BAP Spray and Plastic Container Responses on *Asparagus officinalis* L. Crown Growth

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Abstract: The aims of this work were to study the effect of two different plastic containers on asparagus growth and the effect of early applied 6-benzilaminepurine (BAP) on crown growth during the first two years after seed germination. Although there was not found a root restriction effect with the use of plastic containers, there were significant differences between plants grown in plastic seedbed or single pots which suggesting an unusual and unexpected asparagus autotoxicity. The results showed that crown fresh weight, total dry weight, relative growth ratio (RGR_{root}), root:shoot ratio and photosynthetic shoot number increased in BAP-sprayed plants over the controls without treatment as a result of a change in photosynthate partitioning towards the root system. The BAP sprays seem to be a greater effect under conditions with favor autotoxicity such as the seedbed than in single pot crown-grown.

Key words: Autotoxicity, biomass partitioning, cytokinins, propagation, root restriction.

1. Introduction

Asparagus (Asparagus officinalis) is an important perennial vegetable crop; the persistent crown acts as a storage organ, with fleshy roots acting as the plant carbohydrate storage reserve. Current photosynthesis does not directly contribute to spear growth, under field conditions it is regulated by both genetic and environmental factors that influence photo assimilate production and partitioning [1, 2]. Yield depends on the ability of accumulated carbohydrates in the store roots elaborated by fern activity in the previous season before spear harvest and, possibly also in preceding years [3]. Dufault [4, 5] and Dufault and Ward [6] suggest that the capacity for aged fern to produce carbohydrates was severely reduced.

One- or two-year old asparagus crowns, produced from seeds in high-density nursery plantings, are

commonly dug and planted into commercial production fields during the spring. Studies in asparagus physiology have shown a close relationship between the amount of root storage carbohydrate and spear yield [7, 8]; only a limited number of studies have been conducted on the root growth of asparagus and all have been focused on the growth after crown transplant [9]. Asparagus growth studies have investigated changes in fern and root dry weight and bud numbers during the first year after plant establishment [10] but, biomass accumulation during the time before transplant has been received a very low attention.

Asparagus seed germination, usually made at field bed seeds, has been substituted during the last years by plug trays. For improving asparagus crown growth, seedlings would be cropped in plastic containers under greenhouse facilities [11]. However, the common root restriction found in small containers [12] has not been evaluated up to now for asparagus crown growth.

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Benziladenine (BAP) stimulated shoot emergence in all three asparagus cultivars tested (including UC 157F2) by Mahotiere et al. [13]. The progressive emergence of asparagus spears is caused by apical dominance shoot inhibition. Applied cytokinins may affect apical dominance by releasing lateral shoot buds from inhibition [14, 15]; however, there was not a clear relationship between cytokinin application and yield in field grown asparagus plants. It has been found [16] that a single BAP foliar spray in fall promoted spear sprouting after the application and enhanced thickness of spears; however, the effect was not observed in the following spring. A BAP spray to young spears in spring also promoted spear sprouting after the application, although an effect on thickness of new spears was not observed. The effects of three successive yearly BAP sprays were almost the same as those of a single year application. Spear sprouting simulation was only observed just after the application, but not in the following seasons; no significant influence on crown growth was observed following successive application [13].

The aims of this work were to study the effect of two different plastic containers seedbeds on asparagus growth and the effect of applied BAP on crown growth during the first two years after seed germination.

2. Materials and Methods

Asparagus F-2 “UC157” seeds were germinated and grown in 50 plastic plug trays filled with a high peat-based growing media (Klasmann 411[®]). Experiment was developed under greenhouse facilities located on the University of Buenos Aires campus, Argentina (34°28'S) from October 30th, 2009 to June 4th, 2011. Greenhouse was kept open during autumn and winter to allow crowns became dormant. Plants were transplanted at December 18th, 2009 to (a) individual 3,000 cm³ pots (0.16 m top diameter × 0.15 m tall) and (b) 45 dm³ plastic seedbed (0.54 m long × 0.32 m wide × 0.26 m tall). A density of 25 plants·m⁻²

for both plastic seedbed and individual pots was used. In both container treatments a high peat-based (Klasmann 411[®]) growing media were used. Seedlings were sprayed to runoff with different BAP (6-benzilaminopurine) (SIGMA EC 214-927-5) solutions (0, 5, 50, 100 and 200 mg·L⁻¹) when the 1st true leaf was developed.

Plants were irrigated as needed with high quality tap water (pH: 6.64 and electrical conductivity of 0.486 dS·m⁻¹) using intermittent overhead mist. A weekly soil fertilization (1 N:0.5 P:1 K:0.5 Ca, v/v): Plug tray: 50-100 mg·L⁻¹ N; post-transplant pot: 150 mg·L⁻¹ N was included. Both, the irrigation volume and fertilization practices varied according to the media volume of each container; they were approximately 40% higher for individual pots than for seedbed containers.

Mean temperatures (°C) and photosynthetic active radiation (mol·photons·m⁻²·day⁻¹) for the different experiments were recorded with a HOBO sensor (Onset Computer Corporation, Massachusetts, USA) connected to a HOBO H8 data logger. Mean temperatures ranged between 13.39-20.02 °C, 12.72-14.55 °C, 18.26-24.80 °C and 25.87-30.57 °C during autumn, winter, spring and summer, respectively; while photosynthetic active radiation (mol·photons·m⁻²·day⁻¹) ranged between 5.0-7.01, 3.95-5.93, 8.78-10.71 and 9.46-11.16 respectively for the same periods.

Plants were harvested at the transplant stage (60 days) and at 200, 340, 460 and 580 days from sowing (ten replicates each); roots were washing and both roots and shoots were dried at 80 °C for 48 h and weighed to determine the dry biomass (ten replicates·block⁻¹). The root relative growth rates on a fresh- and dry-weight base were calculated as the slope of the regression of the natural logarithm of root fresh-dry weight vs time. The allometric relationships between roots and shoots were performed using a straight-line regression analysis between the natural logarithm root dry weight and the natural logarithm shoot dry weight during the experiment.

The experimental design was a randomized factorial with 3 blocks of 10 single-pot replications of each treatment combination (BAP $\times$ container type). Data were subjected to a two way analysis of variance and means were separated by Tukey tests ($P < 0.05$).

3. Results

3.1 Shoot Number

Photosynthetic shoot number per plant increased through the experiment (although those expanded in spring and summer died under the winter) (data not shown) and reached, at the end of the experiment, lower values for the control plants grown in plastic seedbed than in single pots (Table 1). An increase in shoot number when different BAP solutions were used was found, but the effect go on in single pot-grown plants until 460 days from sowing. Single (BAP; Container type) and double (BAP $\times$ container type) effects in the ANOVA test showed highly significant differences ($P < 0.001$).

3.2 Biomass Accumulation

Plants grown in plastic seedbed or single pots and sprayed with different BAP solutions increased both root and shoot dry weight, as shown in Fig. 1. Although an increase in root:shoot ratio was found too, the absolute values were always higher for plants grown at single pots. Single (BAP, Container type) for both root and shoot effect in the ANOVA test showed highly significant differences ($P < 0.001$). Double (BAP $\times$ container type) effects showed highly significant differences ($P < 0.001$) too for root and significant differences ($P < 0.05$) for shoots dry weight accumulation.

Crown biomass accumulation on a fresh weight-base was ever higher for plants grown at single pots (Fig. 2B) than for plastic seedbed (Fig. 2A). Crown fresh weight was significantly increased when *A. officinalis* plants which were grown in either plastic seedbed or single pots were sprayed with 5, 50, 100 and 200 mg·L⁻¹ BAP ten days after emergence.

However, the crown relative fresh weight BAP-increase was higher in plastic seedbed (30.65%) than in single pots (16.09%). The straight-line regression used showed highest determination coefficient (r^2) values which ranging between 0.925-0.969 and 0.856-0.919 for plastic seedbed and single pots, respectively (Table 2).

The root relative growth rate (RGR_{root}) on a dry weight base was newly higher for single pot than for plastic seedbed; but, when a single BAP spray was applied, the relative increase was significantly higher for plastic seedbed-grown plants (22.45%) than for single pot-grown plants (14.15%). By the other hand, although single pot-grown plants showed the highest shoot relative growth rates, there were no significant differences between control and BAP-sprayed plants in both container types. The highest RGR_{root}-BAP response was achieved with 50 mg·L⁻¹ and 200 mg·L⁻¹ for single pots and plastic seedbed, respectively (Table 3).

3.3 Photosynthate Partitioning

Table 4 showed the allometric relationships between shoots and roots for *A. officinalis* grown under plastic seedbed and single pots and sprayed with different BAP solutions. The slopes of the

Table 1 Photosynthetic shoot number for asparagus plants sprayed with different BAP solutions grown at plastic seedbed or single pots at the end of the last summer before field transplant (460 days from sowing). Standard errors are indicated. Significance from the analysis of variance (ANOVA): *** 0.001.

BAP (mg·L ⁻¹)	Photosynthetic shoot number	
	Seedbed	Single pots
0	8.20 $\pm$ 0.20	11.06 $\pm$ 0.41
5	8.07 $\pm$ 0.35	12.84 $\pm$ 0.55
50	9.11 $\pm$ 0.51	12.96 $\pm$ 0.34
100	9.40 $\pm$ 0.38	12.77 $\pm$ 0.53
200	9.60 $\pm$ 0.33	12.89 $\pm$ 0.51
ANOVA		
BAP	***	
Container type	***	
BAP $\times$ Container type	***

	ANOVA	
	Root	Shoot
BAP	***	**
Container type	***	***
BAP × Container type	***	**

Fig. 1 Dry weight biomass (root and shoot) accumulation 460 days after sowing for *A. officinalis* plants grown at plastic seedbed (A) or single pots (B). Standard error and root-shoot ratio on the bars are indicated. Significance: *** 0.001 and ** 0.01.

straight-lines which relate natural logarithm root dry weight and natural logarithm shoot dry weight showed that control plants (0 mg·L⁻¹ BAP) assigned a higher photosynthate proportion to roots, although a more balanced ratio between roots and shoots for single pot plants was found. When plants were sprayed with

different BAP concentrations, an increasing high proportion of dry weight towards roots was found. The effect was higher, mainly at the lower BAP doses, when plants were grown at single pots. The determination coefficient (r^2) values ranging between 0.854 and 0.945.

Fig. 2 Changes in root fresh weight in asparagus plants sprayed with different BAP solutions grown at plastic seedbed (A) or single pots (B) using a straight-line regression analysis.

Table 2 Changes in root fresh weight in asparagus plants sprayed with different BAP solutions grown at plastic seedbed or single pots using a straight-line regression analysis between natural logarithm root fresh weight and time from sowing in days. Standard errors for the slope (β) are indicated. The intercept straight-line (α) and the coefficients of determination (r^2) are indicated too.

BAP (mg·L ⁻¹)	Plastic seedbed			Single pot		
	α	β	r^2	α	β	r^2
0	-1.02	0.0062 ± 0.00039	0.937	-0.61	0.0087 ± 0.00051	0.856
5	-1.39	0.0077 ± 0.00042	0.933	-0.65	0.0094 ± 0.00072	0.919
50	-1.28	0.0078 ± 0.00047	0.969	-0.76	0.0101 ± 0.00014	0.880
100	-1.02	0.0075 ± 0.00052	0.925	-0.40	0.0091 ± 0.00070	0.918
200	-1.22	0.0081 ± 0.00062	0.954	-0.76	0.0098 ± 0.00042	0.909

Table 3 RGR (relative growth rate) for both crown roots and shoots on a dry weight base for plants sprayed with different BAP solutions and grown in container or single pots. Standard error is indicated.

BAP (mg·L ⁻¹)	RGR (g·g ⁻¹ ·day ⁻¹)			
	Plastic seedbed		Single pots	
	Root	Shoot	Root	Shoot
0	0.00735 ± 0.00075	0.0112 ± 0.00097	0.0106 ± 0.00091	0.0132 ± 0.00152
5	0.00825 ± 0.00069	0.0111 ± 0.00081	0.0112 ± 0.00084	0.0132 ± 0.00149
50	0.00807 ± 0.00073	0.0109 ± 0.00095	0.0121 ± 0.00089	0.0128 ± 0.00146
100	0.00797 ± 0.00080	0.0099 ± 0.00101	0.0109 ± 0.00086	0.0117 ± 0.00130
200	0.00900 ± 0.00074	0.0117 ± 0.00088	0.0106 ± 0.00101	0.0120 ± 0.00142

Table 4 Changes in allometric relationships between roots and shoots for plants of *A. officinalis* grown at plastic seedbed or single pots and sprayed with different BAP solutions using a straight-line regression between natural logarithm root dry weight and natural logarithm shoots dry weight from the transplant to the end of the experiment. Standard errors for the slope (β) are indicated. The intercept straight-line (α) and the coefficients of determination (r^2) are indicated too.

BAP (mg·L ⁻¹)	Plastic seedbed			Single pot		
	α	β	r^2	α	β	r^2
0	-0.503	0.662 ± 0.052	0.854	0.551	0.916 ± 0.043	0.941
5	-0.443	0.692 ± 0.051	0.860	0.634	0.922 ± 0.042	0.945
50	-0.229	0.862 ± 0.041	0.933	0.604	1.060 ± 0.052	0.936
100	-0.139	0.844 ± 0.046	0.923	0.658	1.029 ± 0.051	0.936
200	-0.307	0.909 ± 0.043	0.940	0.435	1.074 ± 0.053	0.937

4. Discussion

4.1 Root Restriction

The use of plastic plug tray for germination-emergence has the advantage that no manual handling of the plants is necessary, and a short span of time (a few seconds) passes between lifting and transplanting of the seedlings by a machine developed for this purpose. However, plug tray and plastic seedbed for crown growth until the transplant would determine a root restriction because the underground root system exhibits a branching habit, which, as lateral buds develop, extends the crown in new directions. This restriction to root growth usually results, among other effects, in a limited production of cytokinins [12]. A reduction in cytokinin production negatively affects the development of the aerial part [17-20]. However, asparagus root restriction would not be involved in the lowest root dry weight (Fig. 1) and shoot number (Table 1) showed in plants grown in plastic seedbeds; although the volume available for each plant grown in seedbed was 40% lower than for

single pots, water and nutrient availability could be excluded as a limiting growth factor.

4.2 Autotoxicity

The unexpected fresh (Fig. 2, Table 2) and dry (Fig. 1) weight results would be probably associated with asparagus autotoxicity which has been extensively investigated [21-23]. There are numerous reports of declining productivity from old asparagus plantings and extracts from asparagus tissue that had been dead for at least 8 months in the field still contained substances that retarded development of asparagus seedlings, and that the autotoxin was heat stable and water soluble [24, 25]. Crowns of plants grown in plastic seedbed would be increase autotoxicity effects (Fig. 1A; Fig. 2A; Table 2); when plug trays (55.7 cm³·cell⁻¹) or single pots (3.000 cm³·pot⁻¹) (Fig. 1B, Fig. 2B and Table 2) were used, autotoxicity was not observed. Neither mean temperatures, PAR (photosynthetic active radiation) nor watering-fertilization routines would be associated to the observed autotoxicity in the seedbed crown-grown.

4.3 Biomass Accumulation and Photosynthate Partitioning

The crown growth between seed emergence and field transplant takes up a significant proportion of the production cycle and crown needs to achieve a minimum fresh weight before transplant. The results showed that both the higher root fresh weight (Fig. 2, Table 2) or root dry weight (Fig. 1) were achieved for plants growth in single pots as a result of a higher crown relative growth rate on a fresh-weight base or a dry-weight base (Fig. 2, Tables 2 and 3). Theoretically, a larger root system should support higher productivity; it is well documented that high amount of storage carbohydrates, big crowns and strong ferns prove the yielding potential of the asparagus plant to be high [26-28]. *Asparagus* is a perennial and a plantation is usually exploited for 8-12 years; its yielding starts 2-4 years after seed sowing but, after seven harvest seasons, the accumulated yields ($\text{kg}\cdot\text{ha}^{-1}$) declined [29]; the possibility to achieved the minimum crown fresh weight for transplanting as soon as possible or a higher crown biomass at the usually transplant time would increase total asparagus plantation productivity.

By the other hand, Feller et al. [30] showed that the most important differences between yield cultivars were the number of viable buds and the number of active bud clusters; they observed that the number of harvested spears was not limited by a low carbohydrate status in storage roots but it was apparently due to a lack of viable buds. An increase in photosynthetic shoot number when BAP sprays were applied (Table 1) probably associated to a higher crown size was found.

High and low-yielding asparagus cultivars appear to differ in partitioning between roots and shoots. A higher root:shoot ratio has been found to be associated with high spear yield [31, 32]. Greater root dry weight in high-yielding cultivars may be due to a greater number of storage roots formed [33] or early formation in new storage roots and new buds [34];

certainly, a greater crown biomass will provide a larger carbohydrate pool required during the period of fern establishment. Results during the pre-field transplant stage are in agreement with previous reports after field transplant: total dry weight increased as time after sowing with an increase in crown growth (root:shoot ratio increased too) and an early BAP spray accelerated biomass allocation and crown partition; the BAP sprays seem to be a greater effect under conditions with favor autotoxicity, such as the seedbed than in single pot crown-grown (Fig. 1).

It has been indicated that differences in the agronomic performance of asparagus plants have been attributed to differences in the amount of carbohydrate reserves in the storage roots produced in the previous season [35, 36] or the efficiency of partitioning carbohydrates into storage roots [37]. Both processes would be operated in asparagus plants BAP-sprayed such as was indicated by the slopes of the allometric relationship between roots and shoots plotted in Table 4.

The highest total dry weight (Fig. 1) would be partially explained by the photosynthetic contribution of a higher shoot number (Table 1) but an increase in photosynthetic efficiency would be involved. It has been indicated that cytokinins are synthesized by roots and moved to the apical meristems [38, 39] which regulate both photo assimilate production and partitioning [40, 41]. The results are in agreement with Ref. [13] on the effects of BAP application on stimulation for spear sprouting of *A. officinalis* in spear tips after the transplant stage which including the UC 157F2 hybrid.

5. Conclusions

A single 5 to 50 $\text{mg}\cdot\text{L}^{-1}$ BAP spray at the plug growth stage would be a tool for improving crown growth and rapid transplant and limiting asparagus autotoxicity negative effects when a plastic seedbed is used for the UC 157F2 hybrid. A change of the traditional soil seedbed for crown growth would be a

valid alternative only when single pot for each plant is used. However, new replications of this experiments which included different field climatic condition and other asparagus genotypes are needed before a grower technological recommendation can be developed.

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