

Effect of intercropping and ploughing on Mediterranean managed grasslands invaded by *Oxalis pes-caprae*

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Abstract

Intercropping can increase crop productivity and control invasion by alien plants, giving at times more effective results than mechanical control. *Oxalis pes-caprae* L. (soursob) a South African perennial geophyte reproducing vegetatively by bulbs is invading Mediterranean communities around the world. Two adjacent field experiments were conducted in a grassland infested for a long time by soursob in Menorca (Balearic Islands) to test whether intercropping using the grass *Lolium multiflorum* Lam. (ryegrass) or the legume *Hedysarum coronarium* L. (sulla) alone or in combination increased crop production and reduced invasion by soursob. Winter aboveground biomass removals were also conducted to test whether intercropping was more effective in controlling soursob invasion than mechanical control.

Mixed crops did not have higher production than monospecific crops. Ryegrass plots had the lowest soursob cover. However, this decrease in soursob cover was low and only lasted one season. Aboveground biomass removal by ploughing the plots in January, when the soursob parent bulb is almost depleted and further bulb production has not yet occurred, reduced the soursob cover by more than 70%. Overall, contrary to expectations, intercropping neither increased crop production, nor decreased invasion. Mechanical control was notably more effective than cropping and had a legacy effect.

Keywords: Noxious weed, Bermuda butter-cup (soursob), disturbance, invasion, Menorca, ploughing.

Introduction

Human activities are the source of both the intentional and accidental introductions of exotic plants. Some of these exotics have the potential to spread over large areas (Pyšek *et al.* 2004) causing major ecological and economic impacts. For example, weeds can reduce the quality and the

yield of crops and increase management expenses (Pimentel *et al.* 2001).

One area of research in biological invasions focuses on the ecological factors promoting ecosystem invasibility (i.e. ecological factors determining the survival and spread of invaders within a community) (Lonsdale 1999). Disturbances are an important precursor of invasions since they eliminate or reduce plant competition, change the soil microflora and nutrient pools, the native seed banks and nearby seed sources (Forcella and Harvey 1983, Fox and Fox 1986). Soil management activities are an important human-caused disturbance, which affect large portions of land, for example sowing preparation and soil fertilization can sometimes increase weed populations (Navarrete 1992).

In agroecology, there is debate over whether intercropping (i.e. the planting of two or more crops in mixtures) has advantages over single crops by increasing crop yield and reducing weed growth. Jolliffe (1997) compiled studies that compared monocultures and mixtures and concluded that in general, mixtures had a higher productivity than monocultures especially when one of the species was a nitrogenfixer (Vandermeer 1989, Fukai 1993). Although resource exploitation could be more intense in mixed cropping than in single cropping and it could reduce weed growth, this outcome depends on the identity of the crop and weed species (Natarajan and Willey 1980, Reddy and Willey 1981). In this paper we evaluate if mixing a grass and a legume had advantages over single cropping in productivity and the control of the weed *Oxalis pes-caprae* L.

Oxalis pes-caprae L. (Oxalidaceae) (soursob hereafter) is a small perennial geophyte plant from South Africa recognized as a weed worldwide (Clarke 1934). This species is tristylous and legitimate pollination occurs only between individuals of different floral morphs (Rottenberg and Parker 2004). In the Mediterranean basin only the short style pentaploid morphs are present and plants do not set seed

(Rottenberg and Parker 2004). Soursob grows from underground annual bulbs that remain dormant over summer and sprout in autumn. Plants grow rapidly until spring and senesce in late spring before bulb maturation. Each plant can produce up to 20 bulbs with a very high percentage of sprouting and establishment (Vilà *et al.*, 2006). These bulbs are effectively dispersed underground via extensive roots (Pütz 1994). Disturbances play an important role in the dispersal of bulbs. Bulbs can be dispersed by soil movement, attached to the feet of domestic animals, running water or by agricultural machinery. In Menorca (Balearic Islands, Spain), where the study was conducted, this species invades more than 60% of the grasslands (Gimeno *et al.* 2006). Soursob has a negative effect on livestock because its leaves contain a high concentration of soluble oxalates that are poisonous if consumed in high quantities (Libert and Franceschi 1987) imposing a serious effects on the livestock economy (Raquin and Edward-Jones, unpublished data). In Menorca no specific management against soursob is practised, except for the use of chemical methods (i.e. glyphosate) in years of high levels of infestation. However, the costs of controlling the weed chemically are higher than the costs of the soursob impact (Raquin and Edward-Jones, unpublished data).

The effect of the two crop species *Lolium multiflorum*, a grass and *Hedysarum coronarium*, a legume, was analysed in single and mixed cropping in soils invaded by soursob on vegetation cover, crop production and weed control. The effectiveness of soursob control by mechanical removal was also tested. The hypotheses was that when crops are mixed the total crop production will be larger and the weed growth will be lower than in monoculture. It was also expected that if mechanical control is conducted before soursob produces propagules the infestation could be decreased but not as effectively as by intercropping.

Methods

Study species

Lolium multiflorum Lam. (Poaceae) (ryegrass hereafter) is a winter annual grass that may reach 0.5–1 m in height. Ryegrass establishes fast and has a high yield and thus has high value as livestock forage. In Menorca ryegrass grows in disturbed sites, roadsides, fields and pastures and it is also used in seed mixtures as a quick cover crop (www.cime.es/ca/ccea/4.pdf).

Hedysarum coronarium L. (Papilionaceae) (sulla hereafter) is a shrubby, herbaceous perennial or biennial that typically grows 0.3–1.5 m tall and wide. In Menorca this plant is cultivated for hay and livestock fodder and it also grows in seminatural areas (i.e. old fields, roadsides) of the island (www.cime.es/ca/ccea.44.pdf and www.cime.es/ca/ccea.51.pdf).

Area of study and experimental design

The study was conducted in a fallow field heavily invaded by soursob ($98.2 \pm 0.7\%$ cover) for at least the last 10 years and located at the Technical School of Agrarian Experimentation in Menorca (0606889 N, 4422231 E), (Balearic Islands, Spain). The mean annual temperature is 16.5°C and the annual mean precipitation 600 mm. The soil is a sandy silt loam containing 0.15% N and 1.04% C.

In September 2002 a 2546 m² area of the field was split in two parts. One received a superficial (20 cm deep) ploughing treatment and the other a deep (35 cm deep) ploughing treatment. In each ploughed area twenty 5×4 m plots separated by 3 m were laid out. The following four treatments were randomly assigned within each ploughed area:

- eight plots with ryegrass (ryegrass monoculture)
- four with sulla (sulla monoculture),
- four plots planted with a mixture of ryegrass and sulla (intercropping),
- four untreated plots (control).

The seeding rate was 40 kg ha⁻¹ of ryegrass and 30 kg ha⁻¹ of sulla in single crops and 20 kg ha⁻¹ of ryegrass and 15 kg ha⁻¹ of sulla in mixed crops. Seeding was conducted manually. In the mixed seedling, both types of seeds were planted simultaneously. Before seeding the field was fertilized with 500 kg ha⁻¹ P:N:K (15:15:15).

To test the effect of mechanical control on soursob, four randomly chosen plots seeded with ryegrass received a superficial ploughing in January 2003 when soursob plants had around 23 leaves. At this stage the parent bulbs have been depleted and new bulbs yet to be formed (Chawdhry and Sagan 1973). No measurement were taken in these plots during 2003.

Plant cover and crop production

In March 2003, ryegrass, sulla and soursob cover per plot was estimated, except for those ploughed in January 2003, using the point intercept method based on two diagonal transects 6.4 m long in which the species identity was noted every 0.2 m.

In late May 2003, at the time of maximum aboveground biomass, aboveground biomass in two central 1×1 m areas in each plot was harvested, excepting those ploughed in January 2003. Plant material was separated into: ryegrass, sulla, grasses other than ryegrass and herbs other than sulla. At this time of the year, soursob was completely senesced and therefore it was not possible to estimate its aboveground biomass. Plant material was weighed after drying at 70°C to a constant weight.

In June 2003, after harvesting, all plots were superficially tilled 20 cm in depth. In September 2003 all plots were superficially ploughed and seeded with ryegrass. In order to test the legacy effect of crop treatment and the mechanical control performed in January 2003, in January 2004 cover of soursob, ryegrass and herbs was estimated with the same method as in March 2003.

Statistical analysis

The effect of crop planting on ryegrass, sulla and soursob cover, total aboveground biomass, biomass of ryegrass, biomass of sulla, biomass of other grasses and of other herbs except for sulla was analysed by a one-way ANOVA with crop species as the explanatory variable. A different ANOVA was performed for each ploughing treatment because pseudoreplication precluded the inclusion of this treatment into the analysis. Differences between the four crop treatments, if significant, were compared with a Scheffé test.

The legacy effect of crop treatment and mechanical control on soursob, ryegrass and herb cover was compared by one-way ANOVA with crop species type in the previous year or the January 2003 ploughing as a single explanatory variable. Variables were log transformed if needed to meet the assumptions of parametric analysis.

Results

Cover estimates March 2003

Sulla appeared only where planted and its cover was not significantly different in mixed compared to monospecific plots (Table 1). Ryegrass cover was significantly

greater where planted. In the superficial ploughed area, there were no significant differences between monospecific ryegrass and mixed plots. In the deep ploughed area ryegrass cover was significantly lower in mixed plots than in the ryegrass plots and even lower in the monospecific sulla plots (Scheffé test, $P < 0.001$) (Table 1). Differences in soursob cover were not significant in the superficial ploughing treatment (Table 1). In the deep ploughing treatment the lowest values were found in monospecific ryegrass plots and the highest values in control plots.

Biomass production May 2003

There were significant differences in total aboveground production among plots ($F_{3,12} = 3.85$, $P = 0.038$ and $F_{3,11} = 7.48$, $P = 0.005$ for superficial and deep ploughing treatments, respectively). The pattern was very different between superficially and deep ploughing treatments (Figure 1). The highest total production was found in sulla plots with a deep ploughing treatment (264.32 ± 40.05 g m⁻²). In the superficially ploughed area, mixed plots had a higher total crop production than sulla monoculture plots (Scheffé test, $P < 0.05$) but lower than ryegrass (Scheffé test, $P = 0.05$). In contrast, in the deeply ploughed area, mixed plots had a lower total production than both monocultures (Scheffé test, $P < 0.05$). The ryegrass plots and sulla plots produced more biomass than control plots only in the deep ploughing treatment (Scheffé test, $P = 0.02$).

In the superficial ploughing treatment, there was no significant crop effect for ryegrass, sulla, other grasses or herbs production (Table 2). However, in the deep ploughing treatment, there were significant differences in sulla and ryegrass production among plots (Table 2). Ryegrass production was considerably reduced in mixtures and control plots in comparison with monospecific cropping (Scheffé test, $P = 0.01$) and it was not present in plots planted with sulla. Sulla was not present where it was not seeded and its production was larger in monocultures than in mixed crops (Scheffé test, $P = 0.02$).

Table 1. Percentage species cover (mean \pm standard error) in March 2003 in plots sowed with *Hedysarum coronarium* (sulla), *Lolium multiflorum* (ryegrass), both or nothing (control) in two different ploughing areas.

Superficial ploughing	ryegrass	sulla	ryegrass + sulla	control	df	F	
Sulla	0	4.21 ± 1.13	1.82 ± 1.82	0	1	1.85	ns
Ryegrass	42.82 ± 4.09^A	25.80 ± 4.09^B	37.01 ± 4.09^A	29.06 ± 4.72^B	3	5.12	**
Soursob	49.66 ± 6.43	50.40 ± 7.22	56.20 ± 3.83	66.24 ± 2.12	3	1.38	ns
Deep ploughing	ryegrass	sulla	ryegrass + sulla	control	df	F	
Sulla	0	22.98 ± 3.73	12.97 ± 3.38	0	1	3.95	ns
Ryegrass	52.41 ± 3.62^A	8.65 ± 3.62^B	33.32 ± 4.18^C	23.31 ± 3.62^D	3	23.19	***
Soursob	38.21 ± 5.58^A	52.36 ± 4.26^B	48.85 ± 7.64^B	66.85 ± 5.85^C	3	3.94	**

ns = non-significant differences, * P-value < 0.05 , ** P-value < 0.01 , *** P-value < 0.001 . Values with different superscript letters are significantly different between treatments.

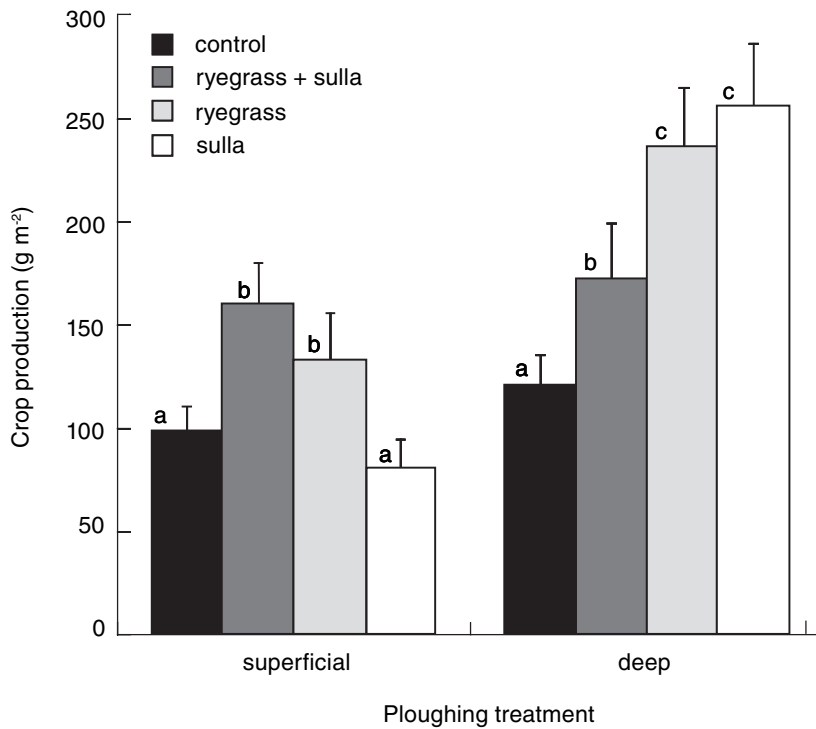


Figure 1. Total crop production (mean + standard error) in plots planted with *Hedysarum coronarium* (sulla), *Lolium multiflorum* (ryegrass), both or nothing (control) in two different ploughing treatments. Different letters above columns indicate significant differences within a ploughing treatment according to the Scheffé test.

Table 2. Production (g m⁻²) of *Lolium multiflorum* (ryegrass), *Hedysarum coronarium* (sulla), other grasses and other herbs in May 2003 in plots sowed with *Hedysarum coronarium* (sulla), *Lolium multiflorum* (ryegrass), both or nothing (control) in two different ploughed areas. Values are mean ± standard error.

Superficial ploughing	ryegrass	sulla	ryegrass + sulla	control	df	F	
Ryegrass	52.78 ± 28.05	0	44.96 ± 17.72	69.05 ± 18.87	2	2.26	ns
Sulla	0	9.11 ± 3.59	2.87 ± 2.87	0	1	2.08	ns
Other grasses	61.68 ± 12.33	59.96 ± 17.68	64.99 ± 16.25	20.19 ± 11.26	3	2.18	ns
Other herbs	2.09 ± 2.09	9.80 ± 9.21	45.63 ± 27.22	8.33 ± 4.20	3	1.81	ns
Deep ploughing	ryegrass	sulla	ryegrass + sulla	control	df	F	
Ryegrass	193.14 ± 26.48 ^A	0	65.03 ± 25.37 ^B	41.17 ± 22.14 ^B	2	18.44	***
Sulla	0	141.95 ± 21.22 ^A	32.39 ± 29.56 ^B	0	1	13.69	***
Other grasses	52.78 ± 28.05	66.64 ± 27.69	60.29 ± 16.38	22.30 ± 12.25	3	0.91	ns
Other herbs	5.51 ± 3.78	60.65 ± 26.46	13.77 ± 6.16	36.28 ± 20.27	3	2.28	ns

ns = non-significant differences, * P-value <0.05, ** P-value <0.01, *** P-value <0.001. Values with different superscript letters are significantly different between treatments.

Table 3. Percentage species cover (mean ± standard error) of herbs, *Lolium multiflorum* (ryegrass) and *Oxalis pes-caprae* (soursob) in January 2004 in plots sowed with ryegrass, *Hedysarum coronarium* (sulla), both or nothing (control) without mechanical control, and in plots sowed with ryegrass and with mechanical control (ploughed in January 2003). These treatments were performed on two distinct ploughed areas.

Superficial ploughing	No mechanical control				Mechanical control		df	F
	ryegrass	sulla	ryegrass + sulla	control	ryegrass			
Herbs	0	3.29 ± 2.49 ^A	4.60 ± 1.65 ^A	3.29 ± 1.26 ^A	22.37 ± 7.25 ^B	3	6.30	**
Ryegrass	23.03 ± 10.10 ^A	27.63 ± 9.15 ^A	20.39 ± 9.33 ^A	34.87 ± 6.74 ^A	94.74 ± 3.22 ^B	4	14.64	***
Soursob	82.90 ± 4.51 ^A	93.42 ± 3.48 ^B	90.13 ± 3.78 ^{AB}	90.79 ± 3.13 ^{AB}	17.76 ± 3.46 ^C	4	20.07	***
Deep ploughing	ryegrass	sulla	ryegrass+ sulla	control	ryegrass	df	F	
Herbs	0	1.97 ± 1.26 ^A	1.97 ± 0.66 ^A	3.95 ± 2.52 ^A	18.42 ± 6.27 ^B	3	5.32	**
Ryegrass	39.47 ± 11.87 ^{AB}	50.66 ± 9.50 ^A	54.61 ± 19.60 ^{AB}	32.89 ± 4.09 ^B	97.37 ± 1.86 ^C	4	4.82	*
Soursob	93.86 ± 3.16 ^A	85.53 ± 6.49 ^A	84.87 ± 6.02 ^A	90.79 ± 1.70 ^A	27.63 ± 5.83 ^B	4	28.77	***

ns = non-significant differences, * P-value <0.05, ** P-value <0.01, *** P-value <0.001. Values with different superscript letters are significantly different between treatments.

Cover estimates after mechanical control
The mechanical control conducted on January 2003 had a very significantly decreased soursob cover and increased ryegrass and herb cover in the following year 2004. This occurred both in plots that had been superficially and deeply ploughed before seeding in 2002 (Table 3).

In the superficially ploughed plots, soursob cover diminished by 80% with mechanical control and ryegrass cover increased by 63%. The cover of herbs other than soursob was five times higher in plots where mechanical control had been applied (Scheffé test, P = 0.003). In the deep ploughing area the same trend was found. The soursob cover was reduced by 70%, ryegrass increased by nearly 50% and herbs increased by 89% (Table 3).

Discussion

Our study did not confirm our hypothesis that mixed plots had larger total crop production than monospecific plots. In the superficial ploughing plots, mixed crops had a total production greater than that of plots planted with sulla but not significantly different to plots planted with ryegrass. The result was more unexpected in deeply ploughed plots, where mixed crops

had lower total production than both monospecific crops. Therefore, our results do not demonstrate that productivity increases with species number in contrast to other experiments in European grasslands where plant cover and aboveground biomass increase with plant species richness (Hector *et al.* 1999).

Furthermore, it was expected that mixed crops would exploit resources more intensively than monospecific crops and should therefore allow less weed growth. However, weed growth in mixtures was not lower than in single crops as has been evidenced in other studies (Mohler and Liebman 1987, Liebman and Dyck 1993). In fact, no significant effect was found in the superficially ploughed treatment. In deeply ploughed plots ryegrass monocultures had lower soursob cover compared to the other crop treatments. The contrasting difference of the two crop species on soursob cover could be related to their different phenology. While ryegrass emergence and growth matches that of soursob, sulla emergence and development is slower and therefore its phenology is different. Nevertheless, this negative effect of ryegrass on soursob was only evident during the first season.

Finally, mechanical control of soursob by superficial ploughing in January was very effective and had a legacy effect. It reduced soursob cover by 80% one year after treatment. This strategy of control was initially proposed by Michael (1965) and it was based on the prevention of bulb production. The success of this technique is related to the effect of removing aboveground biomass at the stage when plants have 20–30 leaves, after the parent bulb is exhausted and before the new bulbs have been formed (Chawdhry and Sagan 1973). It remains to be investigated whether intercropping could have a significant effect in reducing soursob success if conducted the following year after mechanical control when bulb density is much reduced.

Due to logistic constraints, ploughing was not a randomly assigned and replicated treatment in the experimental design. However, the field where the study was conducted was very homogeneous and there is no reason to suspect that other environmental factors besides ploughing could affect the differences between crop treatments. In general, significant differences were found only in deeply ploughed plots. It is known that the ploughing technique is an important factor for seed redistribution in the soil. The timing and intensity of ploughing can change the position of the seeds to a better or worse position for germination (Cousens and Moss 1990). Deep ploughing disturbs the soil surface more than superficial ploughing and thus, the first technique has a major effect on community composition and structure (Smith and Knapp 1999, Stylinski and

Allen 1999). Moreover the deep ploughing technique could favour the development of plant species with deep roots such as sulla. This could explain the differences in sulla production in superficial and deep ploughing treatments. In contrast, the ryegrass production in both treatments was not very different because this species has a superficial root system.

Overall ryegrass interfered more with soursob than sulla but the effect on soursob cover was only significant in deeply ploughed plots. Although intercropping is considered a potential means of maintaining or increasing crop production, and reducing infestation with fewer synthetic fertilizers and pesticides (Horwith 1985), our results showed that for soursob it was not effective at all.

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