Seed survival and predation of *Anoda cristata* in soyabean crops

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Summary

Anoda cristata is a troublesome annual broad-leaved weed in summer crops in the rolling Pampa in Argentina; seeds are the only source of regeneration of this species. Seed persistence or depletion is the result of survival and loss processes, including predation. The objective of this study was to determine survival at two burial depths in undisturbed soil and predation rates of *A. cristata* seeds in soyabean crops in different rotations and tillage systems. Survival was discontinuous and decreased to 25% after 35 months, after which no further reduction in survival was observed to the end of the experiment at 96 months. No differences in seed survival between seeds placed on the soil surface and buried 5 cm below the soil surface were found at 80 months, but at later times survival was lower for seeds placed on the soil surface. Predation rates ranged between 0.3% day^{-1} and 6.7% day^{-1}. Of the models tested, a polynomial regression of the rate of predation with time gave the best representation of seed predation. From January to July, predation was higher in non-tillage plots in the wheat/soyabean rotation. There was no significant difference in predation rates between tillage systems in the soyabean monoculture and no difference between planting densities. Higher crop residue levels in non-tillage plots in the wheat/soyabean rotation was the dominant factor influencing seed predation, probably because such habitat favours the presence of seed predators.

Keywords: Argentina, broad-leaved weed, seedbank, rotation, tillage systems.

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Introduction

Seed survival is one of the processes that explain the particular success of annual weeds in agroecosystems (Harper, 1977; Egley & Chandler, 1983), both for seeds present in the surface and buried in the soil. Long-term studies are required to establish if seed viability remains similar between depths, as many species show higher viability in buried seeds compared with seeds placed on the soil surface (Roberts & Feast, 1972). Natural germination and deterioration due to seed senescence or bacteria and fungal attack (Wilson, 1988) reduce seed survival. Predation also reduces seeds, following attack by insects, birds or mammals (Buhler *et al.*, 1997). There are numerous reports of seed predation in natural ecosystems (Crawley, 1992; Brust, 1994), but the impact of predation in agroecosystems has been examined less often (Cardina *et al.*, 1996).

Anoda cristata (L.) Schlecht is a troublesome annual weed in summer crops in the rolling Pampa in Argentina (Leiva & Ianone, 1994), where it may reduce soyabean yield up to 40% (Puricelli *et al.*, 2003). Compared with most other temperate weeds, *A. cristata* produces fewer, larger seeds (12 g per 1000 seeds, 70–987 seeds produced per plant) (Puricelli *et al.*, 2002). Primary dormancy mechanisms include hard-seededness, with seed coats that are water impermeable (Solano *et al.*, 1976). Per cent viability after burial for 66 months is 30% (Aldrich & Kremer, 1997). In another study, mean viability after burial for 53 months did not differ significantly between

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depths and was 19.5% (Faccini *et al.*, 1992). There are reports about emergence, mortality and seed production of *A. cristata* in soyabean crops (Puricelli *et al.*, 2002), but few demographic studies have focused on other processes involved in *A. cristata* persistence. Predation could be the main source of seed depletion. Tillage may affect the amount and pattern of predation (House & Stinner, 1983), as it modifies the amount of crop residues on the soil surface. Crop rotation may also affect residue levels, as crops differ in the amount of biomass produced during the growing season (Tuesca *et al.*, 2001). Therefore, the objective of this study was to determine survival at two burial depths and predation of *A. cristata* seeds in soyabean crops in different rotations, crop spatial arrangements and their interaction with different tillage systems.

Materials and methods

The experiments were conducted at the 'J.F. Villarino' Experimental Farm at Zavalla (latitude 33°01′S, longitude 60°53′W). The soil was a vertic argiudol with a 0–2% slope. The surface 0–15 cm had 3% organic matter, a pH of 5.8, and a composition of 5% sand, 70% silt and 25% clay.

Survival study

For the survival study, seeds of *A. cristata* were collected in the field at Zavalla at the end of the soyabean growing season in May 1996. The experiment began in May 1996 and finished in May 2004. Lots of 100 *A. cristata* seeds were placed into polypropylene packets (10 cm by 10 cm, 1 mm mesh size) which allowed passage of water, gases and microorganisms, and provided an effective barrier against predators according to another study (Nisensohn & Faccini, 1993). Packets were placed at 0 and 5 cm below the soil surface and were positioned horizontally to avoid overlap. No soil was added inside the packets. The time from harvest to seed burial was 3 days. Plots were arranged as a complete randomized design with three replicates and the area was left undisturbed for the duration of the experiment. Seed packets were exhumed at various intervals over the next 96 months and, following removal from each packet, intact seeds were counted to determine per cent of persistent seeds. To determine germinability, seeds were washed in water and surface sterilized in a sodium hypochlorite solution (1% *V*/*V*), placed on water-saturated blotting paper in 11 by 11 by 4-cm covered plastic boxes. Boxes were placed for 15 days in optimum germination conditions in a dark growth chamber at 25°C (Solano *et al.*, 1976; Faccini *et al.*, 1985). Germinated seeds were counted and removed every 2 days. The blotter paper was kept moist by adding distilled water as needed. Seeds were considered germinated when the radicle had extended at least 1 mm. On day 15, the remaining ungerminated seeds were cut and treated with 0.1% (w/v) tetrazolium chloride to determine viability. Recovered seeds which germinated within 15 days at 25°C were classified as exhibiting enforced dormancy. Seed survival at both depths was compared for each date using a *t*-test.

Predation studies

The effect of crop rotation and tillage system on *A. cristata* seed predation was determined in 1998 and 1999 in a split–split plot design with four blocks. The main factor consisted of two crop rotations, wheat/soybean rotation and soybean monoculture, and the second factor consisted of two tillage systems, minimum tillage and no-tillage (zero tillage).

Crop rotations were: (a) wheat/soybean rotation consisting of double cropping including in wheat and soyabean grown in the same year and, (b) soyabean monoculture grown as a single summer crop per year with a winter fallow. The soyabean cultivar 'Asgrow 640/600', tolerant to glyphosate, was planted in December in the wheat/soybean rotation and in November in the soybean monoculture and harvested in April in both rotations. Soyabean was planted with 70 cm between rows (280 000 plants ha^{-1}). Row spacing was 15 cm for wheat. In the two tillage systems, minimum tillage plots were disked and harrowed to a depth of cultivation of 15 cm prior to planting, while planting was the only soil disturbance in no-tillage plots.

All these treatments were placed within an existing framework of agronomic experimentation initiated 7 years before this study. Plots were 15 by 20 m. In soyabean, glyphosate was applied at the recommended rate for complete control of *A. cristata*, i.e. 1.44 kg a.i. ha^{-1} (Puricelli *et al.*, 2004). Grass weeds were controlled with haloxyfop 0.36 kg a.i. ha^{-1} and broad-leaved weeds, other than *A. cristata*, were hand-weeded prior to sowing in non-tillage plots and post-emergence at 40 days after soyabean sowing. No herbicide was applied in wheat as weed infestation was low and non-competitive.

Predation rate over time was determined during 2-wk intervals by placing 100 *A. cristata* seeds in plastic trays (15 × 15 × 1.5 cm deep) filled with field soil. Five 3-mm holes were drilled in the bottom of each box to allow water to drain. Five open trays and two with complete predation exclosure (plates covered with a piece of 0.1 mm mesh diameter cloth), were located at random in the centre of each plot. The trays were buried so that the surfaces of soil inside and outside were levelled. In no-tillage and reduced tillage plots, residue was scattered over the plates in a manner similar to that of the surrounding plot area. The trays were collected every 2 weeks, taken to the laboratory, where seeds were separated and counted. The next set of trays was replaced in the field at random the same day. Seed predation rate was calculated as follows:

$$P = 100(1 - r^{1/t})$$

where P is the average proportion of seeds recovered, r is the fraction of the remaining seeds and t is the duration of the experiment in days (Mittelbach & Gross, 1984).

Predation rate for each treatment was analysed using polynomial regressions. Parameters were compared using t-tests ($P = 0.05$).

Results

The percentage of seeds recovered showed long periods without seed decay and evident points of discontinuity indicating an abrupt decay for both seeds on the soil surface and buried at 5 cm below the soil surface (Fig. 1). In both treatments during the first 12 months the percentage of intact seeds recovered was about 90%. Rainfall during the period of decay was not different from the average rainfall of the last 30 years in the area. After 12 months, survival decreased remarkably and reached a stable value of *c.* 50% up to 30 months. In month 35, survival decreased to 25% and later, a new period of stable survival was reached. Rainfall during this period of decay was 9% of the average rainfall during the last 30 years. No differences in seed survival between seeds placed on the surface and buried 5 cm below the soil surface were found until 80 months. However, from 80 to 96 months seed survival was lower for seeds placed on the soil surface, as indicated by significant differences between means based on a t-test at $P = 0.05$ (Fig. 1).

During this period of decay rainfall was 34% of the average rainfall during the last 30 years. Throughout the experiment, the intact seeds recovered each month from the packets were 100% viable and 99% of the seeds exhibited dormancy.

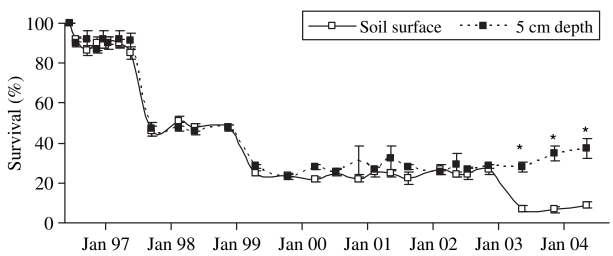

Fig. 1 Seed survival of *A. cristata* at the soil surface and buried 5 cm below the soil surface from 1996 to 2004.

Predation rates ranged between 0.3% day^{-1} and 6.7% day^{-1} (Fig. 2). For both years, regressions were significant ($P = 0.05$) and predation rates tended to decrease, especially in the wheat/soyabean rotation, from January to July and to increase from August to December in all treatments. Parameters of the curves fitted from January to July differed significantly between the wheat/soyabean rotation under no-tillage and the other treatments (Table 1). For the same period in the soyabean monoculture, no significant difference between the parameters was observed between tillage systems (reduced and no-tillage). For the curves fitted from August to December no significant differences were detected between treatments for any parameter. All the seeds placed in the trays with predation exclosure were recovered intact.

Residue levels in no-tillage systems at the beginning of the crop growing season in summer crops averaged (over 4 years) 3210 kg ha^{-1} (SE = 340) in the wheat-soyabean rotation and 1678 kg ha^{-1} (SE = 211) in the soyabean monoculture.

Discussion

Survival of *A. cristata* seeds in soil declined over time, as normally occurs with all species (Roberts, 1964; Cavers & Benoit, 1989), but no seasonal pattern of survival loss was detected throughout the experiment. The first period of decay occurred in winter and the second and third

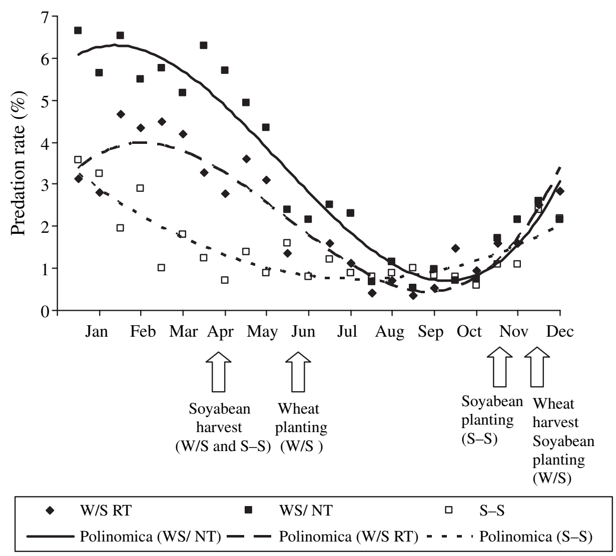

Fig. 2 Predation rate P (%) (Mittelbach & Gross, 1984) of *A. cristata* in the wheat/soyabean (W/S) rotation and soyabean monoculture (S-S) in reduced (RT) and non-tillage (NT) systems in 1998. As RT and NT curves for S-S monoculture were not different, they were fused for clarity. The August period has been recalculated to avoid drawing a discontinuity between the two successive periods of the year.

Year	Condition	10^4a	±95% CL	10^2b	±95% CL	c	±95% CL	r^2
1998 (a)	W/S RT	−1.46	0.471	1.82	1.162	3.27	0.606	0.77
	W/S NT	−1.30	0.441	0.53	0.024	6.21	0.563	0.89
	S-S RT	1.19	0.311	−4.19	0.710	4.35	0.390	0.84
	S-S NT	0.97	0.320	−3.41	0.790	3.88	0.410	0.78
1998 (b)	W/S RT	1.67	1.020	−8.23	5.930	10.72	8.170	0.55
	W/S NT	1.77	1.240	−9.08	7.070	12.41	9.170	0.37
	S-S RT	1.99	0.670	−9.54	3.800	11.93	5.320	0.83
	S-S NT	1.49	0.780	−7.70	4.470	10.56	6.250	0.51
1999 (a)	W/S RT	−1.18	0.320	1.61	0.790	2.27	0.420	0.79
	W/S NT	−1.77	0.310	2.28	0.770	4.55	0.410	0.91
	S-S RT	0.84	0.360	−2.70	0.890	2.79	0.460	0.54
	S-S NT	0.79	0.280	−2.49	0.700	2.51	0.360	0.59
1999 (b)	W/S RT	0.61	0.220	−2.64	1.220	3.36	1.590	0.77
	W/S NT	1.59	0.690	−8.85	3.990	13.21	5.590	0.37
	S-S RT	2.08	0.660	−10.68	3.790	14.43	5.300	0.74
	S-S NT	2.38	1.020	−12.12	4.720	15.84	6.590	0.72

Table 1 Parameter estimates and their 95% confidence limits (CL) of the polynomial regression analysis ($ax^2 + bx + c$, with x the number of day elapsed since crop sowing) for predicting the relationship between *A. cristata* seed predation rate (Mittelbach & Gross, 1984) and time in the wheat/soyabean (W/S) rotation and soyabean monoculture (S-S) in reduced (RT) and non-tillage (NT) systems in 1998 and 1999. (a) Equations fitted from January to August, (b) equations fitted from July to December

periods in spring or summer. The presence of a dry month can be associated with the last two periods of decay. In most survival studies seeds decline in a negative exponential fashion; other studies determined curves showing an initial slow decline followed by a rapid decline (Baskin & Baskin, 1998). In the present study, seed decay was discontinuous. The reduction in the number of seeds recovered may be partially due to germination during those seasons. In another study in the same site, field emergence of *A. cristata* was relatively stable over the 3 years of the study and ranged between 8.5% and 14.7% of the seedbank to a depth of 15 cm (Puricelli *et al.*, 2002). The difference between these two studies is probably due to the soil seedbank of the experimental field reported in 2002 that includes seeds of different ages and different physiological conditions, while in the present survival study all seeds were collected in the same year. This fact may explain the discrepancies between the stable emergence in the field and the discontinuous emergence in the survival study. Seed mortality due to rotting may account for the rest of the reduction observed in the seeds recovered. No senescence was observed in the study, as all the seeds recovered showed high viability.

In the present study the position of the seeds in the soil profile did not affect survival during the first 78 months of the study, which concurs with other studies done in *A. cristata* for *c.* 60 months (Solano *et al.*, 1976; Egley & Chandler, 1983; Faccini *et al.*, 1992) and in another *Malvaceae* weed species (Westra *et al.*, 1996). From 30 to 78 months, seeds buried or placed at the soil surface seem to be insensitive to decay. At 84 months, decay was observed only for seeds placed at the soil surface and < 10% of the seeds survived at the end of the experiment (96 months) in this treatment. Buried seeds showed a higher survival (25%) at the end of the experiment. For many other weed species, survival is lower at the soil surface (Roberts & Feast, 1972; Omami *et al.*, 1999).

The high dormancy of the recovered seeds of *A. cristata* observed throughout the study may be associated with the presence of a hard and impermeable seed coat, which is the main mechanism of dormancy of many species (Stoller & Wax, 1973; Warwick & Black, 1988; Baskin & Baskin, 1998). In another study, *A. cristata* seeds showed high dormancy after seed shed and remained dormant for at least 2 months after collection, even after scarification (Solano *et al.*, 1976).

Predation rates are comparable with those determined in other studies (Mittelbach & Gross, 1984; Brust & House, 1988). Low predation rates during winter can be due to temperatures too low for the activity of arthropods and rodents. Conversely, the high predation in summer may reflect favourable environmental conditions or peak periods of predator populations and low availability of alternative food sources, as was reported by Cardina *et al.* (1996) in *Abutilon theophrasti* Medic. Furthermore, the period of higher predation concurs with *A. cristata* growing season.

As reported in many studies, tillage effect on predation was erratic (House & Brust, 1989; Cardina *et al.*, 1996; Cromar *et al.*, 1999; Nisensohn *et al.*, 1999). The higher levels of crop residue present on the soil surface may favour seed predation, by providing habitat for seed feeding animals, but may also act as an impediment to predator movement on the soil surface (House & Brust, 1989). The higher predation in the wheat/soyabean rotation in no-tillage plots might be due to the presence of greater crop residue levels than in reduced tilled plots. The presence of wheat in the wheat/soyabean rotation accumulates higher residue levels than soyabean monoculture, as was also observed in another study (Tuesca *et al.*, 2001).

Predator species for *A. cristata* were not determined, but for many other weeds, seed predators are rodents (Scopel *et al.*, 1988; Dellafiore & Polop, 1994), birds (Askew & Wilcut, 2001), carabid beetles (House & Stinner, 1983; Nisensohn *et al.*, 1999) and ants (Risch & Carroll, 2001). It is known that carabid beetles did not accept *A. cristata* seeds in a laboratory experiment (Lietti *et al.*, 2000). The absence of seeds in the traps in our study most likely may be attributed to predation by ants, as they were observed carrying seeds in high numbers around the test area. Additional studies are needed to evaluate the exact predation processes for *A. cristata*.

Crop rotation was more of a factor than tillage treatments in determining the number of predated seeds. Higher residue level in non-tillage plots in the wheat/soyabean rotation could be the dominant factor influencing seed predation, probably because such habitat favours the presence of seed predators (House & Stinner, 1983; Cromar *et al.*, 1999). As no-tillage leaves the seeds on the soil surface, this system also has a great impact on *A. cristata* population density, because seed losses due to predation are maximized. The no-tillage wheat/soyabean rotation gives the highest seed losses.

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