grasses

Communication

A New Method for Hybrid Bermuda Grass (*Cynodon dactylon* × *C. transvaalensis* Burtt.-Davy) Vegetative Propagation

Simone Magni [1], Giuliano Sciusco [1,*], Lisa Caturegli [1], Mino Sportelli [1], Tommaso Federighi [1], Marco Fontanelli [1], Alberto Minelli [2], Joseph Scott McElroy [3] and Marco Volterrani [1]

1 Department of Agriculture, Food and Environment, University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy; simone.magni@unipi.it (S.M.); lisa.caturegli@gmail.com (L.C.); mino.sportelli@phd.unipi.it (M.S.); t.federighi@studenti.unipi.it (T.F.); marco.fontanelli@unipi.it (M.F.); marco.volterrani@unipi.it (M.V.)
2 Department of Agricultural and Food Sciences, University of Bologna, 40126 Bologna, Italy; alberto.minelli@unibo.it
3 Department of Crop, Soil, and Environmental Sciences, Auburn University, Auburn, AL 36830, USA; jsm0010@auburn.edu
* Correspondence: giuliano.sciusco@agr.unipi.it

Abstract: Hybrid Bermuda grasses (*Cynodon dactylon* × *C. transvaalensis* Burtt.-Davy) represent one of the greatest contributions to the growing quality of turfgrass in the warm season and transition zone areas of the world. Hybrid Bermuda grass production relies on vegetative propagation from sod or sprigs. In the past, efforts have focused on improving the technique of stolonizing (or sprigging) for establishment in new areas. Such propagation requires bulk harvesting and planting of all rhizomes and stolons. We have developed a novel method of propagation and establishment from a single node harvested from greenhouse grown stolons. Despite a stolon fraction bearing a single node being suitable for effectively propagating a warm-season turfgrass, the technique has been held as economically impractical until now. Our method has been developed to obtain the multiplication of plant material in soilless conditions by harvesting single-node sprigs, propagation of plants from the single nodes, and transplant of single plants in the field. The investigation aimed to identify values for method set-up. Indeed, node and internode size variability with differential between maximum diameters is crucial for discrimination. For Patriot Bermuda grass stolons, nodes exhibited a maximum diameter of 2.43 ± 0.46 mm, while internodes had a maximum diameter of 1.54 ± 0.16 mm. Based on these findings, a 2 mm sieve was selected, achieving an optimal ratio between the node fraction and internode residues. The sieve yielded 87% of node fractions and only 1% of internodes from the initial mix, demonstrating its efficacy. Further results for the transplanting phase indicated that a double release resulted in an average success rate of 98.8%, with only 6.9% blank cells when using a single release. The average was 149 plants per tray over 160 cells, representing a 93.1% success rate. These results underscore the efficiency and acceptability of the overall propagation process in alignment with market references.

Keywords: node propagation; sustainable turfgrass establishment; turf nursery

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1. Introduction

Bermuda grass (*Cynodon dactylon* (L.) Pers.) is extensively grown in the warm season and transition zone areas of the world due to its tolerance to heat and drought [1]. The aggressive lateral growth gives the turf a self-repair ability that makes it the first choice for sports surfaces and worn areas [2]. Based on these main traits, the species has undergone extensive genetic improvement, and a wide number of cultivars are now available to turf growers for specific uses ranging from low-maintenance general-purpose areas to highly manicured golf greens. Sterile hybrid cultivars have become a quality standard for bermudagrass turf and vegetative propagation a related necessity [1]. Even in cultivars that are potentially seed producers, vegetative propagation is adopted to maintain desirable traits true to type [3].

The standard method for Bermuda grass vegetative propagation is based on growing a given cultivar in the field from which sod or stolons and rhizomes, collectively referred to as sprigs, are harvested to establish new areas. This necessitates the establishment and maintenance of turf whose extension and duration in time are set based on the potential market demand. Once the selling target is defined, land allocation, water consumption, machinery assortment, fuel consumption, labor need, fertilizer, and pesticide use are all inputs that become costs with low adaptability to actual market demand [4]. The field production of cultivars with high standards of genetic purity forces producers to adopt stringent protocols to prevent cross contamination of cultivars, weed encroachment, and pathogen infestation, all of which turn into additional inputs or costs [5]. Post-harvest life of the product, either sod or sprigs, is a further source of indirect production costs since moisture and temperature conditions before the transplant can dramatically affect sprig vigor establishment uniformity and can occasionally determine unsuitability for use [6]. Hence, harvest, hauling and transplant must be carefully coordinated to reduce the exposure of plant material to unfavorable environmental conditions, dissipate heat, and encourage rooting at the planting site. Due to these limiting factors, the area served by a single producer strongly depends on the ability to preserve a post-harvest quality that meets customer expectations.

Previous efforts devoted to increasing sprigging efficiency have focused on methods that encourage root and shoot growth, but the actual amount of propagation material and the percentage of its growing points that generate new plants remain poorly defined [7,8]. Based on the constraints of the standard Bermuda grass vegetative propagation and pursuing the "one node-one plant" philosophy, we have developed a system designed to convert single nodes into complete plants and to mechanically transfer these plants into the soil for turf establishment. The system has the potential to dramatically reduce land demand for Bermuda grass production while facilitating weed and pest control as well as maintenance of genetic purity. Furthermore, post-harvest stress occurring during hauling is expected to be reduced to a minimum since propagation material is transported in the form of actively growing, well-watered plants instead of as bulk sprigs or rolled sod. The objective of the current paper is to describe the new propagation system while providing potential benefits for the producer in comparison with the methods traditionally adopted for the same purpose.

2. Materials and Methods

The proposed single-node propagation method has been developed adapting different methods and equipment routinely used for horticulture crops. To test the innovative propagation method, appropriate experiments have been carried out at Azienda Hi-Turf Solution s.r.l., Arena Metato (43.7776° N, 10.3796° E, Pisa, Italy). The single-node propagation consists of three main steps: (i) base production—growing plant base material; (ii) single-plant propagation—production of single-noded sprigs to be converted into plants in honeycomb trays; (iii) transplant and stand development—transplant and post-transplant care. The three main steps are depicted and described hereafter (Figure 1).

2.1. Plant Material Production and Stolons Harvesting

Base plant material consisting of a hybrid Bermuda grass (*Cynodon dactylon* × *transvaalensis* cv. Patriot) was grown in a greenhouse located in Arena Metato (43.7776° N, 10.3796° E, Pisa, Italy) with no heating. At that latitude, the daytime temperatures vary between 0 and 15 °C in winter and 25 to 40 °C in summer. The growing season for Bermuda grass in greenhouse conditions extends from March to October. Plants were raised in plastic nursery pots with 10 cm diameter, 7.7 cm height, and 0.4 L volume filled with a mix of 25:25:50% v/v sphagnum white peat, sphagnum black peat, and coco peat (Gebr. Brill Substrate GmbH & Co. KG, Georgsdorf, Germany) as a growing medium. Pots were arranged in 31.5 cm rows with alleyways 55 cm wide every four rows of pots. Moreover, pots were positioned on benches 90 cm above ground level to facilitate stolon elongation and to avoid contact with the floor.

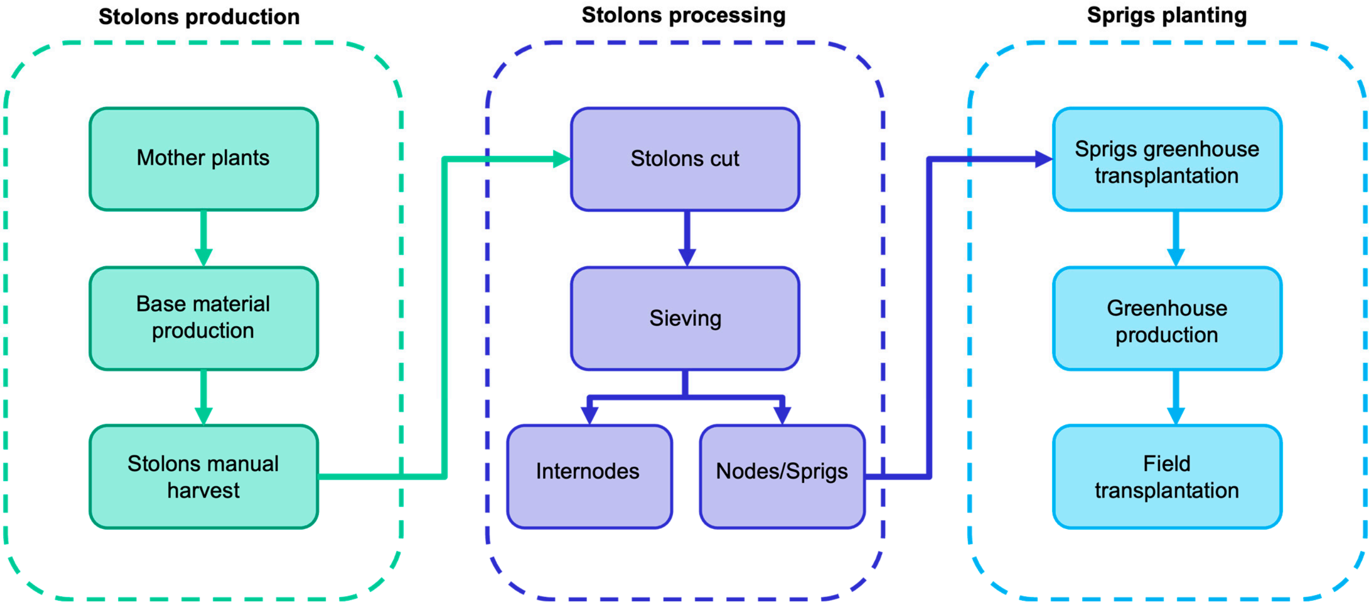

Figure 1. Single-node propagation method conceptual diagram.

Irrigation was carried out with an automated boom system delivering from 1 to 6 mm of water per day according to daily water demand. Nutrients were supplied via fertigation during the active growth period. Fertilizer was applied on a weekly basis using alternatively 30N–4.3P–8.3K and 9N–6.5P–24.9K soluble fertilizers (Grow More, Gardena, CA, USA). During this phase, pots were left unmown, allowing plant stolons to elongate downward on both sides of pot row. As mentioned, the proposed single-node method propagation has three phases: stolon harvesting and processing, stolon planting, and plant development. Stolons were harvested by hand using scissors, and in general, this operation was carried out between May and June. After excision from the donor plants, stolons were collected in bunches and further processed to obtain smaller sprigs. After stolons are harvested, a good practice is to scalp pots to stimulate new leaves and new stolons production. Renovation of growing medium is also suggested to keep aeration, permeability, and water availability as constant as possible. Based on preliminary trials, the best way to maintain these conditions is to pull out potted materials (consisting of growing medium, roots, and rhizome cylinders), cut them into four equal pieces, and transplant them as plugs into pots refilled with fresh growing medium. At this stage, the number of donor plants could be increased by four compared to the initial number or maintained, as in the previous production cycle, by discarding the excess plugs. From July to October, donor plants produce most of the new stolon biomass intended for the following production cycle, with only minor stolon elongation occurring in March and April.

2.2. Stolons Processing

A stolon fraction must contain at least a single node to develop a new plant to be considered a sprig. A total of 800 internode length measurements were conducted, yielding an average of 2.18 ± 0.97 cm (Table 1). Due to the excessive variability, an automated system of cutting should hypothetically adapt its settings for every single operation. In theory, the system may be able to scan the stolon, identify the node and the internode portions, and produce cuts below and above the node. After the stolon-processing phase, two types of products were obtained: stolon fragments containing the node (sprigs) and fragments corresponding to internode tissue, which must be discarded. Fragments with nodes should be separated from the rest of cuttings and collected for use. This process is assumed to be possible only for single stolons. The need for simple mechanical equipment suitable for processing a large number of stolons has led to the adoption of a different approach. Assuming that random cutting of stolons produces fragments with and without nodes, the cut material can subsequently be divided into two fractions based on cross section size. Based on these assumptions, stolon cutting has been carried out with an automated cutting machine (Timatic HerbCut mod. TS 1340, Tecnolab, Spello (PG), Italy) consisting of a conveyor belt that delivers plant materials to an alternate motion blade. Thus, stolons were positioned at a right angle to the cutting line, and the length of cuttings can be adjusted from 6 to 40 mm by modifying the conveyor's pace. The machine has been set to produce fragments with approximately 2.0 cm length to fit into the 2.5 cm diameter cells of the honeycomb trays. The cutting unit settings produce the expected cutting sizes only if ideal geometric asset is maintained (i.e., stolons remained perpendicular to cut line, conveyor belt and plant material stayed in perfect contact, and low plant material thickness). An angled position of the stolons with respect to the cut line, slipping of the plant material on the conveyor belt, and the height of the plant material under the blade cause the actual length of cutting to slightly deviate from the expected value. Due to these factors, a total of 100 random samples of cut stolon fragments were measured after cutting. Based on these nodes and internodes and diameter size distributions, different hypothesis tests were carried out to assess the best sieve size for separating the two resulting stolon fragments.

Table 1. Patriot Bermuda grass internode length and node and internode diameter (Ø) of lateral and vertical sections sizes. Data were average over 800 readings for internode length and over 200 readings for each section.

	Internode Length (cm)	Node Ø (mm)		Internode Ø (mm)	
		Lateral Section	Vertical Section	Lateral Section	Vertical Section
Mean	2.18	2.43	2.15	1.54	1.28
Standard deviation	0.97	0.46	0.35	0.16	0.12
Min	0.95	1.04	1.12	1.08	1.09
Max	3.86	3.58	2.85	2.05	1.51

2.3. Sprigs Planting

After sieving, sprigs are delivered to a vacuum drum seeder (DaRos Seeder SEM100, Sarmede (TV), Italy), which picks up sprigs and lays them in rows aligned with the cells of nursery plastic trays. The rate and spacing of propagation material can be adjusted to different tray sizes and numbers of cells. In order to compensate for blank cells, the equipment can be set to accomplish a multiple release of seed. For testing drum seeder release accuracy, a total of ten 160 cells plastic trays were processed with single- and double-seed release. Success rate was assessed.

To propagate turfgrass plants, plastic nursery trays with 160 to 220 cells were considered suitable and fit most of the machinery involved in the process. With these trays, cell volume and density ranged from 20 to 15 cm^3 and from 934 to 1184 cell m^{-2}, respectively. Tray cells were filled with peat prior to being directed to the drum vacuum seeder. Once the propagation material was laid in the cells, the trays were irrigated and positioned over benches in the greenhouse with the same growing conditions described for the base plant material. Even if kept in ideal conditions for bud sprouting, not all cuttings bearing nodes were expected to generate plants due to variability in plant material. To have an estimation of the overall effect of seeder failures, cutting purity, and plant material viability on the number of plants per tray, 100 trays were prepared and left to grow for a two-week period.

3. Results and Discussion

In Figure 2, different steps of the single-node propagation method proposed are depicted. Hereafter, results from experiments are reported.

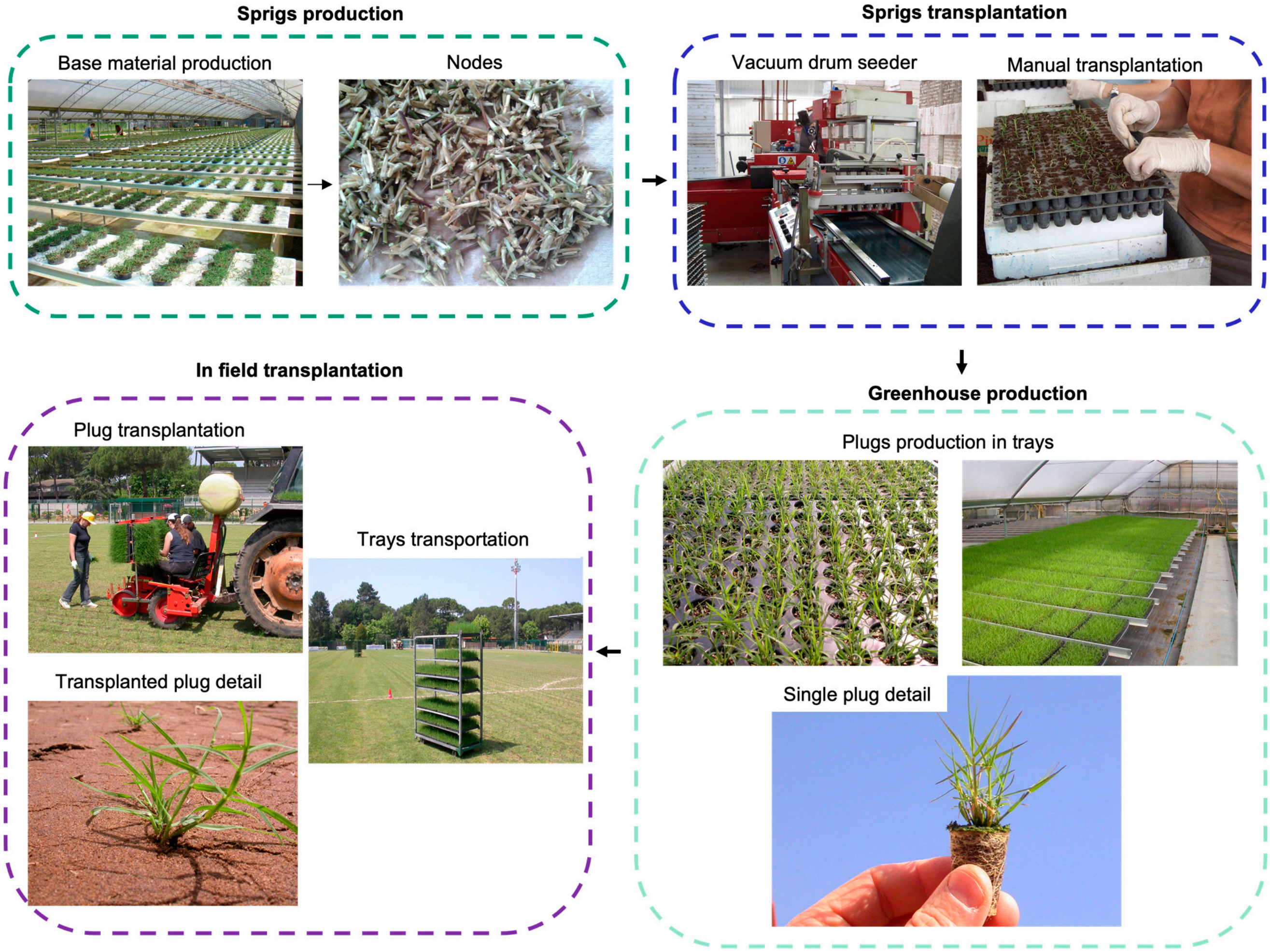

Figure 2. Different phases of the proposed single node propagation method realized both in greenhouse and in field.

A total of 100 random samples of cut stolon fragments were measured after cutting, showing that only 1.5% of the cuttings were longer than 2.0 cm (sporadically longer than 2.5 cm); therefore, cuttings obtained using the cutting unit fall in the suitable size range for use in standard plastic trays. Once cuttings are produced, those bearing at least one node must be separated from the others. Different dimensions between stolon nodes and internodes have been identified as the best method for separating the two fractions via sieving. For internode length, node size and internode size display some degree of variability. To identify the most appropriate sieve for discriminating node and internode size, minimum and maximum diameter (Ø) of node and internodes were measured on a sample of 200 Patriot Bermuda grass stolons for a total of 800 readings. Results indicated that both nodes and internodes have an elliptical shape, and the possibility of separating node relays from internode relays on the differential between maximum diameters, which were 2.43 ± 0.46 mm and 1.54 ± 0.16 mm, respectively (Table 1).

Results of node and internode Ø values were tested on different sieve sizes, and results are presented in Table 2.

Table 2. Patriot Bermuda grass node and internode diameter (Ø) section size proportions tested for different sieving sizes.

Sieve Size (mm)	Node	Internode
	Fragments %	
>1.0	100.00	100.00
>1.5	94.31	60.66
>1.6	93.36	28.44
>1.7	92.89	12.32
>1.8	91.47	5.21
>1.9	89.57	1,90
>2.0	**86.73**	**0.95**
>2.5	46.45	0.00
>3.0	8.06	0.00
>3.5	0.95	0.00
>4.0	0.00	0.00

The bold indicates the best result obtained with a >2.0 mm sieve size. It provides the highest value of node fragments and the lowest value of discarded material (internode fragments).

According to the results (Table 2), a 2 mm sieve was chosen to yield the best ratio between the node fraction and the internode residues: 87% of the node fractions and 1% of internodes of the initial mix.

Results on different seeders' efficacies are reported in Figure 2.

When a double release was adopted, on average, 2 cells over 160 remained blank; 22 had a single stolon fragment; and the remaining had 2 or, occasionally, more fragments, for an average success rate of 98.8% (Figure 3). Assuming that where multiple drops occur, at least one should bear one node and that the propagation material has 90.6% of nodes, two more blank cells are expected to occur.

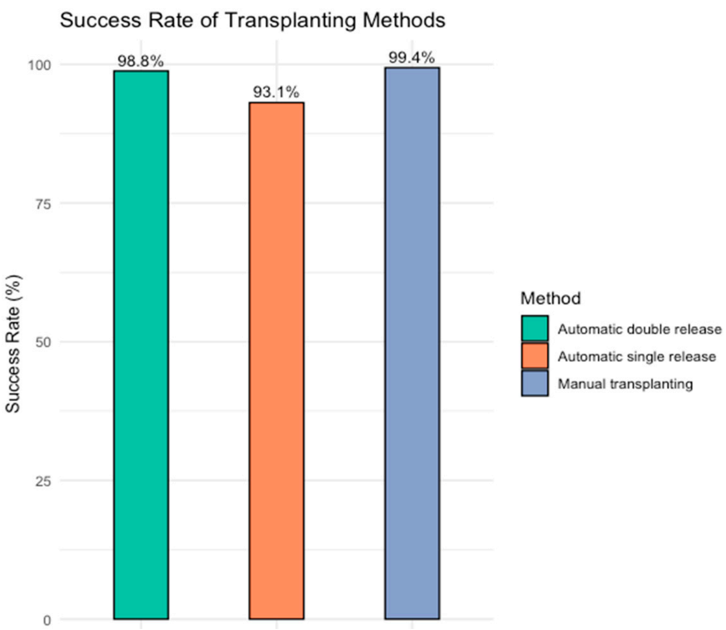

Figure 3. Success rate of Bermuda grass sprig transplantation comparing hand-transplanting and single and double release.

Single release resulted with an average of 149 plants per tray over 160 cells, this representing 6.9% blank cells and an average success rate of 93.1% (Figure 3). With reference to horticulture standards, 10% blanks is considered the upper limit for tray acceptability. Therefore, the overall process of propagation has been considered acceptable and in line with market references.

Full maturity of newly generated plants is typically reached between weeks 5 and 6, when shoots have developed three to five leaves and plugs are firmly held together by the root system. Plug resistance to handling is of primary importance for the subsequent transplant stage, and it is visually evaluated to assess the degree of plant maturity. For the transplant and stand development phase, cell tray hauling and handling and the transplant of seedlings are adapted from common practice in open-field horticulture. Transplanting machinery is available with different technologies depending on what type of crop is to be transplanted and on in what soil conditions the transplant will take place. For the transplanting of cell-propagated Bermuda grass plants, some basic targets were identified to allow plants to establish quickly and uniformly: positioning plugs with the correct orientation, at the correct depth in the soil, with a given spacing on the rows, and with good contact between plugs and soil. Machinery designed for general horticultural purposes and specifically dedicated to grass plants (rice) was tested. Apart from different tray loading capacities, labor requirements, suitabilities to different soil preparation, and other aspects related to the transplanting procedure (not discussed herein), all the equipment compared proved efficient in achieving the basic targets.

Lateral spread of single plants and accomplishment full ground cover has been described in [9,10]. Plant density, turfgrass cultivar, time of year, general weather conditions, and maintenance skill (irrigation, fertilization, mowing, sand dressing, and rolling) all contribute to plants' growth rates, which ultimately leads to establishment speed, but in general, a density of transplant ranging from 15 to 20 plants m^{-2} can be brought to full ground cover in 8 weeks (Figure 4).

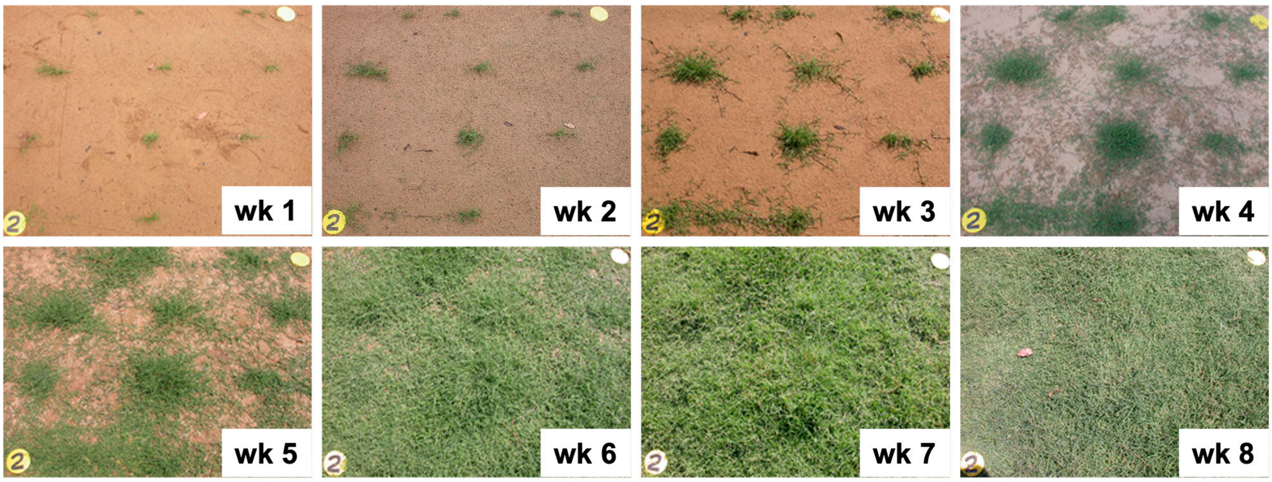

Figure 4. Turf cover over time. Turf development from plugs up to 100% established in eight weeks.

Based on the time needed to obtain full ground cover, there is no major advantage compared to alternative establishment methods, such as sprigging or seeding, but several other aspects can produce direct or indirect benefits that can make single-node propagation competitive. For example, from a production perspective, the potential to propagate every single node of donor plants dramatically reduces the need for surface at the nursery production stage. When sprigging a new surface, the number of bushels recommended to efficiently cover a given area does not tell much of the surface from which the propagation material was harvested. One tenth of the covered surface is generally considered necessary for harvesting sprigs in the nursery, but again, little is known about how many growing points are collected from a given nursery surface and what proportion will generate new plants. From an estimate carried out on Patriot Bermuda grass grown in pots as described above, assuming nodes under 2 mm are discarded and that 50% of nodes are used for double "seeding" to reduce blank cells, a square meter in the greenhouse has the potential to yield enough plants to cover a surface approximately 70-fold greater with a 20 plants m^{-2} density.

The expanded ratio between the surface served and the nursery surface makes it easier for the producer to adapt the farm size to the market demand both in terms of surface and time. On the other hand, sales under the expectations do not produce as high maintenance costs as open-field sod cultivation. Furthermore, greenhouse production extends early and late in the season compared to field conditions, thereby allowing late adjustments to production for future needs.

Greenhouse growing conditions help maintain the purity of a cultivar with low costs for the producer. Once an off type or weed is detected, pots are simply discarded and replaced with new ones, the rest of the nursery being unaffected by the problem. The process of harvesting only stolons growing outside the pots yields plant parts that are clean of soil contamination, and the potential to disseminate weed seeds or propagules is very low. Fungal diseases can be efficiently controlled, and the application of chemicals in an enclosed environment could help reduce aerosol drift or such side effects as soil and water contamination.

Plants raised in cell trays have well-developed root systems, shoots, and leaves and can rely on water and fertilizer stored in the growing medium for a long time. This has great implications on handling, hauling, and post-transplant care. Hauling is accomplished using standard nursery trolleys. For long distances or unpredictable time before transplant, trays are stacked in single layers of four trays on eight shelves per trolley.

Assuming trays with 220 cells with 10% blanks, each tray contains 198 plants, which turns into 6336 plants per trolley, or a final transplanted area of 317 m^2 with a 20 plants m^{-2} density. This arrangement allows maintenance of healthily growing plants for days as long as water can be applied when needed. When hauling is expected to be completed within 12 h, 20 trays can be piled up in 5 layers for each shelf, and trolleys with 5 shelves may contain 100 trays. With same assumptions as above, this arrangement allows the transportation of 19,800 plants—990 m^2 transplanted area—per trolley.

The studied propagation method offers several distinct advantages. Firstly, the integrity of roots and leaves, coupled with water stored in the growing medium, ensures efficient thermoregulation, promoting overall plant health. Unlike sprigs, concerns related to heating and water stress are mitigated. Additionally, immediate post-transplant irrigation is unnecessary, as active growth promptly commences after transplantation provided that light intensity and temperature conditions are favorable. Plant losses in the field primarily result from incorrect irrigation coverage, with irrigation system failures causing only minor delays rather than complete plant desiccation.

The utilization of transplanting machinery further enhances the superiority of the cell-grown plant system. Unlike seeds or sprigs, which require tilling for optimal seed-to-soil or sprig-to-soil contact, transplanting machinery deposits plants into furrows, allowing soil compression around plugs through specialized rolls. This furrow can be created in tilled soil or even in existing sod, a particularly advantageous feature in the conversion of fairways from cool season to warm season turfgrasses. The conventional method of removing cool season sod is both costly and time-consuming, significantly impacting soil shapes and contours. Alternatively, chemical removal of existing turf and sod thinning through scalping and repeated verticutting has proven to be an effective soil preparation method for successful cell-grown Bermuda grass transplant and establishment. Retaining the existing sod and avoiding soil tillage ensures easy machinery access to transplanted areas throughout the establishment period, facilitating growing-in operations and overall maintenance.

Encouraging plant growth is the main aim of post-transplant care. Frequent irrigation and weekly fertilizations are the two main factors that can affect establishment rate. Water application of 5–7 mm day^{-1} are expected to return 100% of daily water loss during summer, while 50 kg ha^{-1} of nitrogen per week is applied to stimulate green tissue production and quick lateral spread. For sprigging, sand top-dressing and rolling are used to smooth the surface and stimulate the rooting of stolons. As soon as the surface is smooth enough, mowing is started, with cutting height being gradually adjusted to the desired value.

Pre-emergent weed control can be used, as can other vegetative propagation methods.

Single-node propagation has the potential to reduce land demand for turfgrass production, have a greater adaptability to market demand, and provide propagation material with enhanced cultivar purity. The system allows better exploitation of the reproductive potential of donor plants, thus reducing the overproduction of base material. Raising a reduced number of complete plants reduces hauling volume and minimizes the risks of heating and loss of product due to inefficient delivery of transplant. Propagation material for a given area is well defined in quality and quantity, and successful establishment is accomplished by virtually all plants. Conversion to a new turfgrass is less invasive and less expensive, with some of the operations being unnecessary for effective establishment.

4. Conclusions

In conclusion, the implementation of the single-node propagation method has proven to be a transformative approach successfully utilized in the conversion of numerous golf courses as well as the establishment of soccer pitches and polo grounds in Italy and neighboring countries. This method has demonstrated its adaptability, particularly in regions in which turfgrass production is not well-established and in which market demands do not warrant the establishment of large nurseries or offer a diverse range of cultivars. Single-node propagation emerges as a valuable opportunity for producers to supply affordable and dependable plant material to meet customer needs. The advantages extend beyond traditional sod production limitations, as the ease of hauling facilitates market expansion beyond conventional coverage areas. Furthermore, the cultivation of base material in pots enhances flexibility, enabling producers to promptly respond to evolving market trends by introducing new cultivars. Looking ahead, future studies will focus on the economic and resource-saving aspects of this innovative propagation method, emphasizing its potential for enhanced use efficiency in the realm of turfgrass production.

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