Research Article

Clonal mechanisms that matter in *Agave fourcroydes* and *A. sisalana* invasions in drylands: implications for their management

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Abstract

Clonal species *Agave fourcroydes* and *A. sisalana* from ancient crops have become invasive in Mediterranean drylands, posing a severe threat to ecologically valuable native plant communities. However, we barely know the relative contribution of clonal mechanisms underlying their invasive behaviour. Therefore, environmental managers must face this problem without scientific evidence on the most effective methods to eradicate or control these invasions. In this study, we monitored populations of *A. fourcroydes* and *A. sisalana* and carried out field and experimental plantings (*in situ* and under controlled environmental conditions) to assess the efficacy of three clonal mechanisms: shoots from rhizomes, shoots from rosettes, and aerial bulbils from floral scapes). Both species showed very similar performance in their clonal expansion. Shoot production from rhizomes was the primary mechanism for densifying *Agave* populations, emerging to the soil 1.5 ± 2.1 shoots per individual. Moreover, we did not observe mortality of new shoots from rhizomes. These new clonal individuals seemed to ensure their survival by connecting to the mother plant (clonal integration). In addition, contractile roots, only recorded in shoots from rhizomes, could represent an advantage allowing them to react to harsh environmental conditions. Regarding shoots from rosettes, individuals produced 0.63 ± 1.01 shoots on average, with no mortality recorded, but without guaranteeing the replacement of mother plants. Finally, bulbil production and their rooting under controlled and favourable conditions were very high, although their establishment success under arid conditions proved irrelevant. These results suggest that management actions should focus on the removal of shoots from rhizomes. This approach could be an efficient, feasible, and cost-effective medium- to long-term habitat management strategy to control these *Agave* invasions, with widespread application worldwide in similar invasion processes.

Key words: aerial bulbils, arid lands, expansion and densification control, invasive plants, habitat management, rhizome, vegetative propagation

Introduction

Biological invasions are considered one of the most significant environmental challenges worldwide (MEA 2005; Vilà et al. 2011). Studies on the ecology of plant invasions reveal that mechanisms that make a plant an invader are complex and synergistic, including plant traits, habitat invasibility, and

invasion stage (Roiloa 2019). Regarding plant traits, clonality, i.e., a vegetative reproduction mechanism in which an individual produces a genetically identical offspring, is a common feature in many plant invaders (Song et al. 2013; Wang et al. 2017; Roiloa 2019). Furthermore, it exists a type of clonality where the new individuals or ramets (shoot-root units) remain physically connected to the mother plant (e.g., by spacers like stolons, rhizomes, or roots) for an extended period (Cornelissen et al. 2014). One of the main processes related to the success of this type of clonality is the capability for physiological or clonal integration (Roiloa 2019), which involves the translocation of resources and the communication between the mother plant and the offspring (Cornelissen et al. 2014). This propagation mechanism allows plants to spread horizontally and colonise harsh environments effectively (Oborny and Cain 1997). In addition, other plants, like succulent rosette plants, can achieve clonal propagation by aerial bulbils produced in the flowering scape (Arizaga and Ezcurra 2002). Therefore, when dealing with the management of invasive species that exhibit these and other clonal characteristics, it is instrumental to know the importance of such traits for the invasive success of the species to control them more efficiently. However, the lack of knowledge on clonal traits (Yu et al. 2016) and the challenge of addressing demographic studies involving clones still represent a difficulty in unravelling the expansion of invasive clonal species populations.

Plant invasions are due to the escape and spread of alien species introduced into a new area by human activities (Mack et al. 2000). These introductions may occur unintentionally (e.g., weeds), or intentionally (e.g., silvicultural, ornamental or agricultural use) (Reichard and White 2001; Dehnen-Schmutz et al. 2007; Pauchard et al. 2016; Hulme 2020). The latter is the case of some clonal species of the genus *Agave* (Agavaceae), which includes species with succulent rosettes. Some of these species are considered invasive in many parts of the world, such as *Agave fourcroydes* Lem. (henequen) and *Agave sisalana* Perrine (sisal) (e.g., Australia, Randall 2007; South Africa, Staatskoerant 2016). Both species produce a high number of ramets by three clonal mechanisms: shoots from rosettes, from rhizomes, and aerial bulbils from floral scapes. Nevertheless, the relative contribution of each different clonal mechanism to the invasion process is so far unknown, which hampers the management of their invasions.

Clonal integration between mother plants and shoots from rhizomes occurs in *Agave* species, allowing their survival in environments where the seedling establishment is infrequent (Tissue and Nobel 1988). Furthermore, studies on *Agave* species propagation in drylands suggest that the most efficient clonal mechanisms involve clonal integration (shoots from rhizomes and shoots from rosettes) (Arizaga and Ezcurra 2002). These studies also indicate that, despite its high aerial bulbil production, bulbil establishment is poor due to the severe environmental conditions imposed

by the prevailing arid climate (Arizaga and Ezcurra 2002; Badano and Pugnaire 2004). Analyses on what factors facilitate bulbil establishment in these high water deficit environments are scarce and inconclusive (Arizaga and Ezcurra 2002). The intrinsic traits of bulbils (e.g., size, number of leaves, and number of preformed roots) could determine their survival capacity during the pre-rooting phase and even the duration of this phase. Traits of the mother plant could also be critical for bulbil rooting success, as parental (environmental) and genetic effects influence the performance of clonal offspring (Dong et al. 2018), although there are no studies on this topic. Finally, there are extrinsic factors that could be significant for bulbil establishment that have not yet been explored: bulbil position in the soil (vertical vs horizontal), and whether the bulbil is buried or not. The position of the bulbil in the soil determines the degree of contact between the preformed roots and the soil, i.e., the more vertical the bulbil, the more contact the roots have with the soil. Whether the bulbil is buried or not will change biotic and abiotic conditions that ultimately will influence its rooting (Dong et al. 2011).

Here, we aimed to disentangle the role of three different clonal mechanisms in the invasion processes of *A. fourcroydes* and *A. sisalana* in Mediterranean drylands. We evaluated the relative contribution of such mechanisms producing aerial bulbils, shoots from rosettes, and shoots from rhizomes during the invasion process. We also explored whether certain bulbil traits improve its establishment success. We implemented monitoring protocols for *Agave* populations and field and experimental plantings. We hypothesized that in arid zones, the successful spread of *A. fourcroydes* and *A. sisalana* would be mainly mediated by the production of ramets connected to the mother plant (i.e., clonal integration). In contrast, aerial bulbils (i.e., ramets produced in the flowering scape without connection to the mother plant) would play a residual role. Factors that would facilitate aerial bulbil rooting are those extrinsic to the bulbil, such as bulbil position (vertical vs horizontal) and the burying of the bulbils, both of which ensure a better contact with the soil. Ultimately, this knowledge will contribute to designing effective control actions where they become invasive.

Materials and methods

Targeted species and study area

Agave fourcroydes and *A. sisalana* are cultivars, probably originated by the Maya civilisation from wild relatives to obtain better quality fibre (Colunga-GarcíaMarín 2003; González et al. 2003). They are spread worldwide (Randall 2007; Forzza et al. 2010; Salinas 2011; PIER 2018; USDA-ARS 2022), mainly because they were cultivated for fibre in the 19th and 20th centuries. Their behaviour as invaders corresponds to the so-called "cacti
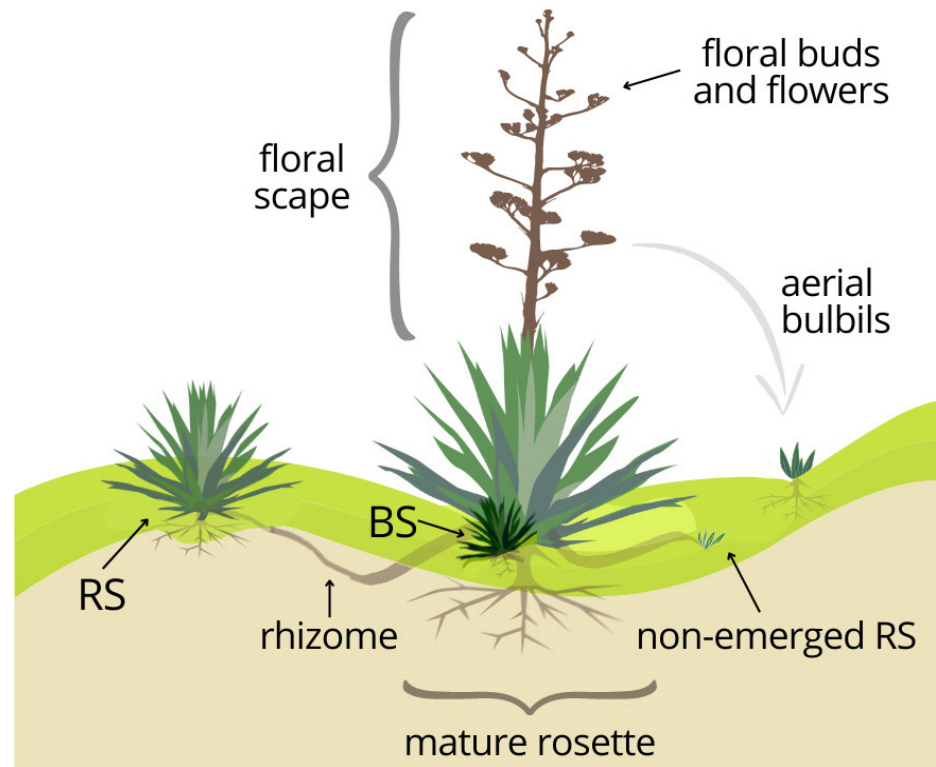
Figure 1. Diagram of a rosette of *Agave fourcroydes* or *A. sisalana* showing their clonal ramet types. BS: basal shoots, born directly from the rosette; RS: shoots from rhizomes, and aerial bulbils from floral scapes.

syndrome" (Novoa et al. 2020), with clonal reproduction resulting in monospecific clusters of impenetrable thickets of spiny individuals. In addition, their CAM-type photosynthesis strategy confers a competitive advantage in drylands by increasing their water-use efficiency (Osmond et al. 2008). They also show a solid capacity to cope with climate change, with high CO_2 uptake and biomass production in warmer climates (Owen et al. 2016). Due to their polyploid and hybrid nature, they produce few seeds by sexual reproduction under natural conditions (Colunga-GarcíaMarín 2003; González et al. 2003; Chaves de Souza et al. 2018). However, when they reach sexual maturity, they develop a tall floral scape from the centre of the rosette. They are monocarpic, i.e., die after reproducing for the first time (Good-Avila et al. 2006). Even so, they multiply successfully through three clonal mechanisms: 1) basal shoots, born directly from the rosette (hereafter BS), 2) shoots from rhizomes (hereafter RS), and 3) aerial bulbils from the floral scape (Figure 1).

We conducted the study in the arid coastal plain of the Cabo de Gata-Níjar Natural Park, Almería, Spain (36°49′43″N; 2°17′30″W). The climate is Arid Mediterranean, with an average annual temperature of 18.1 °C, mild winter temperatures (mean winter minimum of 8.0 °C), and hot summers (mean summer maximum of 30.3 °C). Mean annual rainfall is low (200 mm) and seasonally irregular (Torres-García et al. 2021). Soils are sandy, fixed by vegetation on a Quaternary glacis (Tirado and Pugnaire 2003). The native

vegetation is a mosaic of small shrubs interrupted by bare soil and patches of arborescent shrubs. These patches are dominated by *Ziziphus lotus* (L.) Lam. (Rhamnaceae), a large deciduous shrub up to 3–5 m in height recognised as an ecosystem engineer (Tirado 2009) as its deep roots reach the aquifer (Torres-García et al. 2021). This native ecosystem is under conservation concern, making it a Priority Habitat in Europe (habitat 5220*, European Community 1992). A large part of its extent in the study area, which corresponds to the main population of *Z. lotus* in Europe, is invaded by *A. fourcroydes* and *A. sisalana* (Badano and Pugnaire 2004; García-de-Lomas et al. 2018). These species were introduced into southeastern Spain during the 1950s as cultivated plants for fibre production (Navarro 2005). After the abandonment of crops due to the worldwide development of synthetic fibres and their low productivity (Martín-Galindo 1988; Provansal and Molina 1989; Wells 2006), plants began to reproduce until they dominated the ecosystem. Today, the landscape comprises of native vegetation coexisting with high-density *Agave* populations. These invasive species have developed dense monospecific stands that prevent the establishment of native vegetation and its associated animal assemblages, disrupting the functioning of the ecosystem (Badano and Pugnaire 2004). Sandy soils favour the spread and densification of *Agave* species in this area because this texture improves the efficiency of their clonal reproduction mechanisms (Badano and Pugnaire 2004).

Monitoring and assessment of clonal mechanisms

We evaluated the phenology, production, and establishment rates of the three types of ramets (aerial bulbils, BS and RS). We applied several monitoring strategies based on both individuals and plots for the two species (Table 1), and performed field and experimental planting *in situ* and under controlled environmental conditions to estimate aerial bulbil establishment.

Ramets phenology and production

To estimate aerial bulbil production, we identified the time of the year of maximum bulbil production and assessed plants' capacity to produce such bulbils. First, we randomly selected 60 individuals (30 from each species) with incipient floral scapes and monitored them until they died (February 2015–May 2016). Monthly, we estimated the percentage of floral scape branches in each flowering stage (floral buds, flowers, bulbils, fruits, and fallen bulbils) as an indicator of the flowering stage of each individual (Table 1). We consider a branch in a specific stage when more than 50% of its reproductive structures are in this stage. Second, once we identified the peak of aerial bulbil production, we randomly selected 60 new reproductive individuals (30 from each species, October 2015) to estimate the number of

Table 1. Sampling goals, work stages and summary of data collected.

Sampling goal	Phenology of flowering and bulbils and production of basal shoots	Estimation of bulbil production[*]	Estimation of shoot production from rhizomes	Estimation of rooting rates of bulbils under natural conditions	Estimation of the rooting rates of bulbils in experimental plots (greenhouse and natural conditions)[*]	Phenology of shoots production and rate of establishment of new individuals (rooted bulbils vs shoots from rhizomes)
Sampling unit	60 individuals (30 from *Agave fourcroydes* and 30 from | *A. sisalana*)	60 individuals (30 from *Agave fourcroydes* and 30 from *A. sisalana*)	8 individuals (4 from *Agave fourcroydes*, 4 from *A. sisalana*)	1,184 bulbils (582 from *Agave fourcroydes*, 602 from *A. sisalana*; 590 from early fall, 594 from late fall)	1,440 bulbils, 24 from each of 60 individuals (30 from *Agave fourcroydes*, and 30 from *A. sisalana*)	10 plots 10 × 10 m
Monitored individual nature	Reproductive individuals with incipient floral scapes emerging from the rosette	Reproductive individuals with floral scapes at the stage of maximum bulbils production	Juvenile, i.e., prominent but not reproductive individuals	Mature bulbils fallen from the floral scapes	Mature bulbils fallen from the floral scapes	New young individuals
Traits measured	Height and diameter of the rosette (cm) Height and diameter of the floral scape (cm) Number of scape branches % of branches with floral buds % of branches with flowers % of branches with bulbils % of branches with fruits % of branches with fallen bulbils Number of basal shoots (born directly from the rosette)	Height and diameter of the rosette (cm) Height and diameter of the floral scape (cm) Number of bulbils by scape branches Number of fallen bulbils	Number of rhizomes Number of emerged shoots from rhizomes	Height (cm) Diameter (cm) Number of leaves Number of preformed roots Position (vertical or horizontal) Buried or not Rooted	Height (cm) Diameter (cm) Number of leaves Number of preformed roots Rooted	Origin: from rhizomes or rooted bulbils Presence of contractile roots
Monitoring dates and frequency	February 2015–May 2016 (monthly)	October 2015	May–July 2015	October 2015–October 2016 (monthly)	January 2016–May 2016	February 2015–February 2016 (monthly)

* The individuals selected for these two studies were the same.

bulbils on the branches and those fallen on the ground. Finally, we measured all monitored reproductive individuals' rosette and floral scape sizes.

We recorded the number of BS born directly from the rosette of the individuals monitored for phenology regarding basal shoot production. To estimate RS production, we randomly selected eight prominent but non-reproductive individuals (4 × 2 species, May to July 2015, Table 1). We made a profile of the root system by carefully digging in the sand only on one side of the individual. When we found a rhizome born from the rosette, we dug it out completely to check if it emerged to produce an apical shoot. We made the profile only for a quadrant around the plant;

then, we multiplied the number of rhizomes and emerging shoots registered per profile by 4 to estimate RS production per individual.

Aerial bulbils establishment rates and the factors influencing them

To estimate bulbil establishment rates under natural conditions, we monitored 1,184 fallen bulbils (582 of *A. fourcroydes* and 602 of *A. sisalana*) from the 60 individuals assessed for phenology. We marked 590 early fallen bulbils (October 2015) and 594 late fallen bulbils (December 2015). Since we previously observed bulbils buried and upright rooted more frequently than others under different conditions, we considered these two traits in our experiments. Additionally, we surveyed all bulbils monthly for one year (until October 2016) to check their position (vertical vs horizontal) and whether they were buried—as extrinsic control factors of the establishment—and rooted. To explore environmental factors affecting bulbil rooting success, we also performed two experimental plantings under 1) natural climatic conditions and 2) greenhouse conditions (Mean Temperature = 22.5 ± 8.0 °C, Mean relative humidity = 70.0 ± 10.0%; sand soil with ten topsoil centimetres moisture = 8.8 ± 4.9%). For each experiment (natural and greenhouse conditions), we arranged three plots with 240 bulbils (120 × 2 species) randomly placed (720 bulbils = 240 bulbils × 3 plots, 24 bulbils of 30 individuals by species, 1,440 bulbils in total). We planted the bulbils according to the four combinations we observed in the field: vertical/buried (i.e., the soil covered the base of the bulbil), vertical/not buried, horizontal/buried, and horizontal/not buried. There were 30 bulbils per species for each position in each plot. We carried out the plantings in January 2016 and assessed their rooting in May 2016. In both studies, we recorded bulbil traits such as size, number of leaves and preformed roots to identify intrinsic factors that could determine its establishment success (Table 1).

To estimate the establishment rates of bulbils and RS, we sampled ten invaded plots (10 × 10 m, February 2015–February 2016) and detected new individuals and their origin (bulbils vs RS) (Table 1). The apparent morphological differences between individuals originating from rhizomes and bulbils allowed us to distinguish between them (whereas the rhizome was visible in the basal part of the individuals originating from shoots, the rooted bulbils had roots only in their basal part). We marked the pre-existing shoots in the first month, and recorded the new shoots monthly, identifying them by species. We also noted contractile roots, i.e., anatomically adapted roots to contract longitudinally, pulling the shoots down into the soil (North et al. 2008). These roots, considered an adaptation to protect plants from harsh environmental conditions, have been reported in Agavaceae species, especially in the *Agave* genus, including *A. sisalana* (North et al. 2008). The morphological differences between contractile and non-contractile roots are unambiguous: contractile roots have transverse wrinkles in the

outer tissues and greater thickness in the basal zone than in the mid zone, and do not produce lateral roots within 3 cm of the shoot base (North et al. 2008).

Data analyses

Because the data sets did not meet the assumptions of normality and homoscedasticity, we used non-parametrical tests. First, we performed the Wilcoxon signed-rank test, a method for comparing paired samples (Wilcoxon 1945), to test for species differences in variables related to floral scape and shoots from rhizomes (i.e., % of branches with floral buds, % of branches with flowers, % of branches with bulbils, % of branches with fallen bulbils, and % of shoots from rhizomes, Table 1). Second, we ran Mann-Whitney tests to detect differences between species in aerial bulbil and BS production, and root profile traits. Third, we used Fisher's exact tests (Fisher 1922) to test for differences in bulbil rooting rates, because some samples data did not meet the conditions for the application of the $\chi 2$ test (e.g., that the expected values of at least 80% of the cells in a contingency table are higher than 5, Little 1989). Finally, to identify factors affecting bulbil rooting rates, we performed Generalized Linear Mixed Models (GLMMs), excluding explanatory variables correlated with others (correlation coefficients > 0.7, Moore et al. 2012). We considered as predictor variables: rosette diameter and scape height (intrinsic mother plant traits), height, number of preformed roots, number of leaves (intrinsic bulbil traits), and position (vertical/horizontal) and buried (yes/no) as extrinsic bulbil traits. In the GLMMs, we included species identity as a random factor in all three data sets (i.e., monitored under natural conditions, plots in greenhouse, and plots under natural conditions), and the plot in the experimental essays. As the response variable (rooting success) is binary (rooted/not rooted), we used the binomial function for the distribution error and the complementary log-log function as the link function. This link function is the recommended when the response variable is highly skewed towards very high values of a state (Aldrich and Nelson 1984). We made the statistical analyses using R tools (http://www.R-project.org/) version 3.4.1 and the GLMMs on the IBM SPSS Statistics version 26 statistical software.

Results

Development of ramets throughout the growing season

Flowering and aerial bulbil phenology in both species were very similar (Wilcoxon tests showed no statistical differences in the variables compared, Figure 2). Plants began the flowering period in February, and reached the maximum scape branches with mainly floral buds throughout mid-March and April (means of 93.3% and 92.4% for *A. fourcroydes* and *A. sisalana*, respectively). Flowers-dominated branches appeared between April and July,
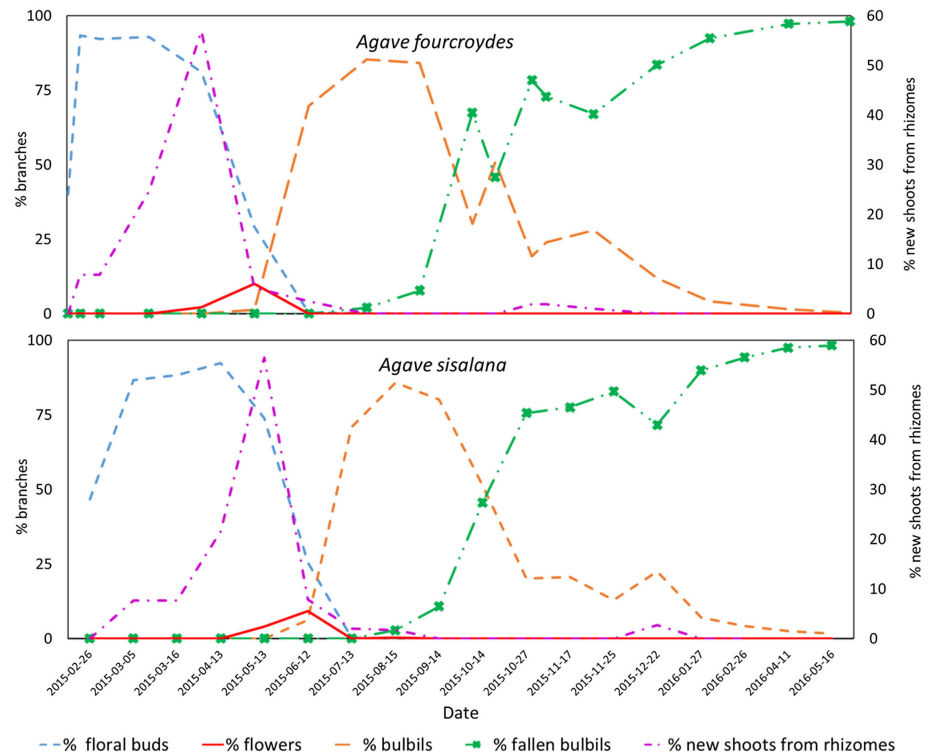
Figure 2. Flowering, aerial bulbil, and shoot from rhizomes phenology of *Agave fourcroydes* (AF) and *A. sisalana* (AS) in the study zone. Wilcoxon signed-rank tests showed no statistical differences between species in any variable. Mean % floral buds: W = 29.0, *p-value* = 0.141; Mean % flowers: W = 2.0, *p-value* = 0.789; Mean % bulbils: W = 49.0, *p-value* = 0.834; Mean % fallen bulbils: W = 13.0, *p-value* = 0.083; Mean % new shoots from rhizomes: W = 16.0, *p-value* = 0.834. W = Wilcoxon statistic.

with the highest rates in June (means of 10.1% and 9.3% for *A. fourcroydes* and *A. sisalana*, respectively). The highest production of bulbils was from July to September, with a peak in August (average rates reached 85.3% and 85.8% for *A. fourcroydes* and *A. sisalana*, respectively). The fall of bulbils started in August, with most bulbils falling between October and January. This phase lasted until May of the following year. We did not find any fruit.

The emergence of new RS was most abundant from April to May, with the majority (56.9% in *A. fourcroydes* and 56.5% in *A. sisalana*) emerging during May, extending into mid-August, with a slight increase in November and December. All new individuals encountered in the surveyed plots throughout the monitoring period originated from RS. Additionally, we found contractile roots in all the RS analysed but not in any BS or the rooted bulbils.

Ramets production rates and mortality of BS and RS

None of the variables analysed differed between species, so we also show the aggregated data. Aerial bulbils per individual ranged from 185 to 2,765 (Figure 3, Supplementary material Table S1), with a mean production of bulbils per individual of 752 ± 357 (n = 60). The mean number of BS produced per individual was below one (0.63 ± 1.01, n = 60), ranging from 0–4 (Figure 3, Table S1), with zero being the most frequent data. The mean
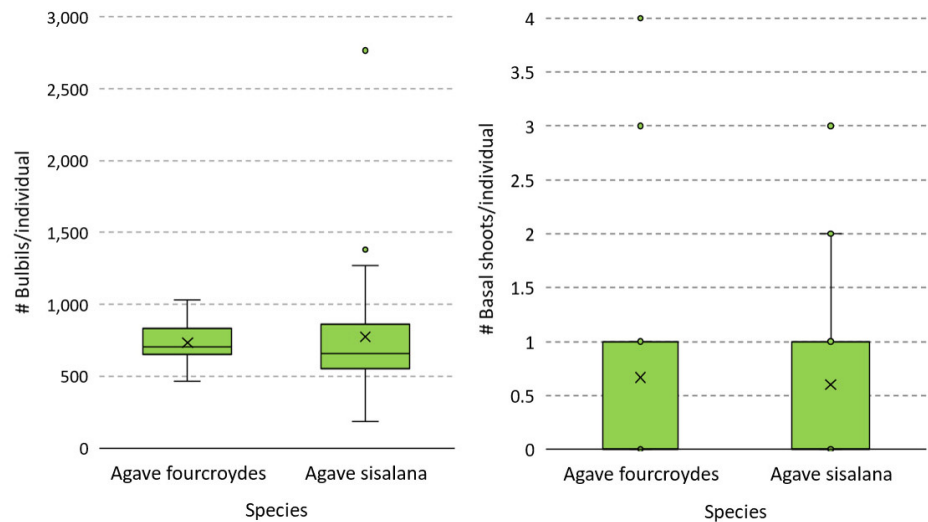
Figure 3. Box-and-whisker plots for total aerial bulbil and basal shoot production per reproductive individual registered in *Agave fourcroydes* and *A. sisalana*. Box represents median and 25th and 75th percentile levels, crosses are the means, and whiskers are the ranges. N = 30 for each species. Non-parametric Mann-Whitney U tests showed no significant differences between species (U = 403.5 and 449.0, and *p-values* = 0.492 and 0.986 for bulbil and basal shoot production, respectively). U = U statistic.

number of rhizomes per individual was 9.0 ± 5.1 (n = 8), with a mean number of RS emerging to the surface of 1.5 ± 2.1 (Table S2). We did not record mortality in either BS or existing and new RS.

Aerial bulbil rooting rates under natural and experimental conditions

Aerial bulbil rooting rates under natural conditions were very low, with only 2.7% of the total number of controlled fallen bulbils rooting during the monitoring period (n = 1,184; Table S3). The rooting rate of *A. sisalana* bulbils (4.0%) was twice that of *A. fourcroydes* (1.4%). Early fall bulbils were more successful in rooting than late fall ones in *A. sisalana* (7.6% vs 0.3%) and taking the data for the two species together (4.9% vs 0.5%) (Table S3). It is worth mentioning that the most critical rainfall event during the monitoring period was just in October, which resulted in the partial or total burial of most of the early fall bulbils (Figure S1). We found no differences in the rooting rates between species concerning the bulbils' extrinsic conditions (similar in both species, Table S4). Therefore, we pooled species data. Rooting was more frequent in buried bulbils (87.5%) than in unburied, with no differences between vertical and horizontal positions (Table S3). Ultimately, all the rooted bulbils died in summer.

Concerning experimental plots, as Fisher's exact tests showed no statistical differences between species, data were taken together. Bulbil rooting rates in the greenhouse plots were higher than under natural conditions, regardless of planting conditions (Figure 4). In both experiments, we observed the highest rooting rates in the buried bulbils.
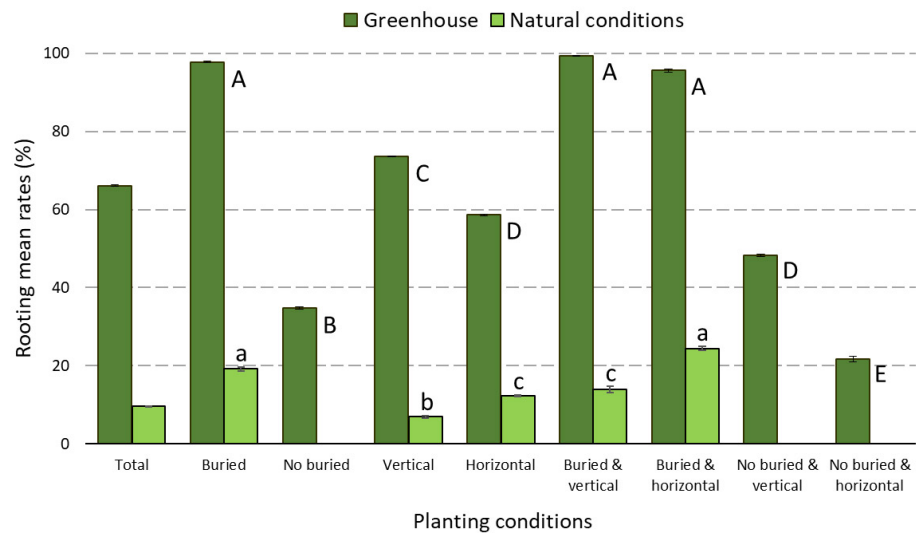
Figure 4. Mean and standard deviation of rooting rates of *Agave fourcroydes* and *A. sisalana* bulbils under experimental plots (greenhouse and natural conditions). Different letters indicate statistical differences (*p-values* < 0.001) between planting conditions for Fisher's exact tests. The lower case letters correspond to the comparisons in the experiment under natural conditions, and the upper case letters refer to those in the greenhouse experiment.

Table 2. Standardized coefficients of the best-fitting Generalized Linear Mixed Models (GLMMs) explaining bulbil rooting success under the monitored conditions (natural conditions, and experimental plots). Three predictor variable types were considered (intrinsic mother plant and bulbil traits, and extrinsic bulbil traits). Species identity and plots were included as random factors. The best model was ranked according to AIC (Akaike Information Criterion) value (See also Table S5). Non-data cells indicate variables not included in a particular model.

Sets of bulbils data	Intercept	Intrinsic mother plant traits		Extrinsic bulbil traits	Extrinsic bulbil traits		Random factor significance	
		Rosette diameter	Scape height	Height	Position	Buried	Species identity	Plot
Monitored under natural conditions	0.936***		−0.064	0.034		0.799***	ns	
Greenhouse plots	−3.805***				0.835***	3.427***	ns	ns
Natural conditions plots	−0.481***	0.124				1.897***	ns	ns

Significance: ****p-values* < 0.001. ns: no significance

Traits influencing the rooting of aerial bulbils

The GLMMs of the three aerial bulbil data sets showed that only extrinsic bulbil traits influenced rooting success (Tables 2 and S5). Bulbils' position was only relevant for bulbils in greenhouse plots. All models agreed that partial burial of the bulbils was the factor explaining bulbil rooting success.

Discussion

The ability of *A. fourcroydes* and *A. sisalana* to produce different ramets in their native area is well known (Colunga-GarcíaMarín 2003; González et al. 2003). However, no scientific evidence on their contribution to the invasive potential of these species has been available so far. Our results help better understand the effectiveness of clonal mechanisms of these species during the invasion process and provide valuable insights to address their control in areas where they develop as invaders. Here, we showed that the clonal mechanisms that produce ramets connected with the mother plant (i.e.,

clonal integration) are more successful than aerial bulbil production during the invasion process in drylands. This knowledge will contribute to design actions to control them where they become invasive.

Agave fourcroydes and *A. sisalana* showed similar reproductive behaviour, both in quantity and phenology of flowering and ramets production, probably for various reasons. First, they are phylogenetically very close, and their origin shares the same purpose as human-made varieties for fibre production (Colunga-GarcíaMarín 2003; Jiménez-Barrón et al. 2020). *A. fourcroydes* originated from a selection from *A. angustifolia* (Gentry 1982), while *A. sisalana* is a hybrid between *A. angustifolia* and *A. kewensis* (Colunga-GarcíaMarín 2003). Second, both species have undergone deep genetic erosion throughout this selection, which guarantees the intended germplasm (Colunga-GarcíaMarín and May-Pat 1993). In this process, similar traits were sought, mainly more significant fibrousness, lesser thorniness, low sexual reproductive capacity, and gigantism (Colunga-García-Marín 2003). This comparable performance offers an advantage to their control, as it allows managers to deal with invasion events for both species with the same strategy.

The clonal mechanisms showed differences in terms of efficiency and phenology. The production of bulbils by reproductive individuals was high compared to other *Agave* species (Arizaga and Ezcurra 2002). Nevertheless, despite the excellent multiplication potential of the bulbils—with high rooting capacity under suitable environmental conditions—their establishment was virtually null in the invaded area during the study period. The climatic conditions prevailing in autumn and winter (low temperatures, short photoperiod, and high air humidity; AEMET 2012) and the partial burial of the bulbils produced by the rainfalls, would increase soil water availability, and bulbil rooting odds. However, the harsh environmental conditions of summer resulted in the death of all rooted bulbils. Our results agree with those of Badano and Pugnaire (2004) in the same study area, where they found a low rate of bulbil establishment (1.3%), and with those from *A. macrocantha* (2.0%) in its native arid zones of Mexico (Arizaga and Ezcurra 2002). In this study, bulbil establishment only occurs under nurse plants and in rainy years (Arizaga and Ezcurra 2002). The great effort that these *Agave* species invest in bulbil production is paradoxical. Arizaga and Ezcurra (2002) justify it as an adaptive trait that, despite the significant failure, any chance should be considered. In our cases, we must take into account the artificial origin of these species, with helpful propagation mechanisms to growers, thus maintained throughout their selection (DAFF 2015). Therefore, although bulbil production is a conspicuous and highly productive mechanism of very efficient ramets, the environmental conditions for their establishment, particularly soil water supply, predetermine this low efficiency in drylands (Arizaga and Ezcurra 2002). The establishment of bulbils will only be achieved under exceptionally wetter conditions than

those that usually exist in arid areas. Consequently, this mechanism would not explain the invasive behaviour of *A. fourcroydes* and *A. sisalana*.

Our data indicated that these *Agave* species produced RS at least from medium-size juveniles, and probably from smaller (younger) individuals than those studied, which would also have this multiplication capacity. The predominance of young individuals in the study zone supported these results (Badano and Pugnaire 2004). Furthermore, the 1.5 RS emerged from soil per medium-size individual would increase *Agave* populations. In addition to our data, Badano and Pugnaire (2004) also supported that this mechanism was highly effective for the spread of these species and the increase of their invasiveness. This type of clonality, in which the offspring remains connected to the mother plant for a prolonged period, would allow for the so-called clonal integration, which involves the translocation of resources and the communication between the mother plant and the offspring (Cornelissen et al. 2014). There is evidence that clonal integration is involved in the invasiveness of clonal plants (Liu et al. 2016). Since clonal integration mitigates local resource shortage (Liu et al. 2016), there is likely at least some alleviation of water scarcity in these *Agave* species, ensuring the survival of all new individuals. Besides, succulence and advantages of CAM nature in hot, arid conditions (Owen et al. 2016) are traits that native species do not have. Such traits could contribute to *Agave* shoots surviving— we recorded no RS mortality—and being active during the driest and warmest season. Significantly, the extended activity of RS allows them to colonise temporarily empty niches during the dormant period of native species (Godoy et al. 2009), which complete their cycle in early spring (Matesanz and Valladares 2014). Overall, the different phenology of these *Agave* species in growth, flowering and bulbil production compared to coexisting native species is a frequently documented feature of invasive species invading the Mediterranean area (Matesanz and Valladares 2014). Exploiting temporary empty niches offers low competition for resources to invasive species against natives (Godoy et al. 2009), which would confer competitive advantages to the exotics. These facts would support the fluctuating resources hypothesis, according to which an alien plant species could benefit from a temporarily (phenologically) empty niche (Davis 2009) by using momentarily available resources (space, soil water, nutrients) (Wolkovich and Cleland 2011). In addition, the contractile roots of RS would provide them additional survival benefits, as they would allow the shoots to partly react to limited water availability (North et al. 2008). In *Agave deserti*, a species close to *A. fourcroydes* and *A. sisalana* (Good-Avila et al. 2006), these roots show zones with increased water permeability (North and Baker 2007), which may also contribute to improving environmental water uptake, apart from the supply provided by the mother plant. Finally, BS production would not guarantee the persistence of *Agave* populations in the study area since, although we did not observe BS mortality (which

argues that clonal integration would also be present), the production was less than one BS per individual. This clonal mechanism would instead promote the production of monospecific clusters of impenetrable thickets that characterize the "cacti syndrome" (Novoa et al. 2020).

Our outcomes support that RS production was the most effective clonal mechanism for the performance of *A. fourcroydes* and *A. sisalana* in drylands. This finding may have implications for the management of invaded native plant communities. *Agave*'s experimental mechanical removal shows that mature individual elimination is insufficient to control or eradicate it. RS colonised the gap left only one year after removal, requiring additional manual action for control (García-de-Lomas et al. 2018). This double-action procedure is costly and arduous (García-de-Lomas et al. 2014), resulting in low efficiency. Since the success of these invasions seems to derive mainly from the RS and that mature individuals will eventually flourish and die (and the bulbils they will produce pose no threat), we suggest focusing efforts on the uprooting of RS from their emergence, along with monitoring the eventual positive response of the agaves to this action. This strategy could be an exportable measure, more effective and less costly than the removal of prominent individuals and could also be considered a type of habitat management tool to address these invasions worldwide. Habitat management has been successfully used to handle biological invasions (e.g., Didham et al. 2007; Kueffer et al. 2013), with strategies that depend on the nature of the interacting elements such as the invasive species, the receiver habitats, the native species, and the phase of the invasion (Davis 2009).

Conclusions

Agave fourcroydes and *A. sisalana* display a similar strategy of clonal propagation that comprises a set of very efficient mechanisms to cover all possibilities of establishment throughout the year (production of RS and BS during most of the year and bulbils during rainy periods), taking advantage of any environmental window of opportunity that may arise. These traits translate into a high potential for invasion. In particular, the propagation-related traits identified as key to their invasiveness are 1) the production of RS as the primary mechanism of clonal multiplication, 2) the clonal integration between the mother plants and RS, which seems to provide new shoots with a complete probability of survival, even during the extreme environmental conditions of summer, and 3) the ability to develop and spread such shoots when native plants are dormant, favouring their expansion through temporarily empty niches. Although the rooting potential of bulbils is very high and bulbil establishment might be one of the primary expansion mechanisms during wet years, their success of rooting and establishment in arid ("usual") conditions is negligible. Therefore,

medium- and long-term efforts to control the invasion should focus on eliminating RS rather than investing resources in removing prominent reproductive individuals or collecting bulbils. The similar propagation behaviour of the two *Agave* species is advantageous for managers, as eradication or control strategies can be the same.

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Authors' contribution

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Supplementary material

The following supplementary material is available for this article:

Table S1. Total aerial bulbil and basal shoot production per reproductive individual of *Agave fourcroydes* and *A. sisalana*.

Table S2. Detailed root profile data from 8 individuals of *Agave fourcroydes* and *A. sisalana*.

Table S3. Rooting rates of bulbils of *Agave fourcroydes* and *A. sisalana* monitored under natural conditions.

Table S4. Rates of bulbil conditions of *Agave fourcroydes* and *A. sisalana* monitored under natural conditions.

Table S5. Standardized coefficients of the best-fitting Generalized Linear Mixed Models (GLMMs) explaining bulbil rooting success under the monitored conditions (natural conditions, and experimental plots).

Figure S1. Daily rainfall (l/m^2) and daily maximum and minimum temperatures (°C) in the study zone along the monitoring period.

This material is available as part of online article from:

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