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Comparative Success of Budding and Grafting *Citrus sinensis*: Effect of Scion's Number of Buds on Bud Take, Growth and Sturdiness of Seedlings

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Abstract

A greenhouse study was conducted at the Lilongwe University of Agriculture and Natural Resources (LUANAR) in Malawi to determine the effectiveness of budding and grafting of *Citrus sinensis*. The study aimed at establishing the effect of number of buds on scion wood on bud take and growth of seedlings. A total of 6 treatments were arranged in randomized complete block design with 3 replicates. Treatments were 1-bud budding and grafting with scion having 1-5 buds. Budding and grafting were conducted in June, 2014 and monitored for a period of 120 days. Data were analyzed using GenStat statistical package, 15th edition and R 3.4.1. Results revealed that grafting treatments had significantly higher bud takes on average while budding had the least bud take. Within grafted seedlings, number of buds had no significant effect on bud take and height. Similarly, number of buds did not affect sturdiness quotients of seedlings, of which all were within the recommended range. However, number of buds significantly affected shoot girth, number of leaves and number of branches. A significant linear relationship and positive correlation was found between number of branches and number of leaves. The study recommended that grafting should be adopted as an alternative propagation technique for sweet orange and must use scion wood with 3-4 buds for ideal bud take, growth and sturdiness quotients.

Keywords: Bud take; Budding; Grafting; Scion wood; Sturdiness; *Citrus sinensis*

Introduction

Sweet orange (*Citrus sinensis*) is a dicotyledenous shallow rooted woody perennial tree that bears round sweet fruits. It belongs to the family Rutaceae, order Geraniales, genus *Citrus* and species *sinensis* [1]. It is one of the widely produced citrus fruits in Malawi [1,2] and was introduced in Malawi by colonial settlers and Christian missionaries [3]. However production is still on small scale and unorganized [1], principally grown under hut door and backyard gardening systems. It is grown in the medium altitudes of Mwanza, Neno, Ntcheu, Dowa, Nkhatabay and Karonga with an annual rainfall of 1000-2000 mm, altitude of 450-1200 m a.s.l. and temperature range of 25-30°C.

According to Ref.'s [1,4-7] budding has been reported as a recommended method for propagating sweet oranges. In this regard [8], indicated that there are two important phases of successful budding operation i.e. (i) The choice of a suitable stock; and (ii) Selection of a satisfactory bud wood. Selection of budwood requires a careful identification of mother trees and appropriate selection of bud sticks on the chosen tree [8-10]. The American Horticultural Society also stresses that rootstocks of oranges must have desired characteristics such as vigor, growth habit and resistance to soil-borne pests and diseases. The rootstock must be at least one year old, to be large enough to be budded [8]. During budding it is necessary to ensure that the rootstock and the budwood are free from harmful pathogens such as viruses, fungi, or bacteria in order to produce nursery trees which are not susceptible to pests and diseases (Goff, 1989). Stocks for budding citrus varieties are grown from seed. However, in seedless varieties such as the 'Rusk" citrange, cuttings may serve in place of seedlings [11].

There are various methods of budding oranges e.g. chip, patch and T-budding. Selection of the budding method is determined by the season during which the budding will be conducted [8] Patch and T-budding are frequently used in the summer when the bark is slipping easily, while chip budding can be done even when the bark is not slipping easily. Rootstock type has an important role in growth, development, and crop production of citrus [12]. Several species or hybrids have been used as stocks. A satisfactory stock must be congenial with the top budded on it; that is, the two must form a union which permits good growth, long life, good yields and good fruit qualities of the scion variety [13]. Any dwarfing effect is an indication of a certain degree of un-congeniality [14], or of the presence of a systemic disease, although yield may be good for the tree size and fruit quality excellent. Some stocks are superior in one or more of these qualities, but inferior in others, and none is outstandingly superior on all counts [15]. Same species respond similarly to similar rootstocks under given environment [16]. Choice of rootstock therefore must consider stock-scion relationship and environmental conditions [14,16,17].

Sweet orange rootstocks produce large and vigorous trees resistant to tristeza and exhibit good adaptation to well-drained light to medium loam soils [8,18]. However, it is susceptible to gummosis (*Phytophthora* spp.) [8]. As such, most farmers opt to use sour orange (*Citrus aurantium*), due to its vigor, hardiness, deep root system, and resistance to gummosis diseases [19] and high quality, smooth, thin-skinned and juicy fruit produced by the cultivars on it [8,9,20]. Other rootstock species for sweet orange are rough lemon (*Citrus limoni*), trifoliate orange (*Ponirus trifoliate*), Cleopatra mandarin (*C. reticulata*) and many other citrus species [8,21]. An alternative way to budding citrus is grafting. It involves joining two plant parts such that

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the cambial layers match and the tissues unite to form one plant [22-24]. The upper part is called a scion while the lower part is called the rootstock. In this plant union, the scion becomes the new shoot for the plant union, and the rootstock supports the root system and conducts nutrients across the graft junction into the shoot. In both budding and grafting, the following factors must be considered when selecting the parent tree; it must be true to type, with a record of satisfactory production for at least 5 years, free from systemic diseases and must have attained maturity [14].

Citrus production in Malawi remains elusive ascribed to lack of adequate planting materials. Budding requires scion wood to be well developed and round for it to be successfully mounted onto the rootstock. Obtaining such scion wood is challenging. Most sweet orange scion wood is angular due to water stress that most groves go through. This reduces bud take and growth of resulting budded seedlings. Hence there is a need to practice both budding and grafting so as to utilize all sizes and shapes of scion wood. Contrasted from budding, in grafting, a scion wood may have different number of buds, while in budding the scion wood has a single bud only. However, little research has established the effectiveness of grafting in bud take and growth of sweet orange seedlings. Furthermore, this must be substantiated with a recommended optimal number of buds on a scion wood. This study was aimed at determining the effectiveness of budding and grafting sweet oranges and establishing the optimal number of buds on scion wood in enhancing bud take and growth of grafted sweet oranges. As the country is making significant efforts to commercialize fruit production, information on the effectiveness of grafting and number of buds on scion wood is critical for optimization of limited scion stock for sweet oranges. Proper selection of propagation technique and bud wood will increase the chances of bud take and growth, thereby increasing the availability of planting materials for sweet oranges while also utilizing and optimizing use of all bud sizes and shapes.

Materials and Methods

The study was conducted in Malawi at the LUANAR's, department of Horticulture nursery from June 2014 to January 2015. A total of 360 sweet orange scions were grafted and budded using Lemon (*Citrus limoni)* rootstocks at a grafting height of 15-20 cm. Scion wood was collected from high producing true to type and healthy orange mother trees. 5 mother trees were selected for scion wood collection, all of which were 7 years old. The bud wood was mature, round and green obtained from last flush of growth or hardened recent flush. It was collected from peripheral of the canopy where the branches were well exposed to sunlight. Each bundle was labeled correctly and wrapped in a moist piece of Muslim cloth to keep them from drying. These were budded using T-budding and grafted using wedge grafting [25-29] below is an illustration of budding in pictures adapted from Lewis and Alexander (Figure 1) [25].

In order to encourage the scion bud to grow, the upper third of the rootstock at the time of budding was removed. Three weeks later, the remaining rootstock shoot above the bud was pruned off [25,26]. The terminal portion of the bud was bent off, a practice termed lopping/crippling; bending (constriction) or cutting halfway through the rootstock stem above the bud union (Figure 1). This forces out the bud and maintain growth of the budded plant by breaking apical dominance of the more distal/axillary buds thereby encouraging the budded bud to grow [30,31] After 8 weeks from budding the rootstock stem was completely removed.

The experiment was a one factor experiment with 6 treatments arranged in a Randomized Complete Block Design (RCBD) with four replicates. These treatments were budding, 1-bud scion grafting, 2, 3, 4, and 5 bud scion wood grafting. Data were analyzed using GenStat statistical package and R version 3.4.1. [32]. Results that showed significant differences were subjected to multiple comparison tests using Turkey test to compare treatment means (Table 1).

Results and Discussion

Bud take

Bud Takes Percentage (%): In this study, bud take refer to propagated plants with a successful union evident from the growth of the grafted/ budded scion on the rootstock within a period of 3 months after grafting/ budding operation due to matching of cambial layers. There were significant differences (Table 1) in bud take % among treatments (*P<0.05*). Grafted plants had considerably higher bud take relative to budded plants. However, among grafted plants, no significant differences were observed. Both seedlings grafted with 1 and 2-buds had 90.6% buds take while seedlings grafted with 3-5 buds had 100% buds take. On the other hand, budded plants had a bud take of 25%, significantly different from all grafted plants. This finding is not in

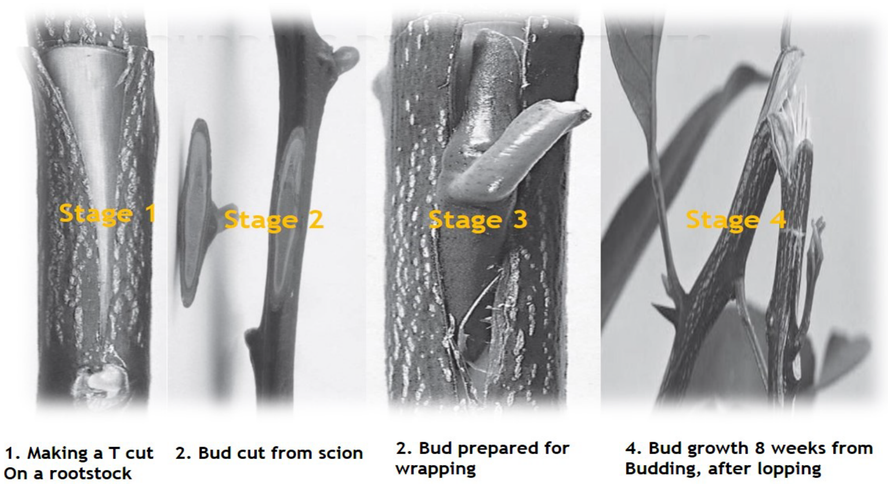

Figure 1: Illustration of T-budding procedures and lopping.

	Parameter					
Treatment	**Bud takes (%)**	**No. of Branches**	**No. of Leaves**	**Shoot height (H) (cm)**	**Shoot stem diameter (D) cm**	**Sturdiness quotient[1] (H_{cm}/D_{mm})**
1-Bud scion grafting	90.6^{b}	1^{a}	13^{a}	12.3	0.41^{b}	3.0
2-Bud scion grafting	90.6^{b}	2^{b}	22^{b}	11.3	0.38^{ab}	3.0
3-Bud scion grafting	100.0^{b}	3^{c}	32^{c}	11.6	0.36^{ab}	3.2
4-Bud scion grafting	100.0^{b}	3^{c}	31^{c}	11.8	0.36^{ab}	3.3
5-Bud scion grafting	100.0^{b}	4^{c}	37^{c}	9.7	0.33^{a}	3.0
1-Bud budding	25.0^{a}	1^{a}	8^{a}	10.4	0.39^{ab}	2.7
CV (%)	4.7	4.6	6.4	7.9	3.4	5.5
F test	***	***	***	ns	*	ns
$LSD_{0.05}$	9.31	0.4165	5.069	2.55	0.045	0.74

*Significantly different at 0.05 level of significance; ***Significantly different at 0.001 level of significance; ns: Not significantly different at 0.05 level of significance. [1]Sturdiness quotient was obtained as a ratio of shoot stem height in cm to diameter mm.

Table 1: Summary of results on growth parameters and bud take at 12 weeks from grafting/budding.

agreement with Lewis and Alexander who reported that budding in sweet oranges achieves 90-100% bud takes under optimal conditions. The mean bud take for grafted plants was compared with that of budding and an independent T-test revealed significant differences (*P <0.05*). It is inferred, therefore, that number of buds do not significantly influence bud take. However, grafting achieves significantly higher bud takes when scion wood is mature enough, round and plump. Therefore, grafting is more effective with regards to bud take when propagating sweet orange relative to budding, and works over a range of sizes and shapes of scion woods [14]. Kumar stressed that bud take is much dependent on the experience and expertise of the grafting personnel [26]. Hartmann et al. further attributes successful bud take to optimal environmental conditions mainly temperature, humidity and water, maturity of the scion and the rootstock among others [6]. Warmer temperatures of about 25-35°C and humidity greater than 90% including pathogen and pest free environment are required which determines the healing of wound and formation of callus, which further influences bud take [6,26,27]. Since grafting has similar ecological requirements irrespective of number of buds, bud take is less likely to differ among grafted plants. On the other hand, T-budding requires that the bark slips easily, achieved when moisture is sufficient, temperatures are warmer, and bud wood is round and plump. Variation in these factors might have played a role in the lower bud take in budded plants. Further attributed to lower bud take in budded seedlings is the angular cross section of seedlings, which compromises union of cambial layers between rootstock and scion [30]. When angular immature buds are used, they remain dormant for several months and eventually burst following spring or autumn growth flush [33].

Time to bud shooting: There were no significant differences (p>0.05) on the number of days taken to form a successful union between the rootstock and the scion among the grafted plants. However, the mean number of days to bud shooting was significantly lower in grafted plants than in budded plants ($F_{0.05}$<0.001). In all grafted treatments, the first bud take was noted after 23 days from grafting while in budding the first bud take was observed after 33 days and the last bud take was after 50 days from the grafting time. Kumar reported that under suitable conditions such as warmer temperatures, mature scion wood and healthy rootstocks takes 2 to 3 weeks (14-21 days) to form a successful union in both budded and grafted plants [26]. Garner also noted that the number of days taken to bud take is invariably a function of health of scion wood and moisture, temperature, humidity and soil nutrients [29]. Since the budding and grafting were conducted in winter season in Malawi, it might not have been conducive enough to facilitate rapid formation of callus [25].

Bud shooting order in grafted plants: This parameter was aimed at answering questions; (i) Do all buds on the scion wood with multiple buds shoot at the same time or not? (ii) If not, what is the order of shooting? In this study, 90% of the plants had their buds shooting at the same time. However, not all the buds are able to shoot and form a branch evident from reduced number of branches (Table 1). Only seedlings having 1, 2 and 3 buds had 100% of the buds shooting up into a branch, while 75 and 80% for those with 4 and 5 buds. On average, only 91% of the buds on the scion wood were able to shoot and form a branch while 9% of the buds did not result into a branch. For the 10% that shot at different times, most shot from the bottom to the top. According to Hartmann, et al., (2011), buds on the lower end are more mature since they are the first to come out on the shoot stem of the scion. Buds on the lower end are in quiescent stage [26], hence when grafted, they are more likely to precede shooting of upper buds.

Growth parameters

Shoot height: Number of buds on the scion wood in grafted sweet orange seedlings did not significantly affect height (Table 1) of the shoot (p>0.05). Shoot height ranged from 12.3 cm for 1-bud scion treatment to 9.7 cm for 5-bud scion wood while budded plants were 10.5 cm tall. Lewis and Alexander argue that growth of a grafted plant is mainly influenced by the vigor of the scion wood, water availability, food and nutrients, temperatures and the success of union. Therefore, number of buds on the scion wood, and propagation type do not affect shoot height [26].

Number of branches and number of leaves: There were significant differences in number of branches ($P_{0.05}$<0.001) and number of leaves ($P_{0.05}$<0.001) among the six treatments (Table 1 and Figure 2). Plants grafted with scion wood with more buds had a proportional increase in number of branches and number of leaves. Consequently, budding had significantly lower number of branches and leaves, along with plants grafted with scion wood with fewer buds. However, this depends on whether all the buds on scions with multiple buds were able to shoot or not, of which 91% did. Notwithstanding the inability of some buds to shoot, more buds on the scion translate into more branches on the plant, inasmuch as there are more buds from which branches will emanate. As a consequence, number of leaves on the plant is dependent on two factors: (i) The average number of leaves per branch, and (ii) The number of branches per plant. In the study, 36 leaves were observed on plants grafted with scion wood with 5 buds whereas only 13 leaves on plants grafted with scion wood with 1 bud. Budded plants had only 8 leaves per plant. Correlation test revealed a strong positive correlation between the two variables, with a correlation coefficient of 0.8980.

There was also a very significant linear relationship between (Figure 3) number of branches and number of leaves ($P_{0.05}<0.001$, $R^2=0.7977$), indicating that almost 80% of the variation in number of leaves was explained by number of branches. The following was the model of the relationship; Y=9.911x+1.168, where Y is the number of leaves on a seedling and X is the number of branches (Figures 2 and 3).

Shoot stem diameter: There was a significant effect (P<0.023*)* of number of buds on the scion wood on the diameter of shoot stems of grafted and budded sweet orange seedlings (Table 1 and Figure 4). Plants propagated with scion with fewer buds were relatively significantly thicker in shoot girth. Higher secondary growth was obtained in plants with 1-bud scion wood (0.41 cm) grafting while the lowest was in 5-bud scion wood plants (0.33 cm) (Figure 4).

Regression analysis revealed a significant negative linear relationship between shoot stem diameter and number of buds ($P_{0.05}<0.001$, $R^2=0.4477$). When number of buds on the scion wood increases, nutrients and photosynthates available in the scion and the rootstock is distributed to the available buds on the scion wood. Consequently, little food is diverted to each branch for the activity of the vascular cambium that is responsible for secondary growth of each shoot. Conversely, when there is one bud, it becomes the sole beneficiary of photosynthates and assimilates for vascular cambium

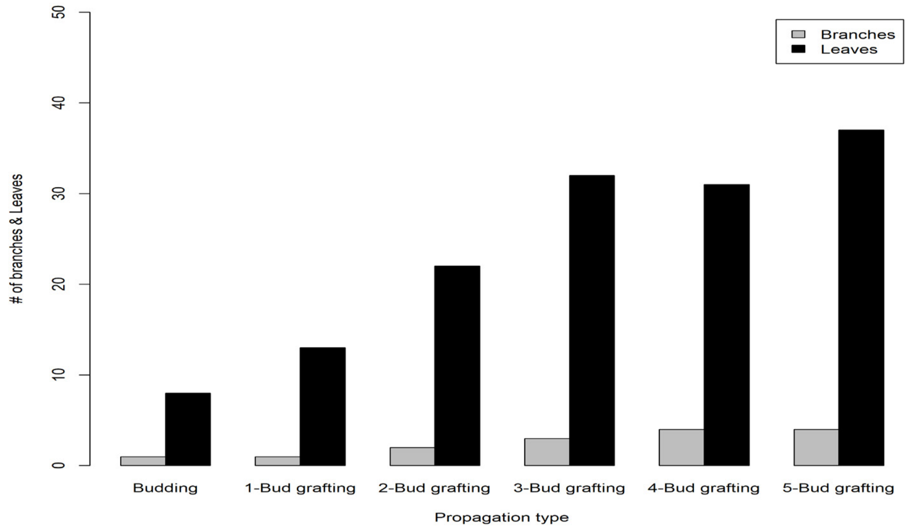

Figure 2: Effect of number of buds and propagation type on number of branches and leaves on the plant.

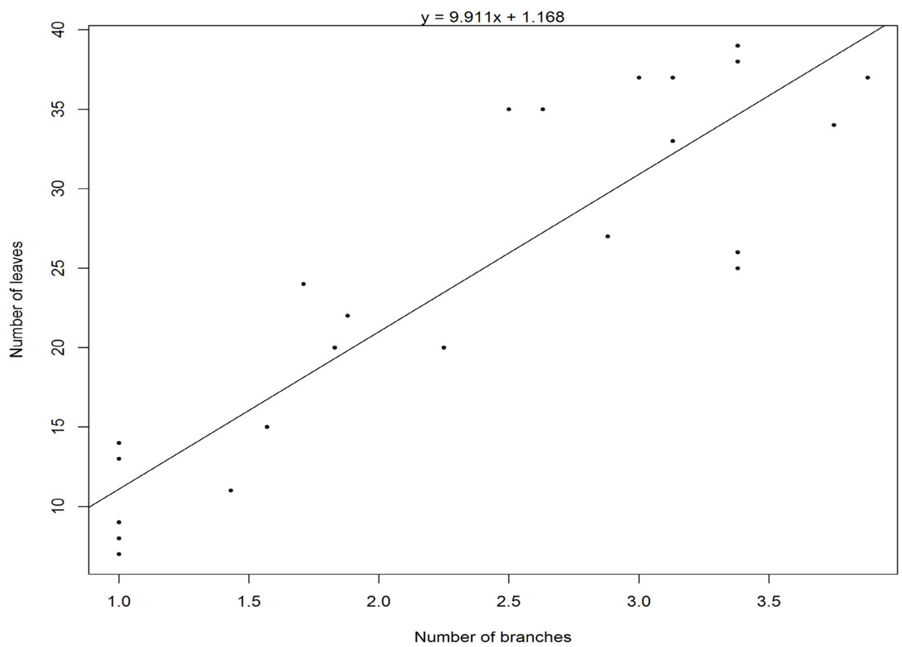

Figure 3: Linear relationship between number of branches and number of leaves on grafted orange seedlings.

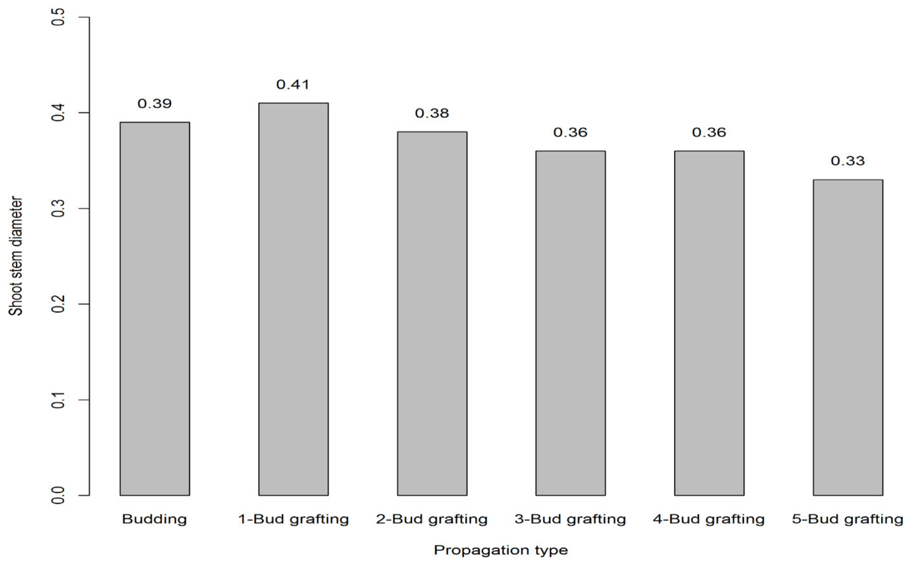

Figure 4: Effect of number of buds and propagation type on shoot stem diameter (cm).

to rapidly multiply producing many cells outwards between the wood (xylem) and the bark (Phloem, cortex and periderm) hence increasing outward growth and consequently exhibiting higher secondary growth [25,26].

Sturdiness quotient: The study revealed that number of buds did not significantly influence sturdiness quotient of seedlings (Table 1). Budding had the lowest sturdiness quotient of 2.7 while 4-bud grafting had the highest sturdiness quotient of 3.3. That notwithstanding, all sturdiness quotients were within the recommended range of ≤6.0. Sturdiness Quotient (SQ) measures the relationship (ratio) between shoot height (cm) and diameter (mm). When a stem has a higher SQ, it is sturdy and relatively less susceptible to transport and planting damage [34,35]. Jaenicke pointed out that seedlings are ideal when sturdiness quotient is less than 6.0, while anything above is termed "lanky" [34]. Seedlings with higher sturdiness are very tall and slender in diameter. Such seedlings are lanky and unlikely to survive in windy and dry conditions [36-40].

Conclusion

The study has established that grafting achieves considerably higher bud takes with less dependence on weather conditions and water availability. Number of buds on scion wood did not influence bud take. However, it significantly affected number of branches, which consequently affects number of leaves. Excessive buds, however, may produce lanky seedlings with higher sturdiness quotients, which are less likely to withstand environmental shock upon out planting. Overall an optimum of 3-4 buds is recommended on scion wood.

Recommendations

The study recommends that farmers should equally use grafting as a method of propagating sweet oranges in addition to budding to enhance bud take and growth and further utilize all sizes and shapes of scion wood. An optimum of 3-4 buds on the scion wood is recommended. It is further recommended that a similar study should be conducted during the summer time (October–December) which should additionally explore other budding types such as patch and chip budding.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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