Cattle dung as vector of spreading seeds of exotic species in the Flooding Pampa grasslands (Buenos Aires, Argentina)

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Emergence of seedlings from cattle dung collected in different seasons from grasslands of the Flooding Pampa was analysed. The objective of this work was to find out (1) how high the proportion of seeds from exotic species in the seeds spread by cattle dung is, and (2) what the forage quality of the species spread through cattle dung is. Fresh dung was collected in late spring and late summer from three grasslands. Seedling emergence from dung was done in greenhouse conditions. Seedling number, forage quality and origin of the species were determined. We found 121 plant species growing in the grasslands. The number of species emerging from dung was 41. Twenty-four and 36 species emerged from dung collected in late spring and late summer, respectively. Nineteen species were common for both seasons. There were 5.33 ± 0.26 and 1.00 ± 0.15 (mean \pm SE) native and exotic species, respectively, per dung sample (22.50 g dry weight) collected in late spring. From the dung collected in late summer emerged 7.70 \pm 0.31 and 2.63 \pm 0.18 native and exotic species per sample, respectively. Carex spp., Cyperus spp. and Juncus spp. (native species) were the most abundant (95%) in dung collected in late spring. In dung collected in late summer Cynodon dactylon (an exotic species) represented 76% and Carex spp., Cyperus spp. and Juncus spp. represented 9%. The most abundant species emerged from dung were of low forage quality and thus are considered weeds in crop fields. Seed propagation through cattle dung helps to explain the increment of exotic species in the Flooding Pampa grasslands under grazing. The results are relevant for the range managers who normally move the cows between stubble crop fields and grasslands, spreading through dung seeds of species of different origin and quality.

Key words: endozoochory, dung, forage, grasslands, seed dispersal, weeds

Introduction

The propagation of plant species into new habitats is strongly dependent on the patterns and mechanisms of seed dispersal (Radosevich *et al.* 2003, Constible *et al.* 2005). Cattle dung depositions provide an opportunity for plant species establishment in grazed grasslands. Dung

depositions generate gaps and provide nutrients and organic matter that facilitate seedling emergence and growth (Janzen 1984, Gardener et al. 1993, Haynes & Williams 1993, Malo & Suárez 1995a, 1995b, Sevilla et al. 1996, Ocumpaugh et al. 1996, Gokbulak & Call 2004). Introduction and dispersal of exotic and native species seeds through cattle dung (endozoochory: ingestion and later excretion of seeds) and its impact on plant communities have been widely studied in different grasslands (Janzen 1984, Welch 1985, Cox et al. 1993, Gardener 1993, Malo & Suárez 1995a, 1995b, Dai 2000, Malo et al. 2000, Pakeman et al. 2002, Constible et al. 2005). For example, on Santa Catalina Island, California, where historically there were no large native grazing animals, propagation of exotic species of Mediterranean origin was facilitated through introduced bison (Bison bison; Constible et al. 2005).

Flooding Pampa grasslands (Buenos Aires Province, Argentina), evolved under a light grazing regime, were heavily disturbed by domestic herbivores after the arrival of European colonists in the 16th century (Sala et al. 1986, Sala 1988). The major effects of grazing upon grassland structure have been on the floristic composition, species diversity, horizontal and vertical distribution of biomass, and specific basal cover (Sala et al. 1986, Chaneton et al. 1988, Sala 1988). The changes in floristic composition are attributed to displacement of native species by exotic ones (Sala et al. 1986, Chaneton et al. 1988, Sala 1988, Oesterheld & Sala 1990). Exotic species inventoried in the Flooding Pampa grasslands are principally annuals of Mediterranean origin (Söyrinki 1991, Perelman et al. 2001, Chaneton et al. 2002). Some exotic species are considered weeds (Marzocca 1976, Montes et al. 2001) and some of them can decrease the forage quality of the grasslands (Cauhépé et al. 1985, Chaneton et al. 1988).

Some exotic species found in the Flooding Pampa grasslands were also recorded in grasslands and pastures of other countries, with cattle dung as vector of propagation (Jones *et al.* 1991, Gardener *et al.* 1993, Malo & Suárez 1995a, Bruun & Poschlod 2006). We believe that the increase of exotic species under grazing conditions in the Flooding Pampa grasslands could be facilitated through cattle dung depositions. Information about which species could be spread via endozoochory by cows in the Flooding Pampa grasslands is so far scarce. We focused the present study on the followingt questions: (1) How high is the proportion of seeds from exotic species in the seeds spread by cattle dung? (2) What is the forage quality of the species spread through cattle dung?

Material and methods

Study sites

The study sites were three grasslands of the Flooding Pampa private rangelands (Ayacucho county, Province of Buenos Aires, Argentina). The rangelands were: San Marcos (37°24'39''S, 58°29'20''W), La Esperanza (37°17'38''S, 58°25'20''W) and Santa Catalina (36°59'25''S, 58°44'32''W), using natural grasslands as forage source. The main activity in these rangelands is cattle breeding (Aberdeen Angus). The rangelands of San Marcos, La Esperanza and Santa Catalina are approximately 260, 500 and 200 ha, respectively, with an annual average stocking rate of 0.6-0.7 animals unit ha-1. Only cattle grazed in our study sites. This region has a subhumid and temperate climate, with a mean annual rainfall of 1008 mm (1971-1991) and mean monthly temperatures ranging from 6.8 °C in July to 21.8 °C in January.

These grasslands present a mosaic vegetation constituted by patches dominated by tall (1.5-2.0 m) tussock grasses of Paspalum quadrifarium and P. exaltatum (locally known as "pajonal") and communities of shorter plants (< 0.40 m). The pajonal is a fragmented landscape with patch size ranging between 0.09 and 1653 ha (Herrera et al. 2005). The communities of shorter plant and pajonal differ in their physiognomy, plant height, green to dry biomass ratio, management, soil types, plant species, and forage quality (Laterra et al. 2003, Herrera el al. 2005). The primary production is seasonal, with the maximum during spring-summer and the minimum in winter. Rainfall is evenly distributed throughout the year but the region is usually affected by winter and spring floods and summer droughts (Chaneton et al. 1988). Plant species found in the grasslands during late spring 2002 are listed in Appendix. There were animals in the same grasslands for at least two months prior to the dung collecting.

Cow dung collecting

The germinable seed content was assessed in fresh dung collected in late spring (December 2002) and late summer (March 2003), when the cool-season and warm-season species were reproducing, respectively (León & Bertiller 1982). Thirty dung samples recently dropped were taken in each grassland at random from short plant communities along a 800-m line transect. In order to avoid contamination by seeds not ingested by cattle, 2-3 cm of the external part of the dung that was in contact with the vegetation and soil surface was discarded. The samples were kept in plastic bags, weighed and stored for 15 hours at 4 °C. Then, the samples were spread out on trays and air-dried in darkness at room temperature (Malo & Suárez 1995a). Dry samples were stored in paper bags at laboratory conditions.

Seedling emergence from dung

Germination took place in a greenhouse of the Unidad Integrada Balcarce (EEA, INTA Balcarce, Facultad de Ciencias Agrarias, UNMdP, Province of Buenos Aires, Argentina, 37°45′36′′S, 58°17′55′′W). During the study period, greenhouse air temperature was very variable — maxima and minima being 23 °C and 11 °C, 19 °C and 5 °C, 23 °C and 9 °C, 28 °C and 14 °C, for autumn, winter, spring and summer, respectively — and capable of breaking the seed dormancy of most species (Baskin & Baskin 1998).

On 11 March 2003, seedling emergence was assessed using 10 dry dung samples taken from each grassland in late spring. Each sample of 22.50 g dry weight was carefully crumbled to avoid seed destruction and spread out in trays (12 cm width \times 20 cm long \times 8 cm depth) on a bed of perlite. Dung sample was spread in layer between 0.5 and 1 cm thick. The trays had trans-

parent plastic covers. To maintain humidity, the samples were watered during the study period. Emerging seedlings were identified according to descriptions of local flora (Cabrera 1970, Marzocca 1976, Montes et al. 2001) and removed. Those species that could not be identified in the seedling stage were transferred into separate pots and grown further. When seedling emergence was not observed for two weeks, the irrigation was interrupted and the samples were left to dry. Later, the dung was stirred and watered to stimulate new emergence pulse. Three control trays with only moist perlite were also set and in those trays seedling emergence did not take place. The emergence pulses were from March 2003 to May 2003, from May 2003 to December 2003, from February 2004 to April 2004, and from May 2004 to August 2004. The trays were periodically shifted from their position to ensure homogeneity in their growing conditions.

Seedling emergence from dung taken in late summer was assessed on 6 May 2003 using 10 dry dung samples of the same weight as in late spring. Emergence pulses were recorded from May 2003 to December 2003 and from February 2004 to April 2004. Emerging seedlings were identified at an early stage and mortality did not take place in the samples.

Data analysis

Forage quality of species recorded in cow dung was evaluated using the Grazing Value Index (GVI) proposed by Daget and Poissonet (1971) and used for different grasslands of the Flooding Pampa (Cauhépé *et al.* 1985, Oesterheld & León 1987, Chaneton *et al.* 1988). The relative contribution of each species and its forage quality were used to estimate the GVI as follows:

$$\text{GVI} = 0.2 \sum_{i=1}^{n} \text{CS}_{i} \times \text{SI}_{i}$$

where *n* is the species number, CS_i is the relative contribution of species *i* to the total number emerged in dung collected in a given grassland, and SI is an index of the forage quality of the species *i*, ranging from 0 to 5. SI_i is determined by nutritional, digestibility and assimilation values. Species with SI = 0 have no forage

quality, and those with SI = 5 have high forage quality. The SI values for different plants species growing in the Flooding Pampa grasslands were proposed by Cauhépé *et al.* (1985).

A non-parametric Mann-Whitney U-test was used to compare the total species numbers between seasons and the numbers of exotic and native species. The results were considered statistically significant at p < 0.05; results are given as means with ±1 SE.

Results

Plant species richness in the grasslands

The total number of plant species found in the grassland was 121 (Appendix).

Origin and forage quality of different species emerged from dung

Due to the difficulty in identifying accurately *Carex* spp., *Cyperus* spp. and *Juncus* spp. at the seedling stage, these species were considered as a group (Table 1). *Atriplex* sp. was not identified. According to the posterior identification the species that emerged from the dung were probably the native *Carex bonariensis*, *Cyperus eragrostis*, *Juncus imbricatus* and *Atriplex montevidense*.

The total number of species that emerged from the dung (22.5 g d.w. \times 10 samples \times 3 grasslands $\times 2$ seasons) was 41 and of those 13 (32%) were exotic. The number of species that emerged from the dung was lower (66%) than the total number of species found in the grasslands (Table 1 and Appendix). The total number $(22.5 \text{ g d.w.} \times 10 \text{ samples})$ of seedlings that emerged in late spring and late summer were not significantly different, 2430 \pm 1152 and 2960 \pm 884, respectively. Twenty-four and 36 species emerged from the dung (22.5 g d.w. \times 10 samples \times 3 grasslands) collected in late spring and late summer, respectively (Table 1). Nineteen species were common for both seasons (Table 1). Carex spp., Cyperus spp. and Juncus spp. were the most abundant species (95%) in the dung samples collected in late spring. In the samples colleted in late summer Cynodon dactylon (76%)

and Carex spp., Cyperus spp. and Juncus spp. (9%) seedlings were the most abundant. Significantly fewer species emerged in late spring than in late summer: 6.26 ± 0.32 and 10.53 ± 0.42 species per dung sample (22.5 g d.w), respectively (Table 1; U = 46, p < 0.0001). The number of native species that emerged from the dung was significantly higher than that of exotic species (Table 1; U = 66, p < 0.0001). The numbers of native and exotic species per dung sample collected in late spring were 5.33 ± 0.26 and 1.00 ± 0.15 , respectively. As per dung sample collected in late summer, 7.70 ± 0.31 native and 2.63 ± 0.18 exotic species emerged. Approximately 80% of the species emerged within the first 52 and 32 days from the beginning of the study from the dung collected in late spring and late summer, respectively (Table 2). The most abundant species that emerged from the dung were of low forage quality. GVI calculated for late-spring species was significantly lower than that for late-summer species: 30.17 ± 0.55 and 36.87 ± 0.28 (*n* = 3, *p* < 0.049), respectively.

Discussion

Exotic species of different forage quality have invaded the Flooding Pampa grasslands at a regional scale as a consequence of grazing by cattle (Sala et al. 1986, Perelman et al. 2001, Chaneton et al. 2002). Although in this study seedling emergence from cattle dung was analyzed in controlled conditions, endozoochory can explain the propagation of these plant species in the Flooding Pampa grasslands. For example Constible et al. (2005) reported that the introduction of bison on Santa Catalina Island, California, where historically there were no large native grazing animals, contributed to the propagation of exotic species through endozoochory and seed-laden hair. Grasslands of the Flooding Pampa were disturbed by domestic cattle after the arrival of European colonists in the 16th century (Sala et al. 1986). Other exotic animals, beside cattle (Ghersa & Matínez 1985, Sevilla et al. 1996), that spread seeds via endozoochory in the Flooding Pampa grasslands are horses (Ansin 2001, O. N. Fernández pers. obs.) and hares (Vignolio & Fernández 2006).

In this study, we recorded 13 exotic species that emerged from dung. However, other exotic species such as *Festuca arundinacea*, *Cardamine hirsuta* and *Medicago polymorpha* have also been found in cattle dung (O. R. Vignolio unpubl. data). Higher numbers of species emerging from dung collected in late summer could be due to the fact that some species flowering in spring prolonged their reproductive period to summer (León & Bertiller 1982) and/ or due to persistence of seeds in flowering stalks. Although approximately 80% of the species emerged within the first 52 and 32 days from the beginning of the study from the dung collected

Table 1. Percentages of emerged species from all cow dung samples collected in late spring and late summer in different grasslands of the Flooding Pampa (Buenos Aires, Argentina). Abbreviations: Forage quality: L = Low, M = Medium, H = High, and W = Weed (Adapted of Cauhépé *et al.* 1985); Origin: E = Exotic, N = Native of (South or North) America, and C = Cosmopolitan (Cabrera 1970, Sala 1988, Söyrinki 1991, Zuloaga & Morrone 1999, Montes *et al.* 2001). SI: index of the forage quality (Cauhépé *et al.* 1985).

Species	SI	Quality	Origin	Spring (%)	Summer (%)
Graminoids					
Agrostis avenacea			E	0.029	0.014
Carex spp., Cyperus spp., Juncus spp.	1.5	L–M	N	93.519	12.676
Chaetotropis elongata	3	Μ	N	0.0	0.026
Cynodon dactylon	2	М	E	0.372	74.334
Diplachne uninervia			N	0.060	0.134
Distichlis spicata	1	L	N	3.206	0.604
Echinochloa crusgalli			E	0	0.028
Hordeum stenostachys	2	Μ	N	0.089	0
Lolium multiflorum	5	Н	E	0.022	0.301
Lolium perenne	5	Н	E	0.183	0.033
Parapholis incurva			E	0	0.064
Paspalum dilatatum	4	Н	N	0	0.055
Paspalum distichum	1	L	Ν	0.105	6.721
Paspalum vaginatum	1	L	N	0.163	2.217
Poa annua			E	0.559	0.007
Puccinellia glaucescens			Ν	0.129	0
Setaria geniculata			N	0.058	0
Sisyrinchium iridifolium	1	L	N	0.007	0
Sporobolus indicus	3	М	N	0.445	0.923
Stenotaphrum secundatum	2	Μ	N	0	0.746
Forbs					
Alternathera philoxeroides			N	0	0.097
Anagallis arvensis			E	0	0.014
Apium leptophyllum			N	0	0.012
Atriplex sp.			N	0	0.007
Eclipta alba			Ν	0	0.014
Eryngium echinatum	1	L	N	0	0.063
Lepidium bonariense			N	0.045	0.040
Lotus tenuis	3	Н	E	0.014	0.184
Ludwigia peploides			N	0	0.007
Lythrum hyssopifolia			С	0	0.039
Lythrum maritimum			Ν	0	0.007
Mentha pulegium	0	W	E	0	0.078
Phyla canescens	0	W	N	0	0.119
Plantago myosuros	2	Μ	Ν	0.691	0.095
Polygonum aviculare			E	0.058	0.254
Polygonum hydropiperoides			N	0.117	0
Portulaca oleracea			С	0	0.036
Rumex crispus			E	0.074	0.012
Trifolium repens	4	Н	E	0.044	0.021

in late spring and late summer, respectively, seedling emergence continued also later. Different genotypes can require different environmental conditions, for example temperature, light, or moisture to break the seed dormancy (Baskin & Baskin 1998, Pezzani & Montaña 2006).

According to our results, endozoochory appears to favour the propagation of some species of low forage quality and weeds of crop fields. For example, *Cynodon dactylon* is an exotic species considered one of the most serious weeds in the world (Cousens & Mortimer 1995). It considerably reduces the crop yield (Fernández *et al.* 2002) and seedling recruitment of forage species such as *Lolium* spp. and *Trifolium repens* in the Flooding Pampa grasslands (Deregibus *et al.* 1986). Besides, it has low forage quality (Cauhépé *et al.* 1985) and can spread through cattle dung (Jones *et al.* 1991, Gardener *et al.* 1993). The short-distance propagation of *Cynodon dactylon* is mainly by stolons and rhizomes (Fernández *et al.* 2002) and seed dispersal through dung may be effective in colonizing news gaps generated in the grasslands. *Carex* spp., *Cyperus* spp. and *Juncus* spp. were also abundant in cattle dung and some of these species are weeds of crop fields (Marzocca 1976). Although other weeds such as *Polygonum aviculare*, *Anagallis arvensis* and *Portulaca oleracea* were present in low percentage, cattle dung can be an important vector of their propagation in grasslands and crop fields (Counsens & Mortimer 1995).

Our results show that the cattle spread through endozoochory a high percentage of low forage quality species. The low GVI values calculated in this study for emerged seedlings are in agreement with those reported for grasslands of low forage quality by studies using data on plant biomass (Cauhépé *et al.* 1985, Cauhépé & Laterra 1998) and cover plant (Chaneton *et al.* 1988). Grasslands with the total GVI of approxi-

 Table 2. Dates of seedling emergence from cow dung collected in late spring (December 2002) and late summer (March 2003).

Emergence date	Species
	Dung collected in late spring (December 2002)
21 March 2003	Cynodon dactylon, Plantago myosuros
25 March 2003	Distichlis spicata, Hordeum stenostachys, Polygonum hydropiperoides, Rumex crispus, Sporobolus indicus
4 April 2003	Lolium multiflorum, Lolium perenne, Poa annua, Setaria geniculata
10 April 2003	Agrostis avenacea, Carex spp., Cyperus spp., Diplachne uninervia, Juncus spp., Paspalum vaginatum, Sysirinchium iridifolium
2 May 2003	Paspalum distichum
6 June 2003	Lotus tenius, Trifolium repens
26 June 2003	Lepidium bonariense
8 July 2003	Polygonum aviculare
5 Aug. 2003	Puccinellia glaucescens
	Dung collected in late summer (March 2003)
19 May 2003	Lolium multiflorum, Lolium perenne, Trifolium repens, Lotus tenius, Rumex crispus, Plantago myosuros
26 May 2003	Cynodon dactylon, Paspalum distichum, Mentha pulegium, Alternathera philoxeroides, Lepidium bonariense, Cyperus spp., Carex spp., Juncus spp., Phyla canescens, Portulaca oleracea, Lytrum maritimum, Poa annua, Stenostaphrum secundatum, Eryngium echinatum, Polygonum aviculare, Apium leptophyllum
2 June 2003	Paspalum vaginatum, Chaetotropis elongata, Diplachne uninervia, Sporobolus indicus, Lythrum hyssopifolia, Eclipta alba, Distichlis spicata, Parapholis incurva
9 June 2003	Atriplex sp., Agrostis avenacea
19 June 2003	Echinochloa crusgalli
8 July 2003	Ludwigia peploides
7 September 2003	Paspalum dilatatum
17 October 2003	Anagallis arvensis

mately 27 were of low forage quality and with 75–55 were of high quality (Cauhépé *et al.* 1985, Chaneton *et al.* 1988). The GVIs recorded in dung collected in late spring were low, due to the higher contribution of *Carex* spp., *Juncus* spp. and *Cyperus* spp. On the other hand, the contribution of those species was low in late summer and the average GVI only increased 19% due to the abundance of *Cynodon dactylon*. Although our results cannot be directly extrapolated to field conditions it is reasonable to assume the seedling recruitment from dung and that these can affect forage quality of the grasslands.

The agricultural aptitude of some soils of the Flooding Pampa grasslands has determined their replacement by crops and pastures. Approximately 40% of these grasslands were converted into croplands and pastures between 1880 and 2000 (Solbrig & Viglizzo 1999, Laterra & Rivas 2005). On the other hand, the stocking rate increased in the Flooding Pampa grasslands from 0.7 to 1.1 animal units ha-1 (Vázquez & Rojas 2006). With the purpose of compensating for the forage shortage, the animals are usually moved from the grasslands to stubble crop fields. In this case, endozoochory through cattle dung is important due to the introduction and spreading of weeds and/or species of low forage value between grasslands and crop fields (Janzen 1984, Ghersa & Martínez 1985, Cousens & Mortimer 1995).

In conclusion, as it has been reported in other grasslands, endozoochory by cows can explain the propagation of exotic plant species in the Flooding Pampa grasslands under grazing conditions. The most abundant species emerged from cattle dung were of low forage quality or weeds of crop fields. The results are relevant for the range managers who normally move the cows to different stubble crop fields and grasslands, spreading through dung seeds of species of different origin and quality.

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Appendix. Plant species recorded in the grasslands of the Flooding Pampa (Buenos Aires, Argentina) (Cabrera 1970, Montes *et al.* 2001).

Adesmia bicolor, Alternanthera philoxeroides, Ambrosia tenuifolia, Ammi majus, Anagallis arvensis, Apium leptophyllum, Apium sellowianum, Asclepias mellodora, Aster squamatus, Baccharis pingraea, Bacopa monnieri, Bothriochloa laguroides, Briza minor, Briza subaristata, Bromus catharticus, Bromus mollis, Bupleurum tenuissimum. Cardamine hirsuta. Carduus acanthoides. Carex spp., Centaurea calcitrapa. Centaurium pulchellum. Chaetotropis elongata, Chenopodium ambrosioides, Cirsium vulgare, Conyza bonariensis, Coronopus didymus, Crepis capillaris, Cynodon dactylon, Cypella herbertii, Cyperus spp., Dichondra microcalyx, Diplachne uninervia, Distichlis spicata, Eleocharis bonariensis, Eryngium echinatum, Festuca arundinacea, Gamochaeta spicata, Geranium molle, Glandularia dissecta, Glyceria multiflora, Hordeum pusillum, Hybanthus parviflorus, Hydrocotyle bonariensis, Hypochaeris grisebachii, Hypochaeris petiolaris, Hypochaeris radicata, Juncus spp., Leontodon nudicaulis, Lepidium aletes, Lepidium bonariense, Lepidium parodii, Lepidium spicatum, Lepidium tandilense, Lolium multiflorum, Lotus tenuis, Ludwigia peploides, Lythrum hyssopifolia, Margyricarpus pinnatus, Matricaria chamomilla, Medicago lupulina, Medicago polymorpha, Melica brasiliana, Melilotus indicus, Mentha pulegium, Micranthemum tweediei, Modiola caroliniana, Nothoscordum bonariense, Nothoscordum montevidense, Oxalis articulata, Oxalis cordobensis, Pamphalea bupleurifolia, Parapholis incurva, Paspalum exaltatum, Paspalum dilatatum, Paspalum distichum, Paspalum quadrifarium, Paspalum vaginatum, Phalaris angusta, Phyla canescens, Picrosia longifolia, Piptochaetium bicolor, Piptochaetium grisebachii, Piptochaetium stipoides, Plantago lanceolata, Plantago myosuros, Pluchea sagittalis, Poa annua, Poa compressa, Poa lanigera, Polygonum aviculare, Polygonum hydropiperoides, Portulaca oleracea, Rorippa bonariensis, Rumex crispus, Scutellaria racemosa, Senecio bonariensis, Senecio madagascariensis, Silene gallica, Sisyrinchium iridifolium, Sisyrinchium platense, Solanum commersonii, Solanum glaucophyllum, Solanum gracilius, Solidago chilensis, Soliva pterosperma, Sonchus asper, Spergularia ramosa, Spilanthes decumbens, Sporobolus indicus, Stellaria media, Stenotaphrum secundatum, Stipa neesiana, Stipa philippii, Stipa trichotoma, Taraxacum officinale, Trifolium bonanni, Trifolium repens, Veronica arvensis, Vicia graminea, Vulpia dertonensis