona Aprile, Teresa Tuttolomondo, Maria Cristina Gennaro, Claudio Leto, Salvatore La Bella and Mario Licata, to be submitted as a new full length article to Scientia Horticulturae. All co-authors have seen, read and agree with the contents of the manuscript and there is no financial interest to report. The paper has not been published in whole or in part elsewhere. I attest to the validity and legitimacy of the data and its interpretation and I agree to its submission to the mentioned journal.

This paper shows the results obtained by a series of tests carried out between 2011 and 2012 at the CREA-DC Research farm in Bagheria (Sicily, Italy) on a simulated green roof using 3 wooden platforms placed at ground level. Each platform was isolated from the soil, covered in polystyrene, mulched with non-woven fabric and contained 6 test plots. The aim of the study was to demonstrate how two cutting-types of *Sedum sediforme* (Jacq.) Pau., directed spreading on a green roof system at three plant densities, can affect both the rooting and ground cover percentages. Over the tests period, rooting percentage of *Sedum sediforme* cuttings and increasing percentage in ground cover were determined. In the initial stage, the effect of cutting-type was evident, on the contrary plant density was found not to affect the rooting percentage of the species. Particularly, stem-tip cuttings were quicker to root and showed significantly higher rooting percentages than nodal cuttings. However ground cover percentage was positively affected by nodal cuttings compared to stem-tip cuttings. The density factor showed greater ground cover percentage in the treatments with higher cutting density. An analysis of the combined effects of the two factors highlighted that good ground cover percentages could be reached with greater density and nodal cuttings.

We believe that our findings could be of interest to the readers of Scientia Horticulturae as they highlight the effects of cutting-type of *Sedum sediforme* both in the initial establishment of the roof and successfully in the complete green coverage. Moreover the effects of plant density on rooting and growth of an extensive green roof of *Sedum sediforme* were also discussed in the study. Particularly, nodal cuttings of the tested species can permit to establish an homogeneous and continuos green coverage in about 1-year period compared to apical cuttings. This information is very important considering the recent use of direct spreading of various type of cuttings, above all on large-scale green roofs. The findings of different cutting-type of *Sedum sediforme* can also be useful for the creation of *Sedum*-vegetated mats or clods and can added other technical information to the field of study. The study highlights that *Sedum sediforme* is well-suitable to establish the ground cover on a green roof in a time period and with a cover percentage according to Italian standards. However there is no large information in literature, therefore further research is needed considering also various *Sedum* species and/or other plant species of potential interest, in order to improve and increase some aspects of green roof technology in the Mediterranean region.

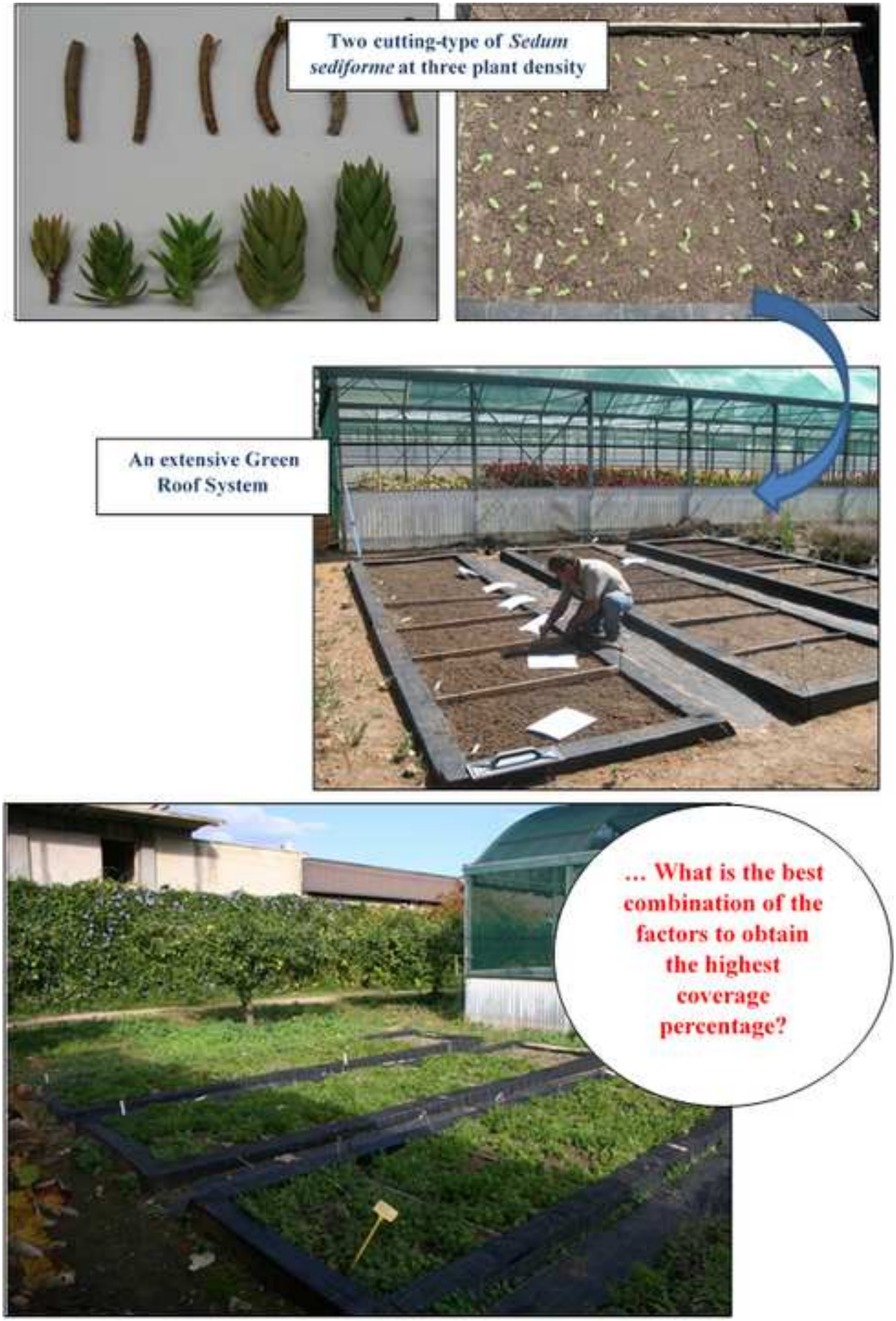
We hope the editorial board will agree on the interest of this study.

Sincerely yours,

Salvatore La Bella on behalf of the authors.

Corresponding author: Prof. Salvatore La Bella, Department of Agricultural, Food and Forest Sciences, Università degli Studi di Palermo, Viale delle Scienze 13 Building 4, 90128 Palermo, Italy. E-mail: salvatore.labella@unipa.it Tel. 003909123862231

Graphical Abstract



RESEARCH HIGHLIGHTS

- The single and combined effects of two cutting-type (stem-tip and nodal) of *Sedum sediforme* (Jacq.) Pau, directed spreading on a green roof systems at three plant densities, significantly affected the rooting and cover percentages of the species.
- Stem-tip cuttings were quicker to root and showed significantly higher rooting percentages than nodal cuttings.
- The interaction “density x cutting-type” highlighted the way in which stem-tip cuttings were able to increase rooting capacity in the treatments with higher density compared to nodal cuttings.
- At equal plant density levels, the greatest ground cover percentages were obtained with nodal cuttings compared to stem-tip cuttings.
- *Sedum sediforme* is well-suitable to establish the ground cover on a green roof in a time period and with a cover percentage, according to Italian standards.

EFFECTS OF PLANT DENSITY AND CUTTING-TYPE ON ROOTING AND GROWTH OF AN EXTENSIVE GREEN ROOF OF *SEDUM SEDIFORME* (JACQ.) PAU IN A MEDITERRANEAN ENVIRONMENT

Simona Aprile[b], Teresa Tuttolomondo[a], Maria Cristina Gennaro[a], Claudio Leto[a], Salvatore La Bella[a]*, Mario Licata[c]

[a]Department of Agricultural, Food and Forest Sciences, Università degli Studi di Palermo, Viale delle Scienze 13 Building 4, 90128 Palermo, Italy

[b]Research Center for Plant Protection and Certification, Council for Agricultural Research and Economics, SS 113 Km 245,500, 90011 Bagheria, Italy

[c]Corissia Research Centre, Via Libertà 203, 90139 Palermo, Italy

*Corresponding author at Department of Agricultural, Food and Forest Sciences, Università degli Studi di Palermo, Viale delle Scienze 13, 90128 Palermo, Italy

The authors contributed equally.

Abstract

Species of the *Sedum* genus are a popular choice for green roofs as they thrive in shallow growth layers and are resilient to extreme environmental conditions; however, they are also easy to propagate from seed, plantlings or plant parts. The use of rooted cuttings is most widespread due to good rates of establishment. In the interest of cost reduction, the direct spreading of *Sedum* cuttings over the roof area, especially over large areas, has attracted attention in recent years. Considering the interest on the above method and the seeming lack of agronomic experimental data available, this study aims to further knowledge in this area. Experimental tests on direct spreading of non-rooted cuttings of *Sedum sediforme* were carried out in order to study the effects of plant density and cutting-type both on rooting and on the growth and cover dynamics in a green roof simulated on the ground. The choice of species was based on previous studies using a number of wild *Sedum* taxa which showed interesting biotechnical characteristics, amongst which *Sedum sediforme.* The results highlight that stem-tip cuttings responded more quickly to rooting and with higher percentages compared to nodal cuttings. However, nodal cutting, when used at greater density, created more continuous plant cover in a shorter period of time.

Keywords: *Crassulaceae*, direct spreading, ground cover, no-rooted cutting, Sicily, vegetative propagation.

1. Introduction

The benefits afforded by green roofs are many and familiar; they include benefits to the environment, in particular through mitigation of the ‘urban heat island’ (Carter and Jackson, 2007; Alexandri et al., 2008), and to the individual building in terms of energy savings (Del Barrio, 1998; Castleton et al., 2010) and lengthening the life span of a flat roof cover. The city also benefits from a series of ecological and aesthetic improvements provided by green roofs (Liesecke, 1998; Liu and Minor 2005; Meng and Hu, 2005; VanWoert et al., 2005a; Villarreal and Bengtsson, 2005; Getter and Rowe, 2006; Simmons and Gardiner, 2007); green roofs are often believed to be a good way to increase the sustainability of buildings and urban areas (Berardi et al., 2014; Blank et al., 2013; Castleton et al., 2010; Cook-Patton and Bauerle, 2012; GhaffarianHoseini et al., 2013). Over the last decade, numerous studies on green roofs have demonstrated advantages linked to runoff-water management (Berndtsson, 2010; Stovin et al., 2012), and the mitigation of air (Yang et al., 2008) and noise pollution (Connelly and Hodgson, 2008; Van Renterghem and Botteldooren, 2009; Van Renterghem et al., 2013). The plant layer in extensive green roofs is particularly important as the plants must be able to live and grow in the extreme conditions of a shallow growth layer, strong insolation and winds, and arid, nutrient poor conditions (Getter and Rowe, 2008). In order to thrive in such conditions, the plant layer needs to be formed by species with specific eco-morphological characteristics: a shallow root system, low water and management needs, perennity, prostrate growth habit, rapid rates of establishment and ease of propagation (White and Snodgrass, 2003; ASTM, 2006; Getter and Rowe, 2006; Snodgrass and Mclntyre, 2010) or high regenerative capacity. Succulents are, undoubtedly, amongst those species that adapt well to the environmental conditions of a roof; the *Sedum* (*Crassulaceae*) genus being a popular choice for green roofs/walls given its ability to thrive in shallow substrates and tolerate drought well (Monterusso et al., 2005; VanWoert et al., 2005b; Rowe et al., 2006). Many *Sedum* species activate CAM (Crassulacean Acid Metabolism) photosynthesis under hot, arid climate and water stress conditions (Kluge, 1977; Terri et al., 1986; Gravatt and Martin, 1992; Lee and Kim, 1994; Sayed et al., 1994; Gravatt, 2003), with the stomata in the leaves remaining shut during the day, thereby reducing both transpiration and water consumption at the same time (Cushman, 2001). Another great advantage of sedums is the fact that they can be established in green roofs either in seed form, as plants or using stem cuttings (Getter and Rowe, 2008). However, vegetative propagation is most used due to good percentages of establishment. Worthy of note is also the recent attention being given to direct spreading of non-rooted cuttings as a means of reducing installation costs in large-scale green roof systems (Zanin et al., 2017). Other key factors in this regard are the establishment period of the plant ground cover – generally considered to be between 12-18 months (UNI 11235 2015) – plant density, management and water requirements, etc. Considering the interest on the above method and the seeming lack of agronomic experimental data available, this study aims to further knowledge in this area. Experimental tests on direct spreading of non-rooted cuttings of *Sedum sediforme* (Jacq.) Pau. were carried out in order to study the effects of plant density and cutting-type both on rooting and on the growth and dynamics of a green roof simulated on the ground in Mediterranean environment. Tests were divided into two stages: i) determination of the rooting percentages of *Sedum sediforme* cuttings; ii) determination of the increase in ground cover. This paper was carried out in the framework of the "Ar.Co.Verde" Italian project focused on exploiting and assessing Mediterranean shrubs and cover crops in view of their use in urban green spaces.

2. Materials and methods

2.1. Test site

Tests were carried out from 2011 to 2012 at the CREA-DC Research farm in Bagheria (Sicily, Italy) (38°05'00" N – 13°30'00" E, 78 m a.s.l.). The climate of the area is typical of Mediterranean coastal areas with mild winters and hot/dry summers.

2.2. Description of the test roof system

Tests were carried out in the open air on a simulated roof using 3 wooden platforms placed at ground level, isolated from the soil using black mulch. Each platform was covered in polystyrene and black mulch, and contained 6 test plots. The plots, each measuring 2.20 m x 1.50 m x 0.17 m, were separated by wooden dividers and were filled with commercial extensive green roof system (Perligarden®) by PERLITE ITALIANA (Corsico, Italy).

The roof system (Fig. 1) consisted of:

a) a water drainage layer. A horizontal and vertical ECODREN SD5 layer was used, consisting of a 0.5 cm-thick geonet heat-bonded nonwoven geotextile with a filtering action, which directed water towards drainage holes positioned at the joint between the base and walls of the platform;

b) a water accumulation layer. This was made of expanded perlite (AGRILIT®), with grain size of 0.1-1.0 mm, in 5.0 cm-thick calendered nonwoven geotextile bags;

c) a growth medium for light, large-scale, intensive cover (AgriTERRAM® TVS). It consisted of a mix of peat, lapillus, pumice, zeolites and slow-release fertilizers, weed-seed free with a grain size of 0.0-10 mm and a flat bag thickness of 5.0-7.0 cm.

2.3. Plant material

Based on results from a previous study carried out in Sicily (Tuttolomondo et al., 2018), in which the biotechnical performance of various Sicilian *Sedum* accessions was evaluated, *Sedum sediforme* (Jacq.) Pau. was chosen for this test as it had the best performance results. This species is a perennial, bushy succulent (25-60 cm in height). It has one the greatest ranges of adaptability to climate and altitude (Pignatti, 1982) of the taxa as one of the most commonly found in the wild in Italy. In Sicily, the species is extremely widespread and is found growing from 0 to over 1,300 m a.s.l. It generally thrives in rocky or semi-rocky areas; stony or gravelly environments (dolomitic limestone or argillaceous limestone lithosols and regosols). The tests compared 3 plant densities of *Sedum sediforme* using direct spreading: D1 (100 cuttings m^{-2}), D2 (150 cuttings m^{-2}) and D3 (200 cuttings m^{-2}) and 2 types of cuttings – stem-tip (C) and nodal (N) using a randomized complete block design (Gomez and Gomez, 1984). The test had 6 treatments and 3 repetitions for a total of 18 plots. Before direct spreading, the cuttings of 3.0 cm in length were placed in a dedicated area at an average temperature of 20 °C with relative moisture level below 40% for partial dehydration of the plant tissue. After 3 days, the cuttings were spreaded over the plots, exerting slight pressure with a wooden roller to ensure adhesion to the substrate. All treatment plots were then irrigated immediately afterwards with 3 L m^{-2} of water using a scale-marked measuring can. During the initial experimental stages, the rooting percentage of the cuttings was determined. Following rooting, the area of plant ground cover was then determined in order to calculate ground cover growth dynamics at the end of the test period.

2.4. Rooting percentage

Following direct spreading of the cuttings, rooting percentages were determined 30-60-90 days after spreading by manually disengaging every observation sample from its soil bed.

2.5. Ground cover percentage

Following rooting, from day 90 to day 330 after spreading, a number of measurements were taken to determine increases in *Sedum sediforme* plant ground cover. During observations, two supplementary irrigation events (July and August) were applied, providing approx. 3 L m^{-2} of water to each plot. Manual weeding was also carried out periodically. At 30-day intervals, all plots were photographed with a digital camera located at a distance of 170 cm from the ground. Shutter speeds were set to 'twilight' in order to avoid shadow and unify contrast. Flash photography was not used. The area of plant cover in each plot was calculated by digital image processing. Adobe Photoshop 5.0 version was used to convert the images into grey-scale where black was the plant cover area and white was the substrate. Plant ground cover percentages were calculated using ImageJ software version 1.38, which provides plant ground cover percentages based on the pixels identified in the photographs (Sendo et al., 2010). 330 days after rooting, this latter was evaluated as a function of the factors considered. Ground cover percentage over time was also noted 90 days after spreading and up to 330 days after rooting.

2.6. Climatic data

Climatic data were collected from a meteorological station belonging to the Sicilian Agro-Meteorological Information Service situated close to the test site. The station was synchronized with GMT in order to operate using synoptic forecast models. It was equipped with a MTX datalogger (model WST1800) and various sensors: wind speed sensor MTX (model Robinson cup VDI with an optoelectronic transducer), global radiation sensor (model Philipp Schenk – 8102 thermopile pyranometer), temperature sensor MTX (model TAM platinum PT100 thermoresistance with anti-radiation screen), relative humidity sensor – MTX (model UAM with capacitive transducer with hygroscopic polymer films and antiradiation screen), rainfall sensor MTX (model PPR with a tipping bucket rain gauge) and leaf wetness sensor MTX (model BFO with PCB). In this study, this equipment provided data on average daily air temperature (°C), total daily rainfall (mm) and rainy days per year (n).

2.7. Statistical analyses

All data were subjected to analysis of variance using the statistical software "Past" (V. 3.16 for Windows). Means for each treatment ($\pm$ SE) were separated using Tukey’s test. Rooting percentage and ground cover percentage for the various study cases were analysed using arcsine percentage transformation (Gomez and Gomez, 1984) prior to analysis of variance.

3. Results

3.1. Microclimatic conditions

Over the test period, average air temperature trends were consistent with the ten-year average, with values never falling below 10 °C (Fig. 2). Maximum average air temperature was 34.1 °C in the first 10-day period of August 2012 and minimum average air temperature was 6.7 °C in the second 10-day period of January 2012. Air temperature trends increased from the beginning of April to the third 10-days of August and decreasing until the end of December. Total rainfall was 966.4 mm. Rainfall was highly concentrated between October and December for both years. In the summer period, average monthly rainfall was 17.1 mm. The most significant cumulative rainfall events were recorded in the first 10-day of October 2011 (78 mm) and first 10-day periods of December 2012 (88 mm).

3.2. Rooting percentage

Figure 3 shows average root growth percentages for *Sedum sediforme* during the tests based on the two types of cutting used. Cutting type determined statistically significant differences. Average rooting percentages of stem-tip cuttings were found to be persistently higher (37.6% - 73.8%) than those of nodal cuttings (23% - 63%) from day 30 to day 90 following spreading. The 'density' factor, however, did not determine statistically significant differences in average rooting percentages for the three test periods (Fig. 4). The statistical significance of the interaction "density x cutting-type" highlighted the way in which stem-tip cuttings are able to increase rooting capacity in the treatments with higher density (D2: 73.3%, D3: 72.6%) compared to nodal cuttings (D2: 60%, D3: 63.6%) (Fig. 5). However, no statistically significant differences were found between the two treatment types at density D1.

3.3. Ground cover percentage

The percentage of ground cover 330 days after rooting was influenced by the type of cutting with average ground cover at 68% for stem-tip cuttings and 90% for nodal cuttings (Fig. 6). As regards the 'density' factor, treatment D2 was found to be statistically different to the less dense treatment (D1) with greater plant ground cover at 80% (Fig. 7). Analysis of combined data regarding the density and cutting-type (Fig. 8) for this latter parameter shows how, at equal plant density levels, the greatest ground cover percentages were obtained with nodal cuttings at 82% (D1) and 92% (D2 and D3), compared to stem-tip cuttings at below 70% for all treatments. An aspect of extreme importance is the time needed to complete the ground cover on a green roof, which is considered to be 12-18 months from planting and with a percentage cover of ≥ 80% (UNI 11235:2015). Figures 9-10 highlight the percentage of ground cover of *Sedum sediforme* over time, starting from 90 days after planting, as a function of the factors in the study. It is worth noting that ground cover percentages from nodal cuttings were higher than stem-tip cuttings very soon after beginning observations, reaching 80% 12 months after planting and over 90% by the end of the test period (14 months from planting), thereby satisfying one of the most important aspects for this type of system within the threshold time limits. The relationship between size development and seasonal trends is also clear from the graphs: during spring and autumn plants entered into growth stage, slowing down during the arid summer months and the colder winter period.

4. Discussion

A number of studies have examined survival capacity and plant growth under differing conditions, such as the type and depth of the substrate, irrigation events and fertilization systems (Wolf and Lundholm, 2008; Lundholm et al., 2010; Thuring et al., 2010; Carpenter and Kaluvakolanu, 2011; Schroll et al., 2011; Getter et al., 2011; MacIvor et al., 2013; Panayiotis et al., 2015; Papafotiou et al., 2016). Other studies have focused on the ecosystem functions of plant cover (Van Mechelen et al., 2015; Lundholm et al., 2015; Lundholm, 2016). There is currently a wealth of information in literature on the ecology of germination and vegetative propagation regarding autochthonous plants (Baskin and Baskin, 2014; Thetford and Miller, 2002); however, few studies have looked at their propagation for use in green roofs (Nagase and Tashiro-Ishii, 2018). In Italy, Benvenuti et al. (2016), Casalini et al. (2017), Zanin et al. (2017) and Tuttolomondo et al. (2018) studied both germination aspects and multiplication in xerophytes and autochthonous succulents for use in green roofs. Understanding propagation methods is essential for further development of native plants in green roof (Nagase and Tashiro-Ishii, 2018). The authors of this paper looked at the effect of some of the factors which influence establishment and creation of a *Sedum sediforme* plant cover for use in extensive green roofs using non-rooted cuttings and direct spreading techniques. Results from this study have allowed us to identify the best-suited cutting type and relative layout density for quicker rooting and more rapid ground cover creation. In consideration of the fact that stem-tip cuttings root more quickly and that nodal cuttings (although delaying root formation) ensure quicker and greater coverage in less than a year, the authors do not exclude the possibility of using the two cutting types together in a higher density layout in order to reach the above aims. This contributes towards more suitable *Sedum sediforme* extensive green roof planning in hot, arid climates, where, in fact, new production systems are currently being developed. This is in agreement with Clark and Zheng (2014) who underlines the fact that, when developing green roofs, production times, cutting type and layout density are those factors linked to specific climate regions. Defining the best production and plant cover creation process, starting from a single species, is essential not only to further knowledge but also to encourage the use of native plants in green roof systems. This is also important for a mix of plants whose combined performance is highly influenced by the combination of the single species used. It is important to understand the characteristics of the single species in order to create the correct mixture (Nagase et al., 2017). In reference to the regulation of green roofs in Italy and in Europe, plant cover creation times determined in this study are consistent with the main Italian regulation on the design, construction, control and maintenance criteria for continuous greenroofing (UNI 11235:2015). These times also place *Sedum sediforme* amongst those plants belonging to low-input technologies already widespread in North-Central Europe, and commonly defined in guidelines, such as the German "Guidelines for the planning, construction, and maintenance of greenroofing" (FLL 2008).

5. Conclusions

This study confirmed the good biotechnical characteristics of *Sedum sediforme* and demonstrated the possibility of employing direct spreading of cuttings. The study would also seem to suggest the use of the higher plant density and the more suitable cutting type in order to allow for quicker rooting and ground cover formation. Further research is needed in order to identify the best combination of the two cutting types for the creation of extensive green roofs in a Mediterranean environment.

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Figure captions

Figure 1. Layout of the roof system.

Figure 2. Rainfall and temperatures during the tests period.

Figure 3. Effect of cutting type on rooting percentage at different stages of the test. Values are means ± SE. For each couple of data, histograms with different letters are significantly different at $p \leq 0.01$ and separeted using Tukey's test.

Figure 4. Effects of plant density on rooting percentage at different stages of the test. Values are means ± SE. For each group of data, histograms with different letters are significantly different at $p \leq 0.05$ and separated using Tukey's test.

Figure 5. Effects of plant density and cutting type on rooting percentage 90 days after spreading of cuttings. Values are means ± SE. For each data, histograms with different letters are significantly different at $p \leq 0.05$ and separated using Tukey's test.

Figure 6. Effects of cutting type on ground cover percentage 330 days after rooting. Values are means ± SE. For each data, histograms with different letters are significantly different at $p \leq 0.05$ and separated using Tukey's test.

Figure 7. Effects of plant density on ground cover percentage, 330 days after rooting. Values are means ± SE. For each data, histograms with different letters are significantly different at $p \leq 0.05$ and separated using Tukey's test.

Figure 8. Effects of plant density and cutting type on ground cover percentage 330 days after rooting. Values are means ± SE. For each data, histograms with different letters are significantly different at $p \leq 0.01$ and separated using Tukey's test.

Figure 9. Ground cover over time as a function of test factors (ground cover % - stem-tip cutting).

Figure 10. Ground cover over time as a function of test factors (ground cover % - nodal cuttings).

Figure 1
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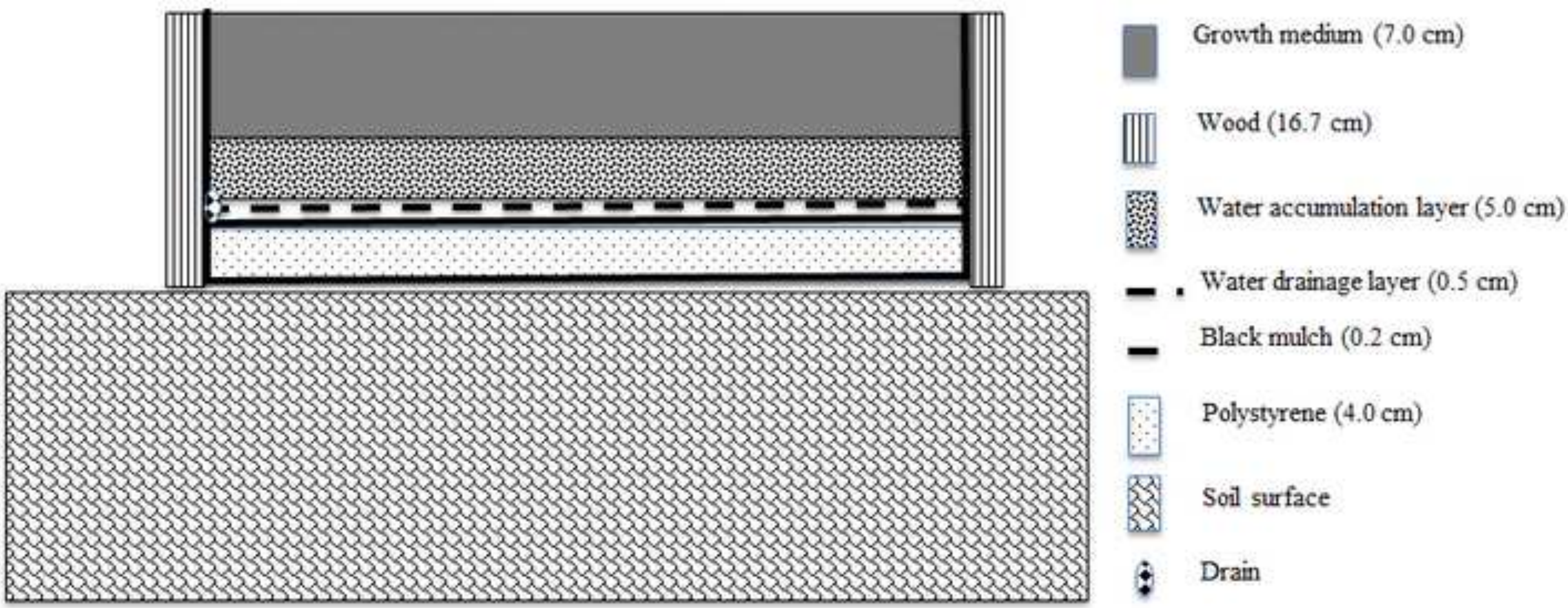

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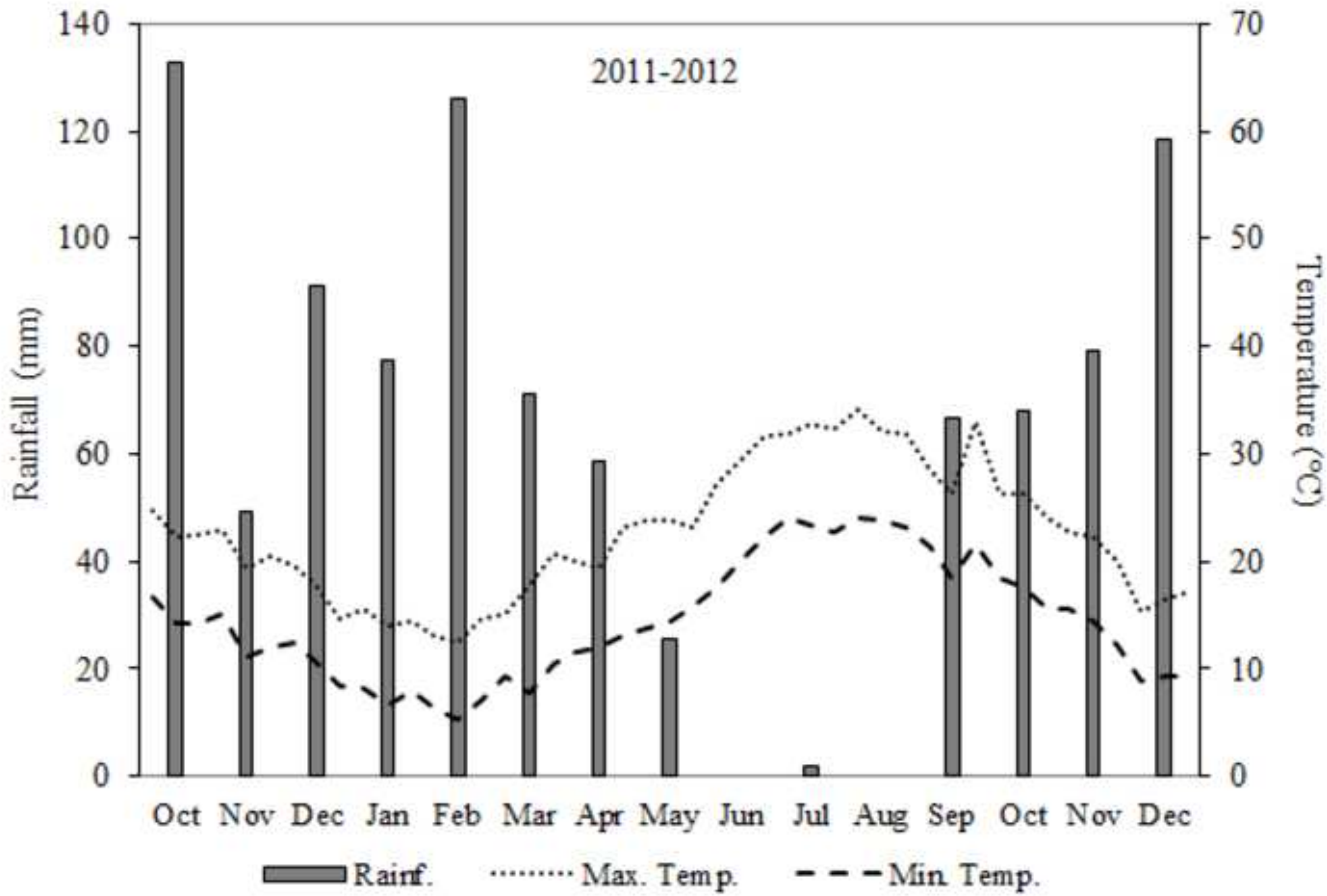

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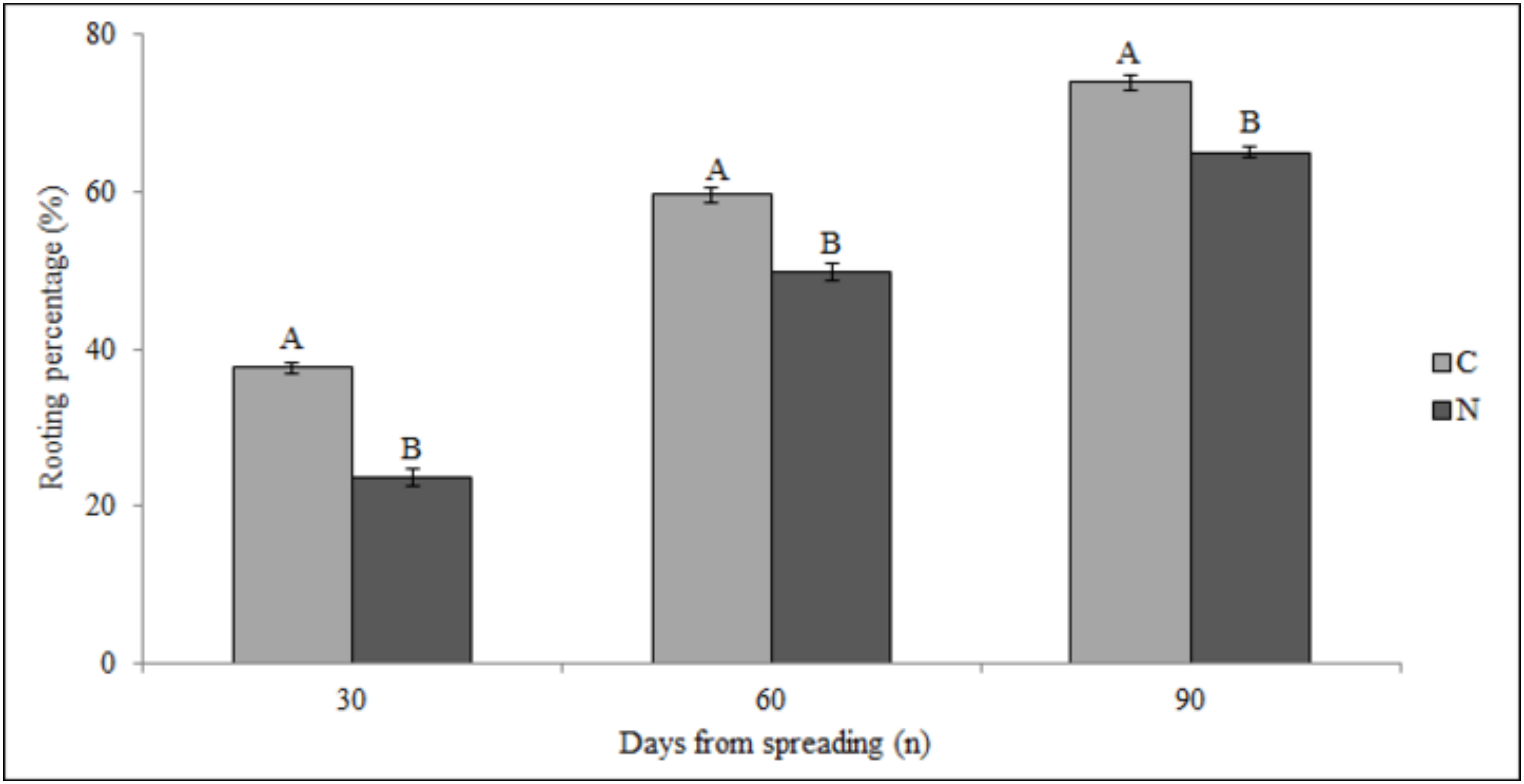

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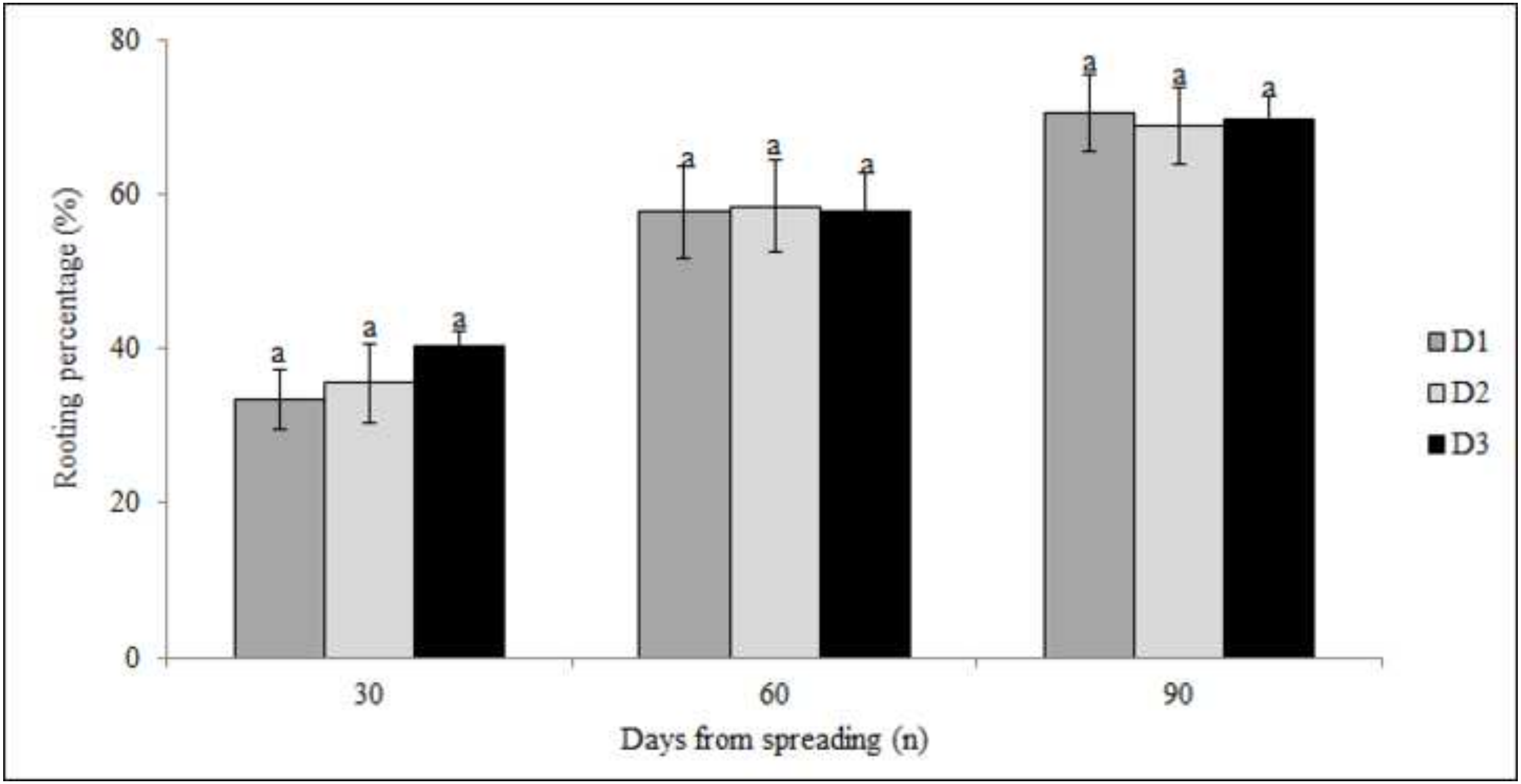

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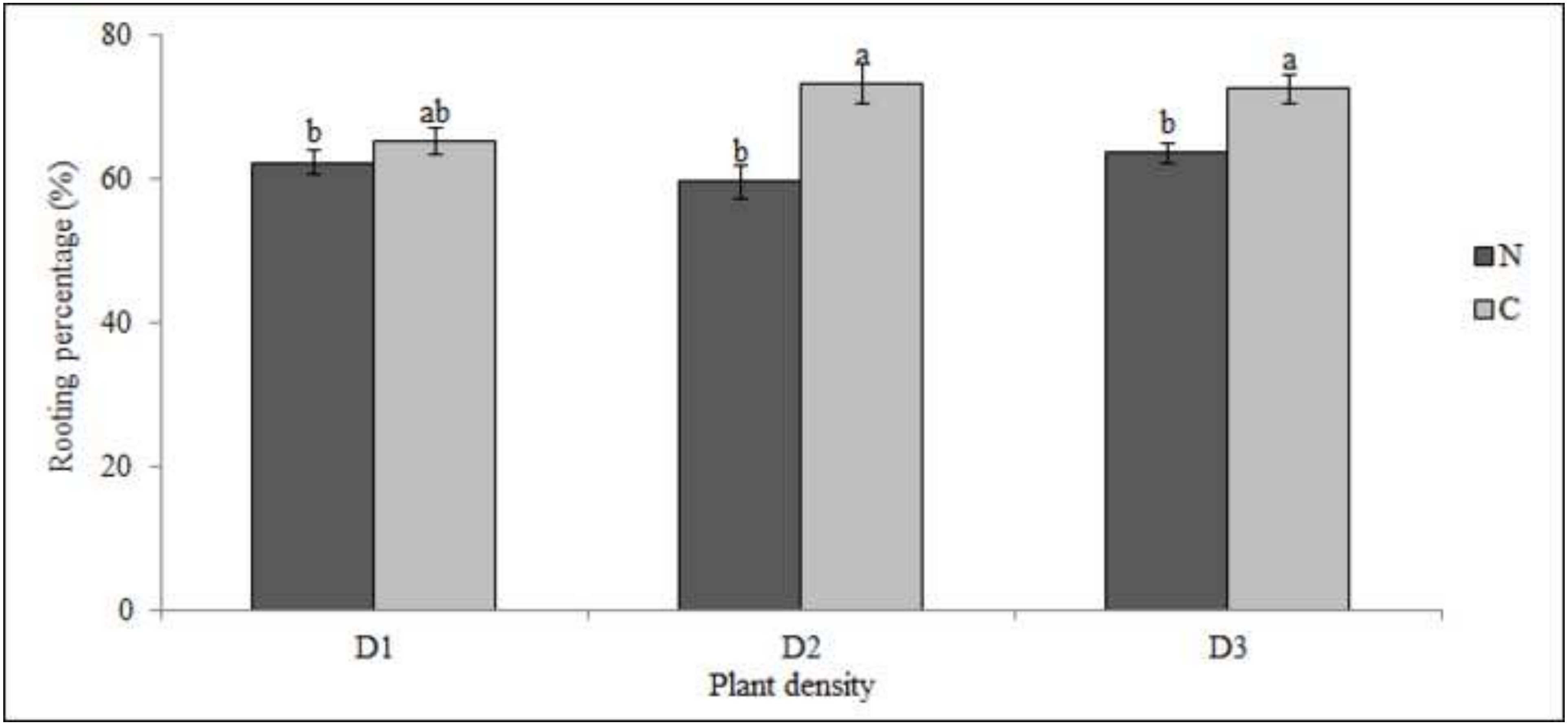

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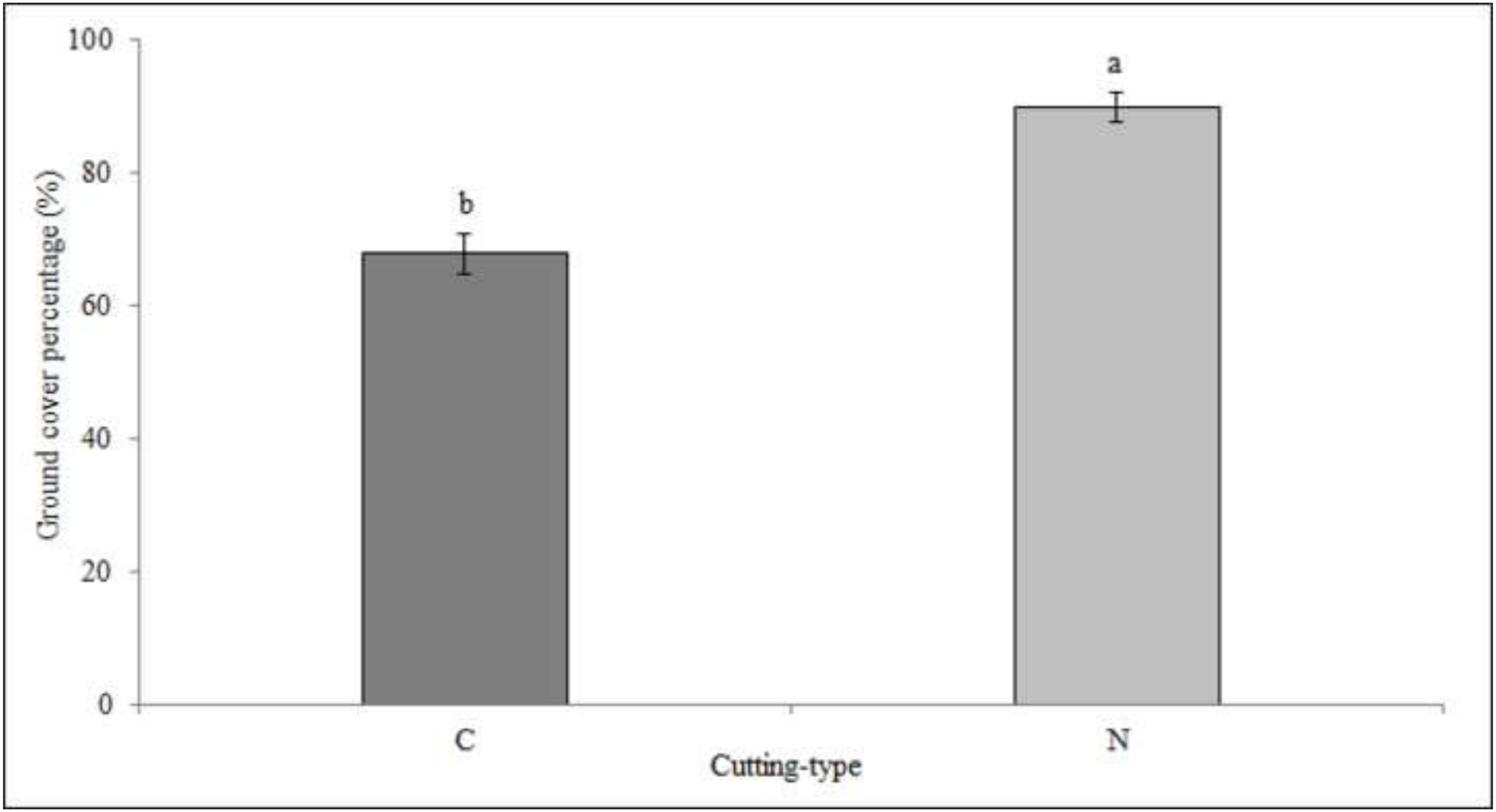

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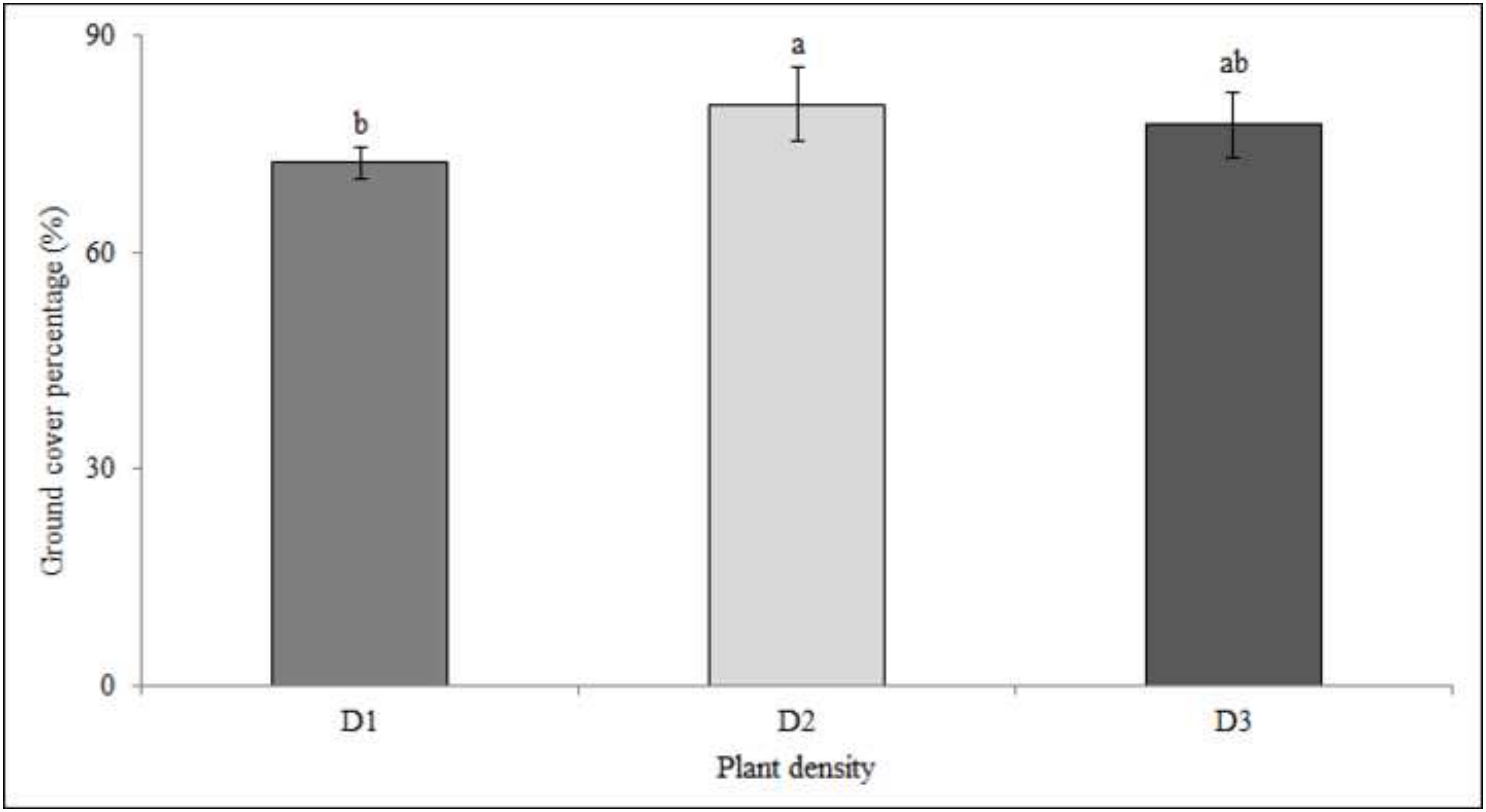

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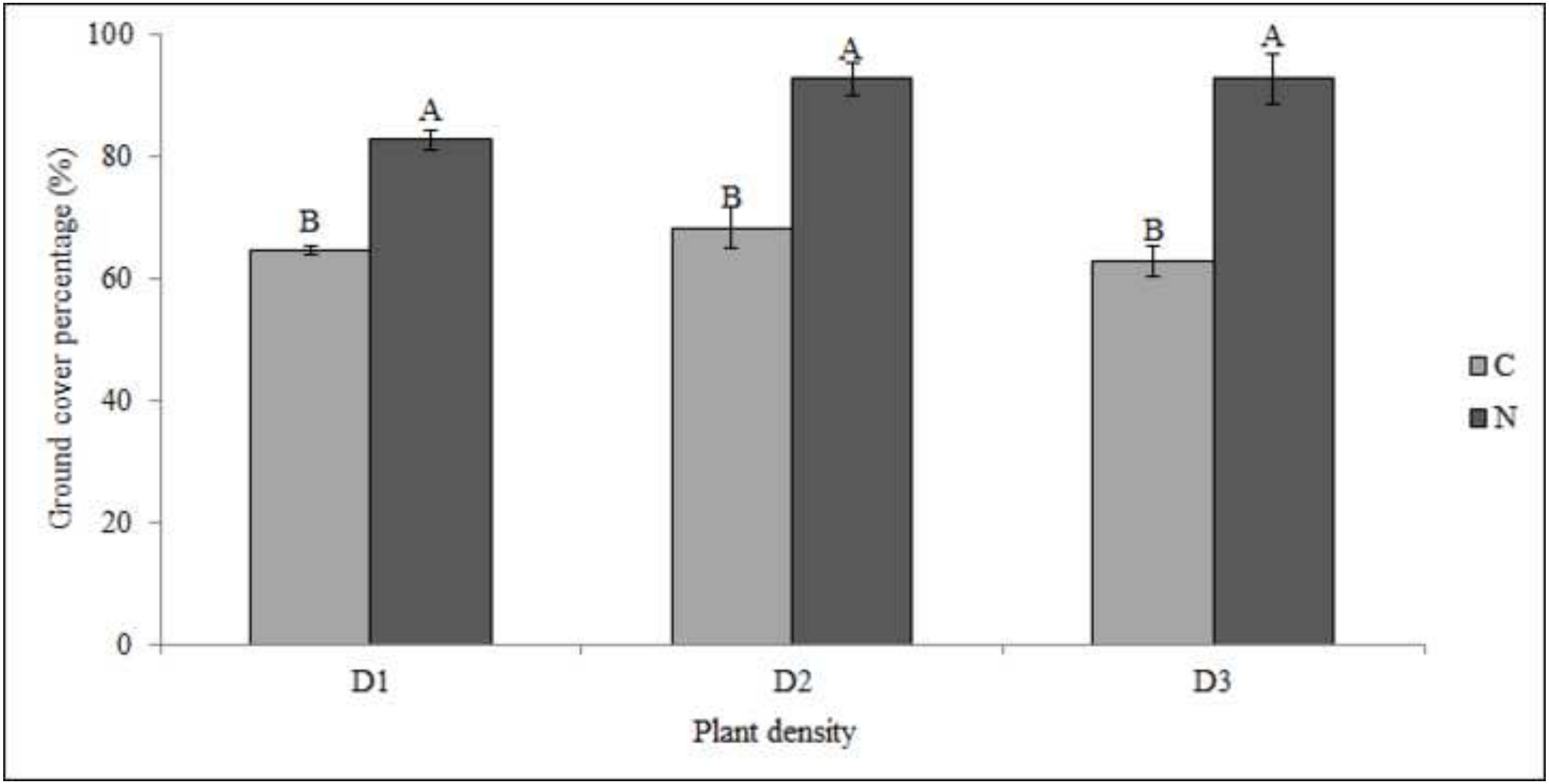

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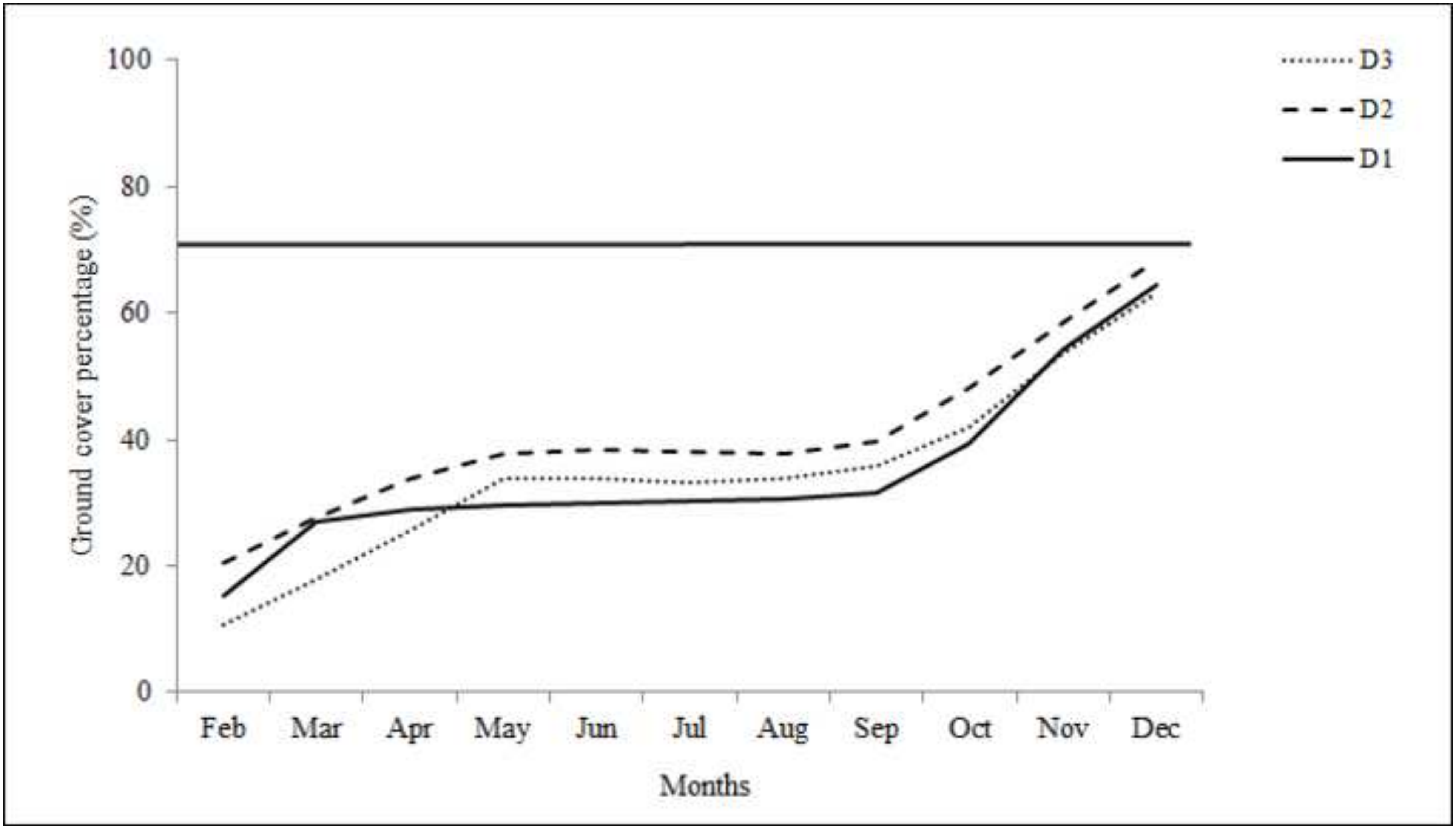

Figure 10
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