Chapter 3

Results of a survey on herbicide resistant *Papaver rhoeas* L. to tribenuron-methyl and to 2.4-D in North-eastern Spain

Summary

A field survey was conducted in Northern Spain focusing on fields with control complains in tribenuron-methyl on *Papaver rhoeas* L. populations. The survey was semi-directed, aiming mainly to detect the areas with real resistance problems.

The detection and classification of the populations was made on seed-based quick-tests in laboratory.

Most of the surveyed *P. rhoeas* populations were resistant: 72% of the tested populations were resistant to tribenuron-methyl and 85% were resistant to 2.4-D. More resistance was detected towards 2.4-D, probably due to the long period of use of this herbicide. Most of the resistant populations, however, were classified as low-degree resistant, while most of the populations resistant to tribenuron-methyl contained a high proportion of resistant plants.

The proportion of susceptible populations was higher in the populations collected with unknown spraying history, as probably non-sprayed fields were included. 58% of the surveyed populations were resistant to both herbicides.

Similar resistance patterns were found between populations collected in Catalonia and in Burgos, the main problematic areas in North-eastern Spain. High degree of resistance to 2.4-D, however, was only found in Catalonia.

Increase, decrease and no change was observed in the proportion of resistant plants to tribenuron-methyl when populations were collected in the same field during several years. The evolution depended on the weed management during the year.

Despite few farmers continue spraying 2.4-D, an increase in the resistance degree was found in different cases when populations were collected in the same field during several years and tribenuron-methyl was sprayed. The reason was probably that the plants resistant to tribenuron-methyl were previously resistant to 2.4-D (multiple resistant) and diminishing the proportion of susceptible plants by spraying tribenuron-methyl increased also the proportion of resistant plants to 2.4-D.

Keywords: herbicide resistance, *Papaver rhoeas* L., tribenuron-methyl, 2.4-D, field survey, quick-test.

Introduction

Problems in controlling *Papaver rhoeas* L. in winter cereal have been quoted since 1992 in Spain (Taberner *et al.*, 1992). The first queries were focused on 2.4-D, a herbicide commonly used in winter cereals since the 1950's in Spain.

Since 1986, the highly active ingredient tribenuron-methyl has been sold in Spain replacing in many cases the use of 2.4-D. The high efficacy of this active ingredient has led to a continuous use imposing a strong selection pressure on *P. rhoeas* in some areas.

Claude et al. (1998) reported the first analysis on a *P. rhoeas* population resistant to 2.4-D and to tribenuron-methyl.

Evolution of herbicide resistance was much faster for tribenuron-methyl than for 2.4-D. Probably this was due to different parameters which play a role in herbicide resistance evolution such as different selection pressures imposed by the herbicides, different initial resistant plant frequency and different resistance mechanisms (Saari *et al.*, 1994). Moreover, 2.4-D metabolism-based resistant populations die with increased field rates, so that farmers hesitated in complaining seriously. In the case of tribenuron-methyl increased dose does not kill any more target-site resistant plants, so that complains arrive much faster.

Similar surveys on resistant weeds have been recently published for resistant *Alopecurus myosuroides, Lolium multiflorum* and *Avena* spp. in United Kingdom (Moss *et al.*, 1999), for resistant *Lolium rigidum* in Central Italy and in Western Australia (Bravin *et al.*, 2001; Llewellyn & Powles, 2001) and in *Raphanus raphanistrum* in Western Australia (Walsh *et al.*, 2001) among others.

Moss *et al.* (1999) conducted a directed survey aiming to find how many individual farms with resistance problems had been identified until the moment. Therefor most weed seeds came from fields where complains on lack of efficacy occurred and only some were randomly collected. The testing method was in this case glasshouse pot assays conducted by different organisations or companies who had collected the samples previously.

In the case of the Italian survey described by Bravin *et al.* (2001) around 130 populations were collected, but only four were thoroughly tested with five herbicides. Pot tests and a test on agar medium were used for resistance detection.

As resistance is much more widespread in Australia than in Europe and is commonly accepted, studied and prevented, the aim of the survey described by Llewellyn & Powles (2001) was to "take a picture" of the present situation but not to find out if resistance occurred. Therefor, the samples of *L. rigidum* in the Western Australia survey were collected randomly. Testing was done in greenhouse pot trials including the Syngenta Quick-Test spraying on weed re-growth of plants previously sprayed with another herbicide (Boutsalis, 2001).

In the study on *R. raphanistrum* described by Walsh *et al.* (2001) the first survey was directed aiming to detect and to characterise the resistance problem. Afterwards, a random survey was conducted in order to describe the overall widespread of resistant populations. One herbicide was only tested using the Syngenta Quick-Test (Boutsalis, 2001).

The growing queries and confusion between farmers, applicators and solders lead to the present survey of *P. rhoeas* conducted in North-eastern Spain. Main affected areas were winter cereal monoculture in rainfed cropping systems. Also the European Weed Resistance Working Group (EWRWG) showed its interest in describing the present herbicide resistance situation by financing part of the present study.

The aims of this work were:

- To draw a picture on the present situation of *P. rhoeas* herbicide resistance towards tribenuron-methyl and 2.4-D in order to clarify which was the prevalent situation.
- To describe the possible cross- or multiple resistance towards these two herbicides.
- To find out which were the most frequent cases inside the problematic populations.
- To find out if the populations collected in different areas (Catalonia, Navarra and Burgos) followed similar behaviour patterns.

The complete database on the tested populations is related to a geo-referred map allowing consulting the data of each population. The present work represents a summary of the results.

Materials and Methods

A total amount of 172 *P. rhoeas* samples were collected during the years 1990 to 2001, focusing on the years 1997-1999, where 134 populations were sampled. The survey was mainly directed, that means that most of the samples were found after complains on lack of herbicide efficacy, especially towards tribenuron-methyl. This methodology was chosen, as very few confirmed resistant cases were known. Moreover, it was aimed to find out whether complains on lack of efficacy were well based. 32% of the populations were collected randomly without knowing the field owner or their spraying history.

Susceptible standards were searched collecting seeds in non-sprayed areas and in non-cultivated fields with *P. rhoeas* infestations. The characteristics of the collected populations are shown in Table 3.1.

Seeds were collected prior to the cereal harvest all over the fields gathering together a mixed sample of each field. Spots or single lines of weeds, which could have survived due to an incorrect herbicide application, were avoided. As shown in Figure 3.1., the surveyed area was North-eastern Spain, mainly the Catalonia region although 30 samples were also collected in Burgos, Navarra and in Aragón. A hand-held global positioning system unit was used to record latitude and longitude for each site. Seeds were air-dried after harvest and stored in closed plastic pots in a warehouse.

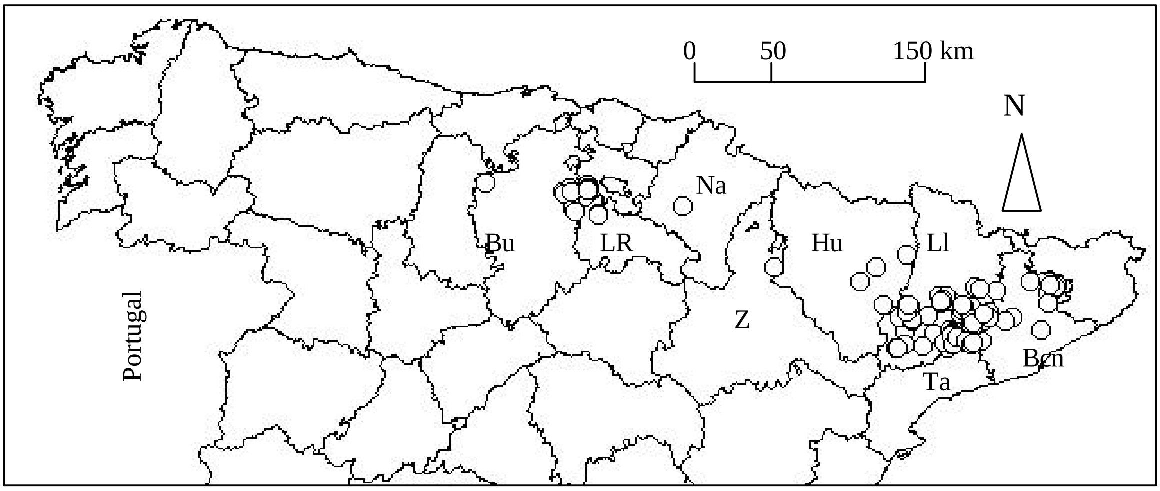

Figure 3.1. Geographical distribution of the collected *Papaver rhoeas* populations in Northern Spain. Each point refers to a sampled population. Bcn: Barcelona, Bu: Burgos, Hu: Huesca, Ll: Lleida, LR: La Rioja, Na: Navarra, Ta: Tarragona, Z: Zaragoza are Spanish provinces.

Table 3.1.: Description of the main characteristics of the collected *Papaver rhoeas* seed samples.

Year of collection	Number of collected samples	Number of samples of new fields	Number of samples with known spraying history	Number of new possible susceptible standards
1990	1	1	0	0
1992	1	1	1	1
1993	7	7	1	1
1995	9	8	8	0
1996	14	8	8	0
1997	24	24	17	1
1998	46	44	38	2
1999	64	56	39	4
2000	6	2	4	1
Total	**172**	**151**	**116**	**10**

Most of the samples came from fields in monoculture situation growing only barley for the last 20 years. In very few fields the crop rotation included wheat, oil-seed rape and peas.

The climatic conditions of some representative observatories in the surveyed area are shown in Table 3.2. As it is reflected in the climatic regime of Papadakis, Burgos and Navarra are colder in winter and cooler in summer than the other regions. In all the sites most rainfall occurred in autumn and in spring.

Table 3.2.: Climatic data from some representative locations in the surveyed area. In parenthesis: m above sea level. M: Annual mean of the monthly absolute maxima; Mean: Annual mean of the monthly means of mean temperatures ºC). M: Annual mean of the monthly absolute minima; W: winter Sp: spring, Su: summer, A: autumn; n: number of months with mean of minimum temperatures under 7ºC; P: Mean annual precipitation (mm). Seasonal temperatures are means of mean temperatures. Climatic classification following Papadakis. Adapted from De León (1987a, 1987b, 1989, 1991) and Forteza (1981).

	Temperature										Precipitation					Climatic classification
Location	**M**	**Mean**	**m**	**W**	**Sp**	**Su**	**A**	**First frost**	**Last frost**	**n**	**P**	**W**	**Sp**	**Su**	**A**	
Burgos (929 m)	34.8	10.6	-8.9	3.5	9.3	18.0	11.4	17/10	17/04	8	559.7	144.8	164.2	102.5	148.2	Mediterranean temperate
Caldes de Montbui (203 m)	35.9	14.3	-6.6	7.2	12.8	21.8	15.4	27/10	29/04	6	641.4	121.1	178.5	140.8	201.0	Mediterranean continental temperate
Balaguer (233 m)	37.7	14.9	-5.5	6.0	14.4	23.6	15.7	3/11	14/04	5	417.0	78.7	134.7	85.4	119.0	Mediterranean continental temperate
Huesca Monflorite (436 m)	36.6	13.3	-7.0	5.3	12.2	21.5	14.0	29/10	23/04	6	584.8	130.7	170.7	129.1	154.3	Mediterranean continental temperate
Sto. Dgo. de la Calzada (639 m)	33.9	11.3	-6.8	5.6	10.3	17.9	11.9	17/10	10/05	6	581.1	143.2	150.8	116.7	170.4	Mediterranean temperate

Characterisation of the sensibility towards the herbicides in Petri dishes on agar medium

In order to simplify the tests and after having developed and validated seed-based quick-tests for *P. rhoeas* resistance, no greenhouse experiments were conducted.

Towards tribenuron-methyl

The followed methodology for detecting the herbicide resistance towards tribenuron-methyl was the quick-test on agar medium as described by Cirujeda *et al*. (in press). 50 seeds per dish were placed on the 1.3 % agar medium surface containing 0.2 g gibbereline GA^3 L^{-1}, 2 g KNO_3 L^{-1} and 7.68 µM tribenuron-methyl. Three dishes for each population were placed randomly in a growing chamber under 20 µmol S^{-1} m^2 light.

Evaluation was made 14 days after sowing counting well-developed plants and seedlings staying in cotyledon stage. Also dead and too young plants were counted and the results rejected if their proportion was more than 20% of the total plants. Resistant plants continued developing dark green leaves while susceptible plants stayed in cotyledon stage after germinating and took a light green coloration. Percentage of resistant towards total germinated plants was established. In each trial, susceptible and resistant standards were included.

The resistance to tribenuron-methyl in *P. rhoeas* is probably due to a target-site resistance mechanism, as most of the cases reviewed by Saari *et al.* (1994). The methodology used for the detection purpose already demonstrated this characteristic because plants either continued developing or stopped in cotyledon stage regardless of the herbicide dose used. In this case, it was necessary to classify the populations depending on the percentage of resistant plants inside the sample. A similar classification system as the grass-weeds classification system described by Moss *et al.* (1999) was chosen.

The criterion for classification of the different degrees of *P. rhoeas* resistance to tribenuron-methyl was done according to the proportion of resistant plants in the sample, as explained in Table 3.3. This way, populations classified as one-star populations have an incipient resistance problem; in the two-stars populations a considerable proportion of the seeds are resistant; in the three-star populations, almost all the seeds will origin resistant plants.

Table 3.3.: Classification criteria for the *Papaver rhoeas* populations resistant to tribenuron-methyl.

Percentage of resistant plants in the seed sample	Classification	Category
0	S	Susceptible
$0 < \% \leq 10\%$	*	Incipient resistance
$10 < \% \leq 90\%$	**	Moderate until high resistance
> 90	***	Complete resistance

The following results show the data of 148 out of 172 collected samples, excluding the repeated populations collected in several years and too small samples. Other few populations had to be excluded du to seed rot causing too little germination.

Towards 2.4-D

For this herbicide, the dose had an influence on the resistant populations, so that higher dose resulted in bigger efficacy (data not shown). In the quick-test described in chapter 2, a gradual response with increasing dose was found for the whole plant tests and for the Petri dish tests on seedlings. These results suggest that the resistance to 2.4-D in *P. rhoeas* is probably due to a metabolic resistance mechanism as it is supposed for *Carduus nutans* (reviewed by Coupland, 1994) and for *Stellaria media* (reviewed by Holt *et al.* 1993).

Seeds were also placed on 1.3 % agar medium containing 0.2 g GA^3 L^{-1}, 2 g KNO_3 L^{-1} and 3.49 µmol L^{-1} 2.4-D under 40 µmol S^{-1} m^2 light. The average length of the germinated seedlings was determined and a length ratio was calculated dividing the average length of the tested sample with the average length of the susceptible standard population included in each test. This standard population was chosen after analysing several possible susceptible populations. Populations with a ratio bigger than 1 were considered resistant. Resistant populations were classified in three categories, which reflected also the survival of whole plants with increasing dose (chapter 2). Three categories were chosen as proposed by Moss *et al.* (1999) for herbicide resistant *Alopecurus myosuroides*. The classification levels are shown in Table 3.4.

Table 3.4.: Classification criteria for the *Papaver rhoeas* populations resistant to 2.4-D based on the coleoptils' length ratio and referred to the survival of whole plants in greenhouse trials.

Length ratio	Classification	Category
Ratio ≤ 1	S	Susceptible
1 < Ratio ≤1.5	*	Survival of 0.9-1.2 L 2.4-D ha^{-1}
1.5 < Ratio ≤2.0	**	Some survival up to 1.8 L 2.4-D ha^{-1}
Ratio >2.0	***	Remarkable survival at 1.8 L 2.4-D ha^{-1}

Ioxinil + Bromoxinil + MCPP

With the aim of deepening in the multiple and cross-resistance of *P. rhoeas* a greenhouse experiment was conducted testing the efficacy of Oxytril, a widespread herbicide used for *P. rhoeas* control in the surveyed area containing 7.5% ioxinil, 7.5% bromoxinil and 37.5% MCPP. Seeds were sown on an aluminium tray (0.20 m x 0.15 m) containing a 1:1 peat-sand mixture. About 25 seedling were sprayed at a rosette stadium when the plants had a diameter of 3-5 cm. 0.625, 1.25, 2.5 and 5 L ha^{-1} corresponding to a quarter, half, full and double field rate were applied with a constant pressure sprayer. Three replicates of each treatment were placed randomly in a greenhouse with temperatures ranging from 10ºC to 25ºC. Final visual survival evaluation was performed 31 days after treatment.

Results and Discussion

Classification based on the Petri dish quick-test with tribenuron-methyl

Pure resistant, pure susceptible and mixed populations towards tribenuron-methyl were found. The results of the Petri dish quick-test are shown in Table 3.5. and 3.6.

Table 3.5.: Number of *Papaver rhoeas* populations and percentage on the total tested populations found in the different resistance categories. Results of the Petri dish assay on tribenuron-methyl. In case of repeated populations, the oldest sites were chosen for results.

	S	*	**	***
1990	1 (0.7)	0	0	0
1992	1 (0.7)	0	0	0
1993	6 (4.1)	1 (0.7)	0	0
1995	2 (1.4)	1 (0.7)	1 (0.7)	3 (2.0)
1996	4 (2.7)	2 (1.4)	0	2 (1.4)
1997	7 (4.7)	2 (1.4)	7 (4.7)	4 (2.7)
1998	3 (2.0)	9 (6.1)	9 (6.1)	23 (15.5)
1999	14 (9.5)	5 (3.4)	6 (4.1)	31 (20.9)
2000	3 (2.0)	0	1 (0.7)	0
Total	**41 (27.7)**	**20 (13.5)**	**24 (16.2)**	**63 (42.6)**

Most of the tested populations showed a very high resistance proportion to tribenuron-methyl belonging to the three-star group. Moreover, 13% of the tested samples had a pure population of resistant plants. 72.3% of the populations included resistant plants in the sample. This demonstrates that the farmers complains in the surveyed fields are, in most of the cases, justified. Additionally, 13.5% of the tested populations had incipient resistance, suggesting that the problem can increase if resistance is not prevented.

In Figure 3.2. the tested populations are grouped per intervals of resistant plants, showing that most of the populations had a high proportion of resistant plants inside the sample. Slight differences in behaviour were found between the areas. These differences, however, were probably due to that fact that less known susceptible populations were collected out of Catalonia than in Catalonia.

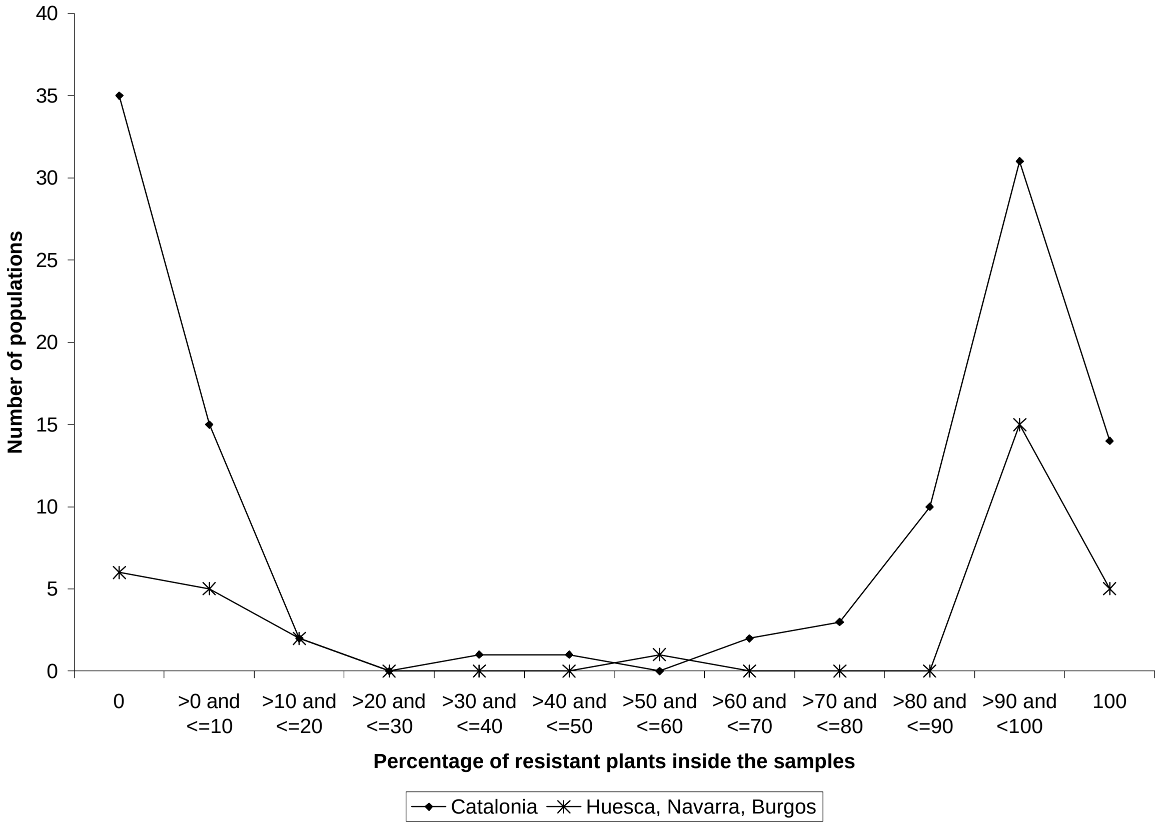

Figure 3.2. Number of *Papaver rhoeas* populations containing different proportion of resistant plants to tribenuron-methyl. Populations were separated following their origin area.

In Figure 3.3. the distribution of the populations in the whole area is represented. The star size is related to the resistance degree towards tribenuron-methyl.

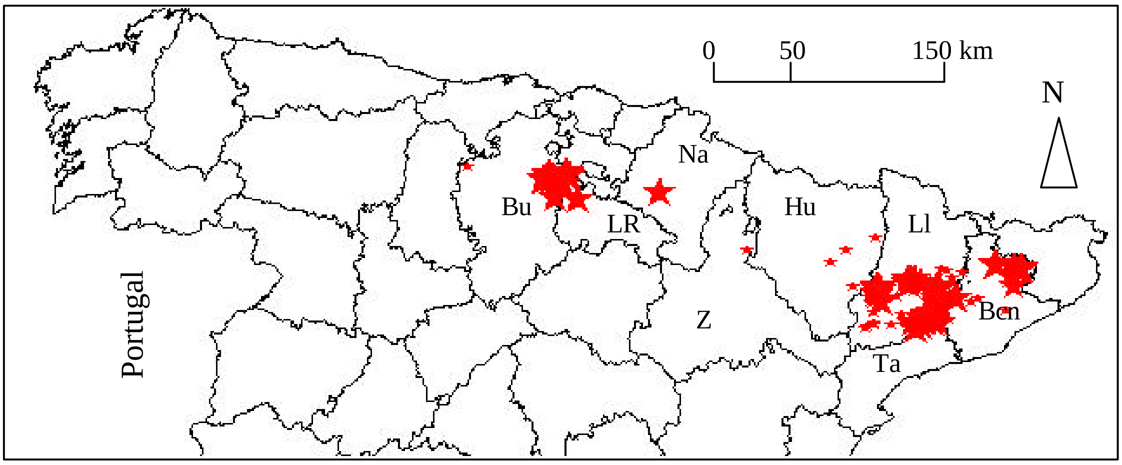

Figure 3.3. Distribution of the *Papaver rhoeas* populations in Northern Spain classified according to their resistance degree to tribenuron-methyl. Bigger stars refer to a bigger proportion of resistant plants inside the seed samples. The smallest stars correspond to susceptible populations. Bcn: Barcelona, Bu: Burgos, Hu: Huesca, Ll: Lleida, LR: La Rioja, Na: Navarra, Ta: Tarragona, Z: Zaragoza are Spanish provinces.

The main affected areas found were Central Catalonia and Eastern Burgos. Figure 3.4. shows the Catalan area in detail. The areas with most the most resistant populations were La Noguera, l'Anoia , La Segarra, l'Urgell and Conca de Barberà. No resistant populations were found in El Solsonés, Bages, Segrià, Les Garrigues, Vallés Oriental and l'Anoia.

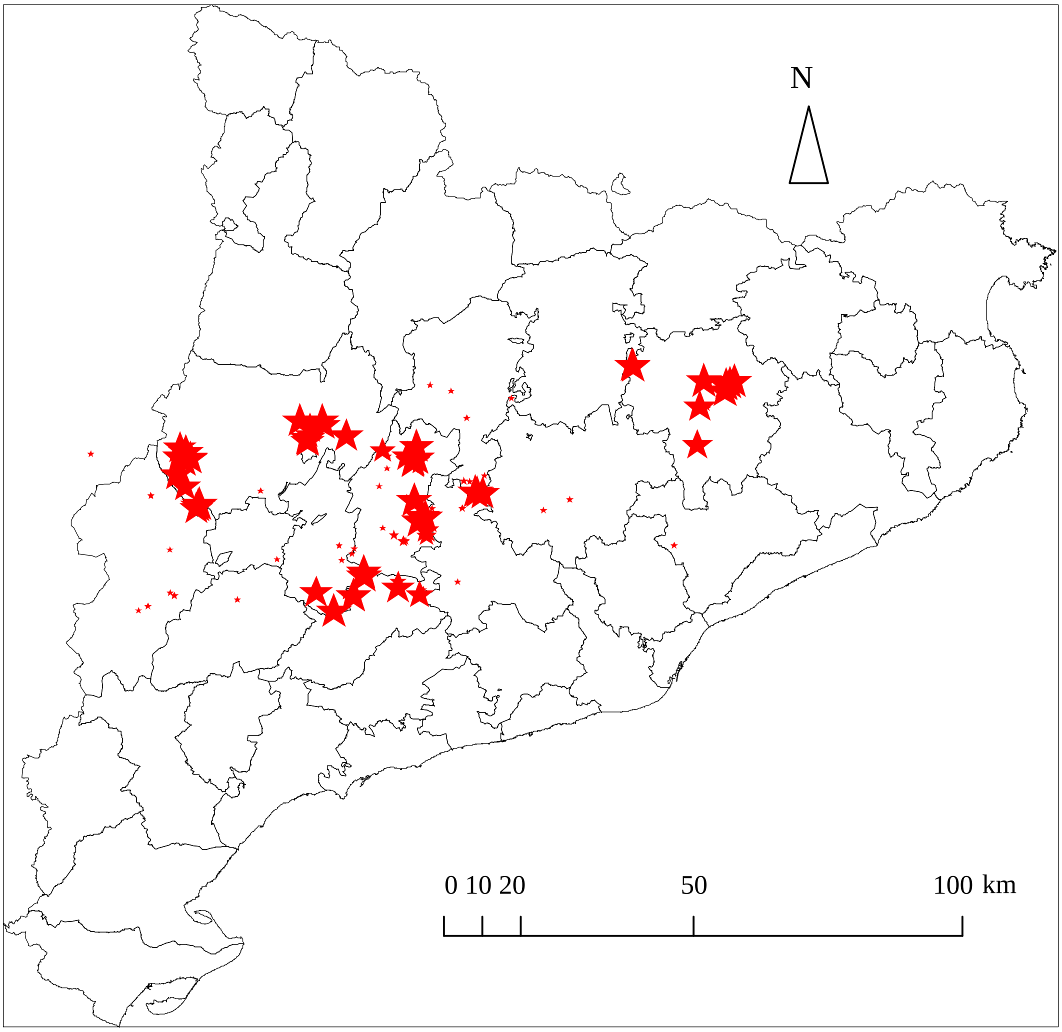

Figure 3.4. Distribution of the *Papaver rhoeas* populations classified in Catalonia according to their resistance degree to tribenuron-methyl. Bigger stars refer to a bigger proportion of resistant plants inside the seed samples. The smallest stars correspond to susceptible populations.

The abundance of the detected resistance is probably higher than the real proportion of resistant populations in the area as the survey was directed to suspicious fields, so that the results refer to resistance appearance in the fields with complains on lack of efficacy. As the seed bank of *P. rhoeas* persists during many years (Barralis *et al.*, 1988; Kjær 1940) regardless to the future weed management these populations will probably continue containing resistant plants during several years, so that the herbicide tribenuron-methyl will probably be non-effective in these fields during a long period.

Table 3.6.: Number of populations and percentage on the total populations of *Papaver rhoeas* susceptible or resistant to tribenuron-methyl taking into account the influence of the known or unknown history of the populations. In case of repeated populations, the oldest sites were chosen for results. The stars refer to the resistance level group classification.

	S	*****	******	*******	**Total**
Known spraying history	19 (12.8)	13 (8.8)	20 (13.5)	42 (28.4)	94 (63.5)
Unknown spraying history	22 (14.9)	7 (4.7)	4 (2.7)	21 (14.2)	54 (36.5)
Total	**41 (27.7)**	**20 (13.5)**	**24 (16.2)**	**63 (42.6)**	**148**

Out of the susceptible samples, more were found in the fields with unknown spraying history (40.7%) than in the other cases (20.2%). Nevertheless, most of the problematic samples showed a very high proportion of resistant plants (44.7% for the known populations and 38.9% for the unknown populations). This demonstrates that the resistant populations can also be found in a random survey. In the present case, more herbicide resistant than susceptible populations were found suggesting that in many cases, big *P. rhoeas* populations are due to herbicide resistance and not to wrong herbicide applications.

In 4% of the 148 analysed populations, complains on efficacy were not justified and the populations were found susceptible to tribenuron-methyl. The other way round, in 2% of the cases, populations thought to be susceptible were found resistant. Thus, in most of the cases, in which the farmers suspected resistance, complains were well founded.

Classification based on the Petri dish quick-test with 2.4-D

As well as in the case of tribenuron-methyl, Susceptible, slightly resistant and very resistant populations were found within the different populations. Table 3.7. shows the results of this quick-test.

Table 3.7.: Number of populations and percentage on the total tested *Papaver rhoeas* populations found in the different resistance categories. Results of the Petri dish assay on 2.4-D. In case of repeated populations, the oldest sites were chosen for results.

	S	*	**	***
1990	0	1 (0.7)	0	0
1992	1 (0.7)	0	0	0
1993	1 (0.7)	3 (2.0)	2 (1.4)	1 (0.7)
1995	0	4 (2.7)	3 (2.0)	0
1996	0	0	0	9 (6.1)
1997	3 (2.0)	14 (9.5)	2 (1.4)	0
1998	7 (4.8)	26 (17.7)	9 (6.1)	3 (2.0)
1999	9 (6.1)	20 (13.6)	17 (11.6)	10 (6.8)
2000	1 (0.7)	2 (1.4)	0	0
Total	**22 (15.0)**	**69 (46.9)**	**33 (22.4)**	**23 (15.6)**

85% of the analysed populations were resistant to some extent to 2.4-D. Most of the tested populations (47%) were only slightly resistant. Following the classification described in chapter 2, these populations would survive 0.9 or 1.2 L 2.4-D ha^{-1} in greenhouse tests while the commercial field rate is 0.6 L 2.4-D ha^{-1}. These doses, however, would probably be too high for a cereal crop causing phytotoxicity. Only 22.4% belonged to the two-star-classification and 15.6% to the three-star-group. These results suggest that herbicide resistance is even more frequent for 2.4-D than for tribenuron-methyl, taking into account that most of the surveyed populations were fields with complains towards tribenuron-methyl and only in very few cases towards 2.4-D.

The most frequent situation found was low-degree resistance. An explanation could be that farmers do not spray very often 2.4-D for *P. rhoeas* control any more, so that the selection pressure diminished.

Figure 3.5. shows the amount of populations with the different length ratios. The most resistant populations towards 2.4-D were found in Catalonia. In the other areas, most of the samples were resistant at lower degree (Figure 3.6.).

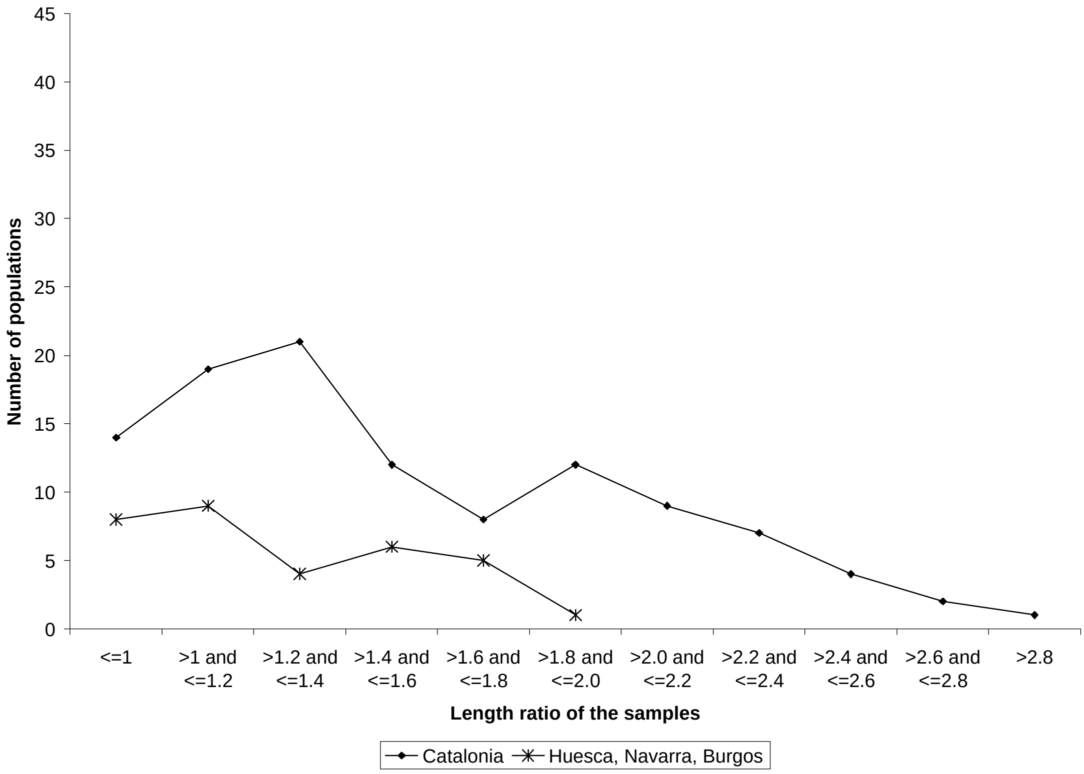

Figure 3.5. Number of *Papaver rhoeas* populations with different length ratio obtained in the quick-test on 2.4-D. Populations were separated following their origin area.

In Figure 3.6. the distribution of the populations is represented. The star sizes refer to the resistance degree towards 2.4-D.

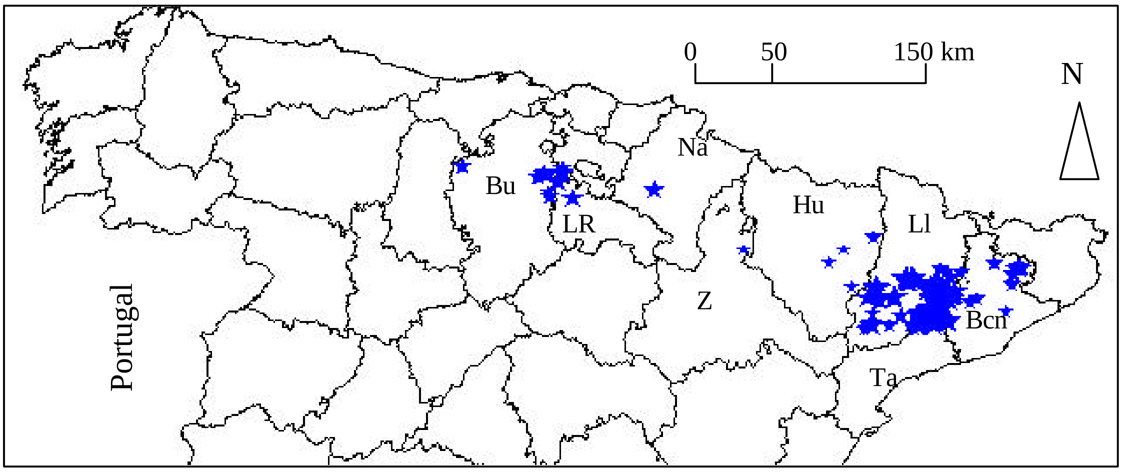

Figure 3.6. Distribution of the *Papaver rhoeas* populations in Northern Spain classified according to their resistance degree to 2.4-D. Bigger stars refer to a bigger ratio based on the longer hipocotyl of the plants in the seed-based quick-test. The smallest stars correspond to susceptible populations. Bcn: Barcelona, Bu: Burgos, Hu: Huesca, Ll: Lleida, LR: La Rioja, Na: Navarra, Ta: Tarragona, Z: Zaragoza are Spanish provinces.

As shown in Figure 3.6. in Catalonia, high-level resistant populations were concentrated in a smaller area of Central Catalonia than the tribenuron-methyl resistant populations, which were more widespread (Figures 3.3., 3.4.). The main affected regions were La Segarra, Conca de Barberá, l'Anoia, l'Urgell and La Noguera.

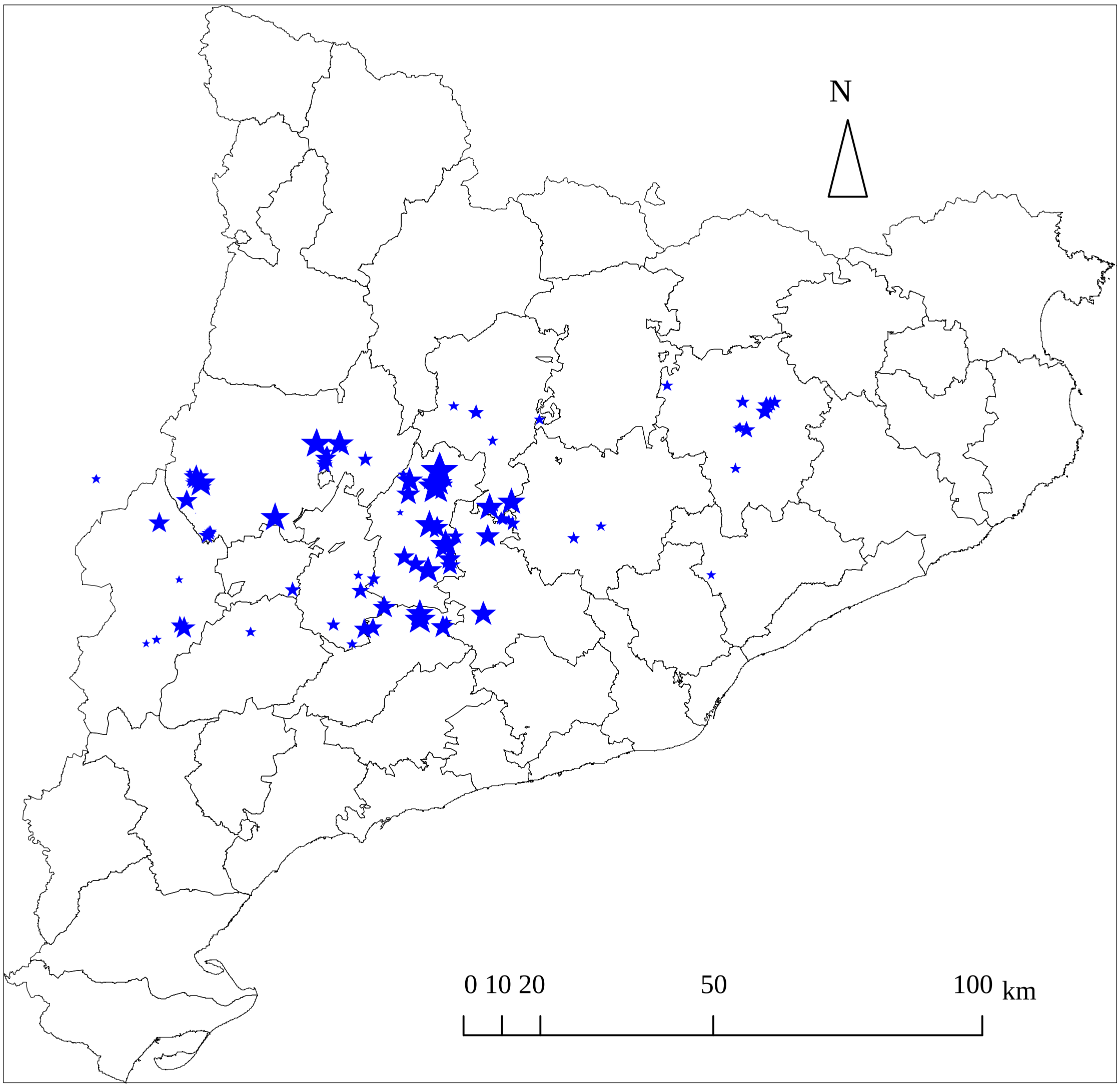

Figure 3.7. Distribution of the *Papaver rhoeas* populations classified in Catalonia according to their resistance degree to 2.4-D. Bigger stars refer to a bigger ratio based on the longer hipocotyl of the plants in the seed-based quick-test. The smallest stars correspond to susceptible populations.

Less resistant and more susceptible samples were found in the randomly collected populations than in samples with known spraying history. In the populations with known spraying history 88.4% resistant samples were detected versus 78.8% resistant samples in the populations with unknown history (Table 3.8.). Nevertheless, the proportion of the resistance degrees was still very similar in both cases.

Table 3.8.: Number of populations and percentage on the total populations of *Papaver rhoeas* susceptible or resistant to 2.4-D taking into account the influence of the known or unknown history of the populations. In case of repeated populations, the oldest sites were chosen for results. The stars refer to the resistance level group classification.

	S	*	**	***	Total
Known spraying history	11 (7.5)	48 (32.7)	22 (15.0)	14 (9.5)	95 (64.6)
Unknown spraying history	11 (7.5)	21 (14.3)	11 (7.5)	9 (6.1)	52 (35.4)
Total	**22 (15.0)**	**69 (46.9)**	**33 (22.4)**	**23 (15.6)**	**147**

If a new survey was conducted focusing on 2.4-D resistance instead of on tribenuron-methyl, probably more strongly resistant populations could be found. Nevertheless, it is shown that there is still a resistance problem towards 2.4-D in the surveyed area. However, other herbicides are provided so that this problem does not cause other attitude change than the use of a different herbicide.

Fields with hard control problems decades ago probably could be well controlled afterwards with other herbicides appearing in the market. Following, high-resistant populations to 2.4-D are less probable to be found nowadays than some years or decades ago.

Multiple resistance to tribenuron-methyl and to 2.4-D

In 136 populations sensibility towards both herbicides were tested. The results are shown in Table 3.9.

Table 3.9.: Overall number of *Papaver rhoeas* populations susceptible or resistant to the different degrees of tribenuron-methyl and 2.4-D collected in Catalonia / collected in the rest of Spain. Populations were collected in Northern Spain. In case of repeated populations, the oldest sites were chosen for results.

2.4-D / **tribenuron-methyl**	**S**	*****	******	*******	**Sum**
S	11 / 3	14 / 0	7 / 0	5 / 0	**37 / 3**
*****	2 / 0	6 / 4	3 / 0	5 / 0	**16 / 4**
******	3 / 1	10 / 2	8 / 0	1 / 0	**22 / 3**
*******	3 / 4	12 / 9	11 / 9	8 / 0	**34 / 22**
Sum	**19 / 8**	**42 / 15**	**29 / 9**	**19 / 0**	**64 / 24**

As shown in Table 3.9. the most frequent situations out of the tested populations collected in Catalonia was one- or two-star-resistance to 2.4-D combined with three-star-resistance towards tribenuron-methyl. These results are consistent with the spraying history in the area, where 2.4-D had been sprayed during decades in a continuous way. At the moment, after the appearance of other herbicides less 2.4-D than tribenuron-methyl is sprayed, so that highly resistant populations to 2.4-D are more difficult to detect. In the case of the populations collected in the rest of Spain, the same tendency was observed.

As explained by several farmers, in many cases tribenuron started to be sprayed as an alternative to 2.4-D as this herbicide needed to be sprayed progressively in higher doses in order to achieve some efficacy. It is then not a surprise to find out that most of the analysed populations were at least to some degree multiple resistant to both herbicides. As it also corresponds to the history of many cases, much more populations were resistant to 2.4-D only than to tribenuron-methyl only.

Despite that the resistance towards tribenuron-methyl is very spectacular due to the fast development and to the complete lack of response by increasing field rates, this herbicide is probably still useful and very active in many fields. More resistant fields towards 2.4-D were found, suggesting that 2.4-D has a shorter commercial life. Thus, tribenuron-methyl seems to be still a good product in more cases than 2.4-D. On the other hand, as the results suggest, once resistance towards tribenuron-methyl has developed, there is a high probability of evolving to high-resistant samples if the product is still used. This way, even if more resistant fields towards 2.4-D were found, resistance was often low-degree so that higher rates of 2.4-D could still control many fields.

Resistance towards ioxinil + bromoxinil + 2.4-D

Out of the 65 tested populations, none survived the double-dose treatment of Oxytril. In ten populations, some plants survived the single-dose treatment (survival between 2 and 8%). In six out of these ten populations the survival was high (between 12 and 51%) when half-rate was applied. Two of these populations were classified as three-star resistant to 2.4-D and four populations as two-star resistant. Table 3.10. shows the multiple resistance characteristics of these ten populations, where some plants survived the single-dose Oxytil treatment.

Table 3.10.: Herbicide resistance towards tribenruon-methyl and 2.4-D of ten *Papaver rhoeas* populations, where some plants survived a 2.5 L ha^{-1} Oxytril treatment.

2.4-D / **tribenuron-methyl**	**S**	*****	******	*******	**Sum**
S	-	-	-	-	-
*****	-	-	2	1	-
******	-	-	1	-	-
*******	-	1	2	3	-
Sum	**-**	**1**	**5**	**4**	**10**

The possible resistance to Oxytril seems, thus, to be related to the herbicide resistance towards 2.4-D. The behaviour of these populations towards tribenuron-methyl was irregular, suggesting that there is no relationship between resistance towards tribenuron-methyl and towards this herbicide mixture. Three of these populations were three-star resistant to tribenuron-methyl, one was two-star resistant and two were one-star resistant.

Evolution of resistance in time inside the same fields

Whenever it was possible, samples of the same fields were taken again in the following years. The results of the quick-tests for the two herbicides are shown in Table 3.11. and Table 3.12., respectively.

Table 3.11.: Percentage of *Papaver rhoeas* plants resistant to tribenuron-methyl inside the seed sample of populations collected in the same field over different years. Data show the evolution of herbicide resistance in these fields. Samples with known spraying history are highlighted.

Sample numbers			**Percentage of resistant plants (following the agar quick-test)**			**Resistance evolution**
3/95	**6/96**	**No seeds in 1998**	**81.8**	**91.5**		**Increase**
5/95	7/96	No seeds in 1998	91.9	88.7		Decrease
6/95	**12/96**	**41/99**	**0**	**0**	**11.9**	**Increase**
8/95	**17/96**	**-**	**92.2**	**97.5**		**Increase**
12/97	**18/98**	**36/99**	**63.4**	**99.1**	**97.6**	**Increase**
1/98	**6/99**	**-**	**98.2**	**92.8**		**Decrease**
5/98	**28/99**	**-**	**5.5**	**92.5**		**Increase**
48/98	**72/99**	**6/00**	**92.8**	**100**	**92.1**	**Stable**
51/98	90/99	-	1.7	37.0		Increase
36/98	**8/00**	**-**	**85.3**	**64.9**		**Decrease**

As shown in Table 3.11., all kind of evolution patterns were observed. The spraying history during the previous years and in the sampling year affects the evolution of the resistance in the samples.

No spraying would increase the proportion of susceptible plants, germinating from the seed bank. As an example, in the case of the populations 36/98 and 8/00 it is known that during the cropping season 1999-00, a different herbicide was sprayed, which controlled efficiently the resistant *P. rhoeas*. Seeds of sample 8/00 were collected from an untreated stripe in which no selection pressure was produced on the plants. The decrease of resistant plants compared to the sample the year before is thus probably due to the lack of selection pressure in that year.

Spraying with the same herbicide again would increase the proportion of resistant plants. An increase in the proportion of resistant plants could probably occur if the same herbicide was sprayed again. As an example, in the case of populations 6/95, 12/96 and 41/99, complains on lack of efficacy towards tribenuron-methyl were already recorded in 1995, but detected only 4 years later. There was probably a combined effect of lack of efficacy due to resistance and non-correct spraying, so that also susceptible plants survived and its seeds were collected and analysed.

Spraying with an effective or non-effective different herbicide would leave the proportion of resistant plants towards tribenuron-methyl the same. A stable evolution occurred in the case of an already very high proportion of resistant plants inside the samples. In the example of the populations 48/98, 72/99 and 6/00, no surviving *P. rhoeas* plants were found in summer 2001, as a very effective herbicide was used in the cropping season 2000-01.

Table 3.12.: Length ratio of *Papaver rhoeas* plants resistant to 2.4-D found by a quick-test inside the seed sample of populations collected in the same field over different years. Data show the evolution of herbicide resistance in these fields. Ratio>1 indicates resistance. Samples with known spraying history are highlighted.

Sample numbers			Hipocotyl length ratio (following the agar quick-test)			Resistance evolution
3/95	**6/96**	**No seeds in 1998**	**1.57**	**1.02**		**Decrease**
5/95	7/96	No seeds in 1998	1.20	1.27		Increase
6/95	**12/96**	**41/99**	**1.54**	**1.52**	**1.83**	**Increase**
8/95	**17/96**	**-**	**2.00**	**2.99**		**Increase**
12/97	**18/98**	**36/99**	**1.52**	**1.51**	**1.59**	**Stable**
1/98	**6/99**	**-**	**1.37**	**1.39**		**Increase**
5/98	**28/99**	**-**	**1.34**	**1.79**		**Increase**
48/98	**72/99**	**6/00**	**2.47**	**2.33**	**1.22**	**Decrease**
51/98	90/99	-	1.74	2.25		Increase
36/98	**8/00**	**-**	**1.26**	**1.10**		**Decrease**

The situation of all populations shown in Tables 3.11. and 3.12. was multiple resistance towards tribenuron-methyl and 2.4-D. No 2.4-D was sprayed any more since 1995 in most of the sampled populations. Despite this, increases in the resistance degree towards 2.4-D were observed. As the ratios were calculated as an average of all the seedlings' length in one Petri dish, a bigger ratio can be caused by both, an increase in the resistance degree of the sample or to the disappearance of susceptible plants, which were in the sample before. So, selecting tribenuron-methyl resistant plants, 2.4-D resistant plants were probably also favoured.

Conclusions

An abundance of resistant populations towards tribenuron-methyl and 2.4-D was detected in the surveyed area.

Most of the complains on lack of efficacy towards the herbicides were justified.

Despite 2.4-D is not very commonly used any more and the populations are subjected to little selection pressure on this herbicide, herbicide resistance towards 2.4-D is still widespread and was found in more samples than resistance towards tribenuron-methyl.

Most of the resistant populations towards tribenuron-methyl had a very high proportion of resistant plants inside the sample but most of the resistant samples towards 2.4-D were slightly resistant.

The most frequent case of the analysed populations was multiple resistance to both herbicides, slight to 2.4-D but high to tribenuron-methyl.

It is unknown how the resistance pattern was like when 2.4-D was still commonly used but this evolution indicates that the populations resistant to tribenuron-methyl will probably also persist during long time. Also the fact that most of the populations found resistant towards tribenuron-methyl have high proportions of resistant plants suggests that the problem will last long.

A starting decline of efficacy was observed in some populations towards the herbicide Oxytril containing ioxinil + bromoxinil + MCPP. The lack of efficacy is probably related to strongly 2.4-D resistant populations.

A new survey could focus on collecting samples from older known populations in order to see the evolution of resistance or on collecting samples randomly in order to characterise the present resistance status of *P. rhoeas*.

The populations collected in Catalonia and in Burgos and Navarra showed similar behaviour, even if the main difference was that the very resistant populations towards 2.4-D came from Catalonia.

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Part B

Management of herbicide resistant *Papaver rhoeas* L. populations in North-eastern Spain

Part B 1

Study of biological characteristics of *Papaver rhoeas* L. in order to develop cultural management strategies

Chapter 4

Germination and survival habits of a susceptible and a herbicide resistant *Papaver rhoeas* L. population in North-eastern Spain (Catalonia)

Germination and survival habits of a susceptible and a herbicide resistant *Papaver rhoeas* L. population in North-eastern Spain (Catalonia)

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Summary

Two types of experiments on *Papaver rhoeas* L. population dynamics were conducted. The first one aimed to describe the germination period and to quantify germination of a susceptible and of a herbicide resistant population in two locations taking into account the population's origin, the effect of tillage, the population's age and the sowing year.

Overall germination was observed between September and April but main germination occurred between October and December. The behaviour was similar for the two tested *P. rhoeas* populations and germination was much bigger in the cooler and moister location. Germination in the second and third year was generally inferior in spite of good climatic conditions for freshly-sown seeds suggesting that secondary dormancy induced in older seeds is more difficult to break.

Tillage stimulated germination in almost all cases. Soil containing seed mixtures from different years showed the same behaviour pattern than seed lots containing uniform aged seeds. The second experiments characterised the plant survival in 18 field trials. Variable initial plant densities were recorded depending on the location and on the year ranging between 161 and 1743 plants m^{-2}.

Plant mortality of 29 up to 84% generally occurred gradually since end of February. Final density values ranged between 49 and 543 plants m^{-2}. Very drastic reductions in some fields as well as almost invariable populations were observed. Different causes were found to be responsible of the mortality making prediction were difficult. The practical application of these findings is discussed.

Keywords: germination period, *Papaver rhoeas* L., survival, plant mortality, dormancy, tillage, herbicide resistance.

Introduction

In order to use cultural weed control methods effectively, it is necessary to deepen in matters of weed biology like weed seed bank dynamics and modelling weed seedling emergence (Bhowmik, 1997). Also Liebman & Gallandt (1997) think that information regarding spatial and temporal patterns of weed abundance besides other matters are necessary to develop weed management strategies based on submitting weeds to multiple and temporary variable stresses.

Studies on germination periods and population dynamics for *Papaver rhoeas* L. have been conducted in Denmark (Kjær, 1940) and in Great Britain (Roberts & Feast (1973), Roberts & Neilson (1981), Roberts & Boddrell (1984), Blair & Green (1993). In the semi-arid conditions of North-eastern Spain a one-year experiment in one location was conducted by Izquierdo & Recasens (1992).

In the present work, a demographic study during three years on *P. rhoeas* seed germination and on *P. rhoeas* plant survival in field conditions is described. These two segments of the weed life cycle are the ones, which can more directly be affected by agricultural practices. Therefore, results of two groups of experiments are described in this paper. The first ones are referred to as *germination habits experiments,* while the others are named *plant survival in field conditions experiments.*

Germination habits experiments

Weed emergence germination periods are important to be known in order to prepare the correct control strategy in time. As germination is highly weather-dependent it is useful to study the germination behaviour throughout several years in the same area in order to observe the general tendency. On the other hand, data on the percentage of fresh seeds germinating in the first cropping season help to give an idea of the needed time to deplete the seed soil bank. Data on these topics are sparse.

The biological cycle of *P. rhoeas* in North-eastern Spain starts with germination in autumn between September and December, following Izquierdo & Recasens, (1992). Nevertheless, some spring germination had also been observed in some cases. As no detailed data is available on this topic for the North-eastern Spanish conditions, a field experiment in two locations was designed in order to describe the germination habits of two *P. rhoeas* populations throughout three years. Vegetative growth continues since March, reaching the flowering stage approximately in April. Mature seed production occurs at the end of May until end of June or beginnings of July, depending on the area and on the moisture and temperature conditions of the season. (Bolòs, 1990). This cycle is very synchronic with the barley or wheat crop cycle in the same region.

As a known amount of seeds was sown out in this experiment, percentage of emergence of fresh seeds, one-year and two-year old seeds after one, two and three years could also be determined. In a second experiment, the germination of a mixture of different-aged seeds was analysed in order to find out if differences with homogeneous-aged seed lots could be observed.

One *P. rhoeas* population used was susceptible to herbicides and the other one was resistant to 2.4-D and to tribenuron-methyl. This way, the possible differences in germination between herbicide resistant and susceptible *P. rhoeas* could be analysed. With the exception of a faster germinating ALS-resistant *Lactuca serriola* population, generally no differences had been quoted between the behaviour of ALS-resistant and susceptible biotypes (Saari *et al*., 1994). Following the review of Coupland (1994) fitness penalty is also rare for 2.4-D resistant species but found in a resistant *Sinapis arvensis* population. For another multiple resistant weed species towards ALS-inhibitors and auxin-type herbicides namely *Galium spurium*, no different behaviour has been quoted either (Hall *et al.*, 1998).

Big differences between the susceptible and the herbicide resistant populations were therefore not expected to be observed but were considered worth to be analysed.

In order to search for a possible cultural control by stimulating *P. rhoeas* germination previously to sowing, the effect of cultivation was also analysed in these experiments in autumn and early winter. It has been observed that late autumn soil cultivation can be followed by abundant *P. rhoeas* germination while post-harvest cultivation of cereal fields in early summer provokes the rise of very few or no *P. rhoeas* seedlings (data not shown).

Understanding seasonal variation of seed germinability may improve strategies for either favouring *P. rhoeas* emergence previous to crop sowing or reducing *P. rhoeas* germination within the crop by preparing the seed-bed in non-optimal germination conditions of the weed. This data could also help deciding if untilled fallow is a possible control strategy or if cultivation in an appropriate moment is more effective reducing the *P. rhoeas* seed bank.

Plant survival in field conditions experiments

In the present study almost mono-specific *P. rhoeas* populations in herbicide resistant fields were the study object, a situation not commonly described previously in Spanish field conditions. In order to face the weed control in this new situation it is necessary to collect as much information on weed dynamics as possible.

Few and variable data is found in the literature regarding the normal *P. rhoeas* population density at the beginning and at the end of the cropping season in winter cereals. Based on previous own studies, McNaughton & Harper (1964) observed that self-thinning commonly reduces population size in United Kingdom to about 750-860 mature plants m^{-2} without describing if this occurred in non-cultivated land or in cereal fields. This density is very high compared to 51.4 plants m^{-2} at the end of the cropping season in a field trial containing also other weed species in the same country by Blair & Green (1993).

The only available data in Spanish conditions was of a field trial conducted in Central Spain, in which 25.8 *P. rhoeas* plants m^{-2} were found in April within a total weed species density of 151 plants m^{-2} of mixed weed species (Lacasta *et al*, 1997).

Within other information, weed population dynamics could provide information on natural plant mortality due to intra- and interspecific competition. If this mortality was find out to be very high also in small weed populations, the plant decrease alone or together with a medium-effective control method might be enough to maintain *P. rhoeas* infestation at acceptable levels in these cases. If plant mortality was found to be high only in big weed populations, this decrease might not be enough and the reduction would be unacceptable. In an integrated weed management system, additive small reductions in weed populations are targeted (Liebman & Gallandt, 1997). This natural mortality could be one more "little hammer" to be accounted.

As several fields were studied during following cropping seasons, *P. rhoeas* germination could also be described from year to year in these cases. The influence of the season's climate could be thus observed. These data can be useful to predict possible weed infestation levels in a field in consecutive years.

Material and Methods

In the first *germination habits experiment*, seeds were collected at mature stage, air-dried during some days and sown out in July imitating natural seed rain. In the second *germination habits experiment*, superficial field soil containing a mixture of different-aged *P. rhoeas* seeds was placed on pots in the field also in July. In the *plant survival in field condition experiments*, evolution of spontaneously emerging *P. rhoeas* plant number m^{-2} in commercial cereal fields was recorded since the establishment of most of the plants (December since February) up to flowering (April since May).

Germination habits experiments

The tested *P. rhoeas* populations were the standard susceptible population towards herbicides codified as A/98 from Algayón (La Llitera region) and the tribenuron-methyl and 2.4-D resistant population codified as B/98 from Baldomar (Noguera region). The Algayón field was a non-cultivated fallow field while in Baldomar barley was grown during all the years under minimum tillage.

The climatic characteristics of these locations during the cropping seasons are shown in Figure 4.1. a, b.

Monthly mean temperatures were very similar in both locations (Figure 4.1.a, b). More rainfall was recorded in Baldomar than in Algayón but the main rain periods were coincident in both locations. Quite long fog periods occurred in Baldomar during all three winters maintaining the humidity during longer periods than in Algayón where the soil dried out more frequently.

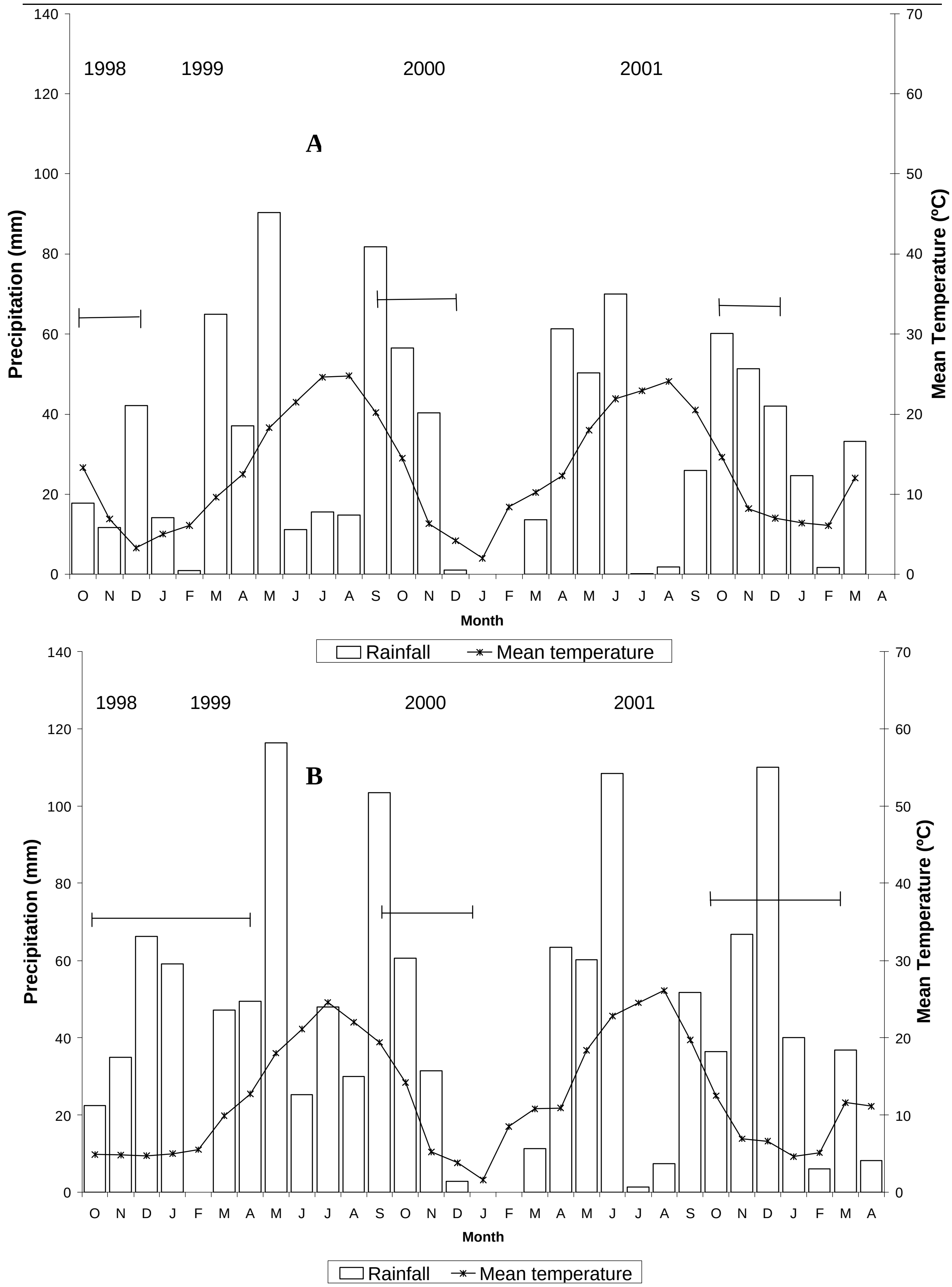


Figure 4.1. Climatic data of the two sites during the duration of the *Germination habits experiments*. ? - | indicates overall *Papaver rhoeas* germination periods found in the germination habits experiments. A) In Algayón (La Llitera, Aragón). Data from the nearby climatic observatory "La Melusa" at 41.780º latitude, 4.0614º longitude, 218 m altitude. B) In Baldomar (La Noguera, Catalonia). Data from the nearby observatory in Vilanova de Meià located at 41.991º latitude, 1.022º longitude and 590 m altitude.

Seed germination of both populations was tested at both sites. The bottom of 18 cm high 19 cm diameter plastic pots was cut off and the pot buried up to surface at the border of both fields. In the first experiment, the free room in the pots was filled with seed-free silt loam soil and approximately 1000 *P. rhoeas* seeds were sown on the surface of each pot and mixed with the first two centimetres of soil. Seeds were collected in the two sites in summer 1998, 1999 and 2000. Initial seed viability was comprised between 90 and 100% in all cases. 1000-seed lots were prepared by weighing after determining the 1000 seed weight of each population separately. The data of the 1000-seed weight is shown in Table 4.1.

In the second experiment, superficial field soil containing an unknown amount of different aged *P. rhoeas* seeds was collected in each field and sown in separate pots in the respective site, only. These pots were established in summer 1998, 1999 and 2000 with the aim of observing if similar germination responses could be expected from a mixture of different aged *P. rhoeas* seeds in comparison to uniform aged seed lots.

In both experiments, half of the pots were kept undisturbed during the testing period, in the other half, autumn cultivation was imitated in the pots in the same dates as the farmer conducted theses operations in the field. The normal farmers' practice was seedbed preparation and a second cultivator pass combined with cereal sowing after raining. In 1998, two cultivation passes were performed in the field before sowing. They were imitated in the pots, including soil disturbance imitating sowing. In 1999, seedbed preparation and sowing were conducted in dry conditions.

Rainfall didn't arrive since April and no additional soil movement was imitated. In 2000 seedbed preparation was performed in humid conditions and sowing done afterwards. Timing of the operations is shown in Table 4.1. Due to removal of the pots placed in July 2000 in Algayón at beginnings of September by unknown people *P. rhoeas* seeds had to be re-sown afterwards.

Seedling emergence was recorded monthly since sowing and plants were removed afterwards. Barley was sown around the pots in order to shade the pots imitating the crop situation. Since end of June to August, pots were covered with a fine net preventing seed rain from surrounding *P. rhoeas* plants.

Table 4.1.: Sequence of cultural operations on the outside pot experiments conducted in Algayón and in Baldomar. Due to the drought, cultivation was conducted more times in the first year. New established pots in 2000 in Algayón were removed by unknown people forcing to place them again at beginnings of September. 1000 seed weight in g ± SD.

	1998	**1999**	**2000**
Algayón			
1000-seed weight	0.1251 ± 0.00163	0.0827 ± 0.00336	0.0921 ± 0.00384
Papaver rhoeas *s*owing date	27/07	16/07	19/09
Cultivation 1	09/09	-	-
Cultivation 2	07/10	-	-
Imitating cereal sowing	27/10	21/09	10/10
Baldomar			
1000-seed weight	0.1053 ± 0.00181	0.0881 ± 0.00330	0.1150 ± 0.00182
Papaver rhoeas *s*owing date	28/07	16/07	19/07
Cultivation 1	09/09	-	-
Cultivation 2	06/10	-	-
Imitating cereal sowing	26/10	21/09	07/09

Statistical analysis of germination data were performed submitting data to a Standard ANOVA using the SAS system (SAS Institute 1991). In case of significant differences, the Duncan mean separation test was conducted.

Plant survival in field conditions

19 field trials on chemical and mechanical *P. rhoeas* control were established during the years 1998-2001 on commercial winter cereal fields. In most of them, high populations of herbicide resistant *P. rhoeas* made weed control difficult. In the field of Bellprat and of Nalec *P. rhoeas* was herbicide resistant to 2.4-D, only, while in all the other cases the weed was resistant to 2.4-D and to tribenuron-methyl. Plots measured 2 m x 5 m and were randomly distributed in three blocks. Evolution of *P. rhoeas* plant number was recorded periodically in three different untreated plots of each trial. Therefor, three 0.10 m^2 frames were randomly counted in each of the plots.

In the location Baldomar, field trials were established during four cropping seasons. In Torrelameu and Nalec fields were kept during two cropping seasons while Sanaüja, Bellprat and Sta. Coloma de Queralt were studied during one year only (Table 4.2.). First evaluations were conducted from December until March, depending on the field conditions. Last assessments were conducted from March until beginnings of May corresponding to the earing stage of the cereal crop and to early flowering of *P. rhoeas*. Statistical analysis were done on the mean of each plot and means were separated with the Duncan test inside each trial. Differences were considered significant at P<0.01.

Table 4.2.: Characteristics of the chosen field sites for the plant survival experiments. a, b refer to two field trials inside the same field.

Location	Cropping seasons	Abbreviation	Number of fields in the location	Soil type	Altitude (m)
Baldomar	1997-98 1998-99 1999-00 2000-01	B 98 1a,b B 99 2 B 00 2 B 01 2a, b	2	Sandy loam	590
Torrelameu	1999-00 2000-01	T 99 1a, b T 00 1a, b	1	Loam	218
Nalec	1999-00 2000-01	Na 00 1a, b Na 01 1a, b	1	Silty loam	420
Sanaüja	2000-01	San 01 1a, b	1	Silty loam	315
Santa Coloma de Queralt	2000-01	Sta 01	1	Loam	718
Bellprat	2000-01	Bell 01	1	Loam	718

The ombrothermic diagrams of the different locations are shown in Figure 4.2. a, b, c, d, e, f. The main differences between locations and between years within the same location were the distribution and amount of precipitation. The amount of the precipitation in the cropping season comprising October to June is shown in Table 4.3.

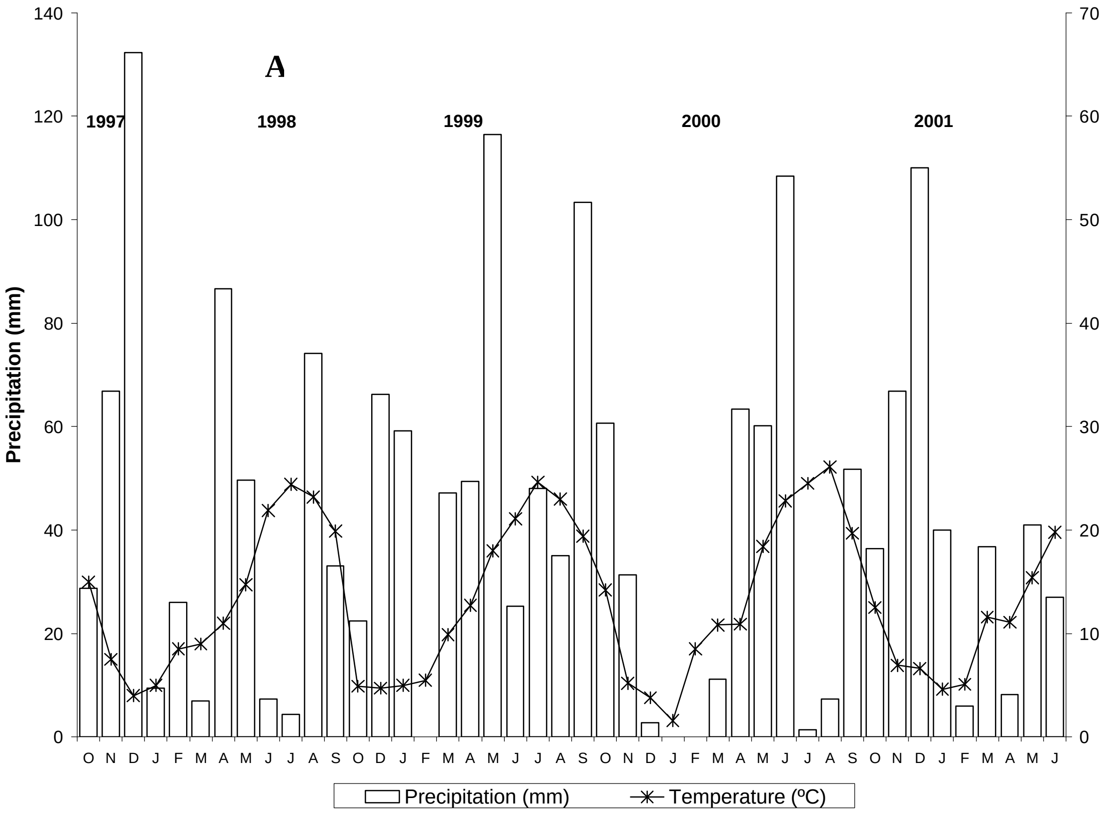


Figure 4.2. a. Climatic data of Baldomar (La Noguera, Catalonia). Data from the nearby observatory in Vilanova de Meià located at 41.991° latitude, 1.022° longitude and 590 m altitude.

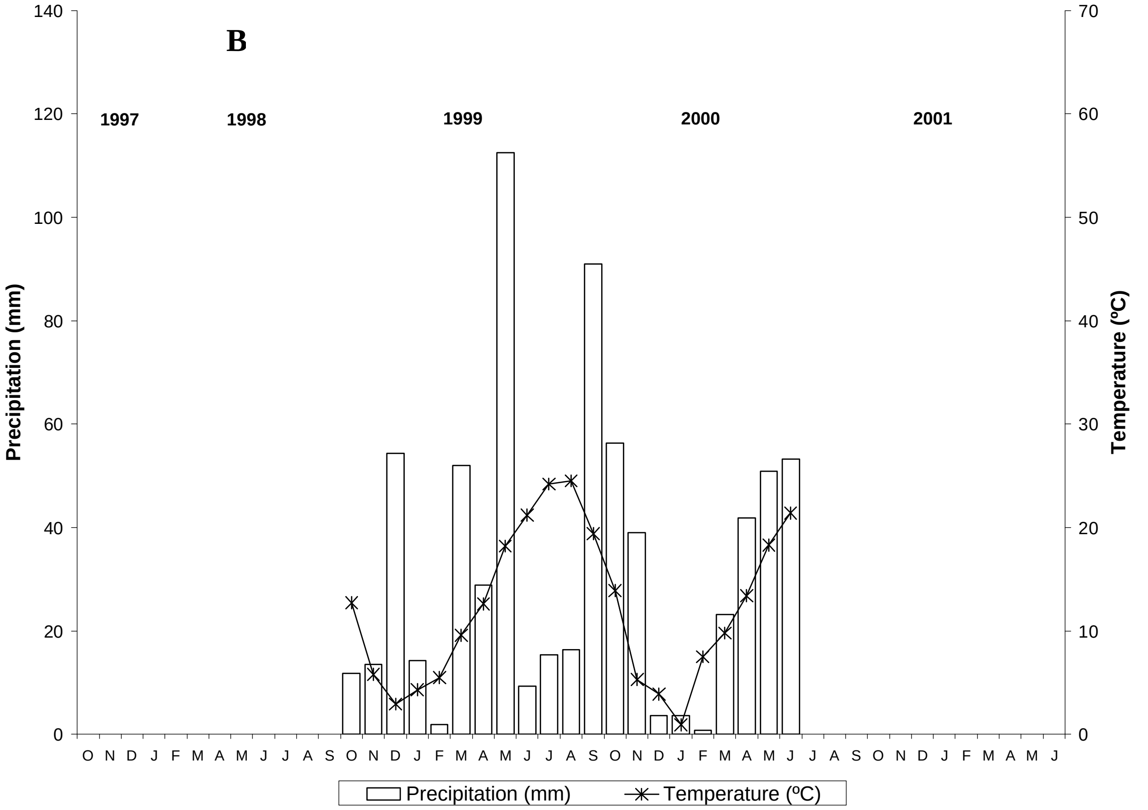


Figure 4.2. b. Climatic data of Torrelameu (Segrià, Catalonia). Data from the nearby observatory in Vilanova de Segrià located at 41.715º latitude, 0.629º longitude and 218 m altitude.

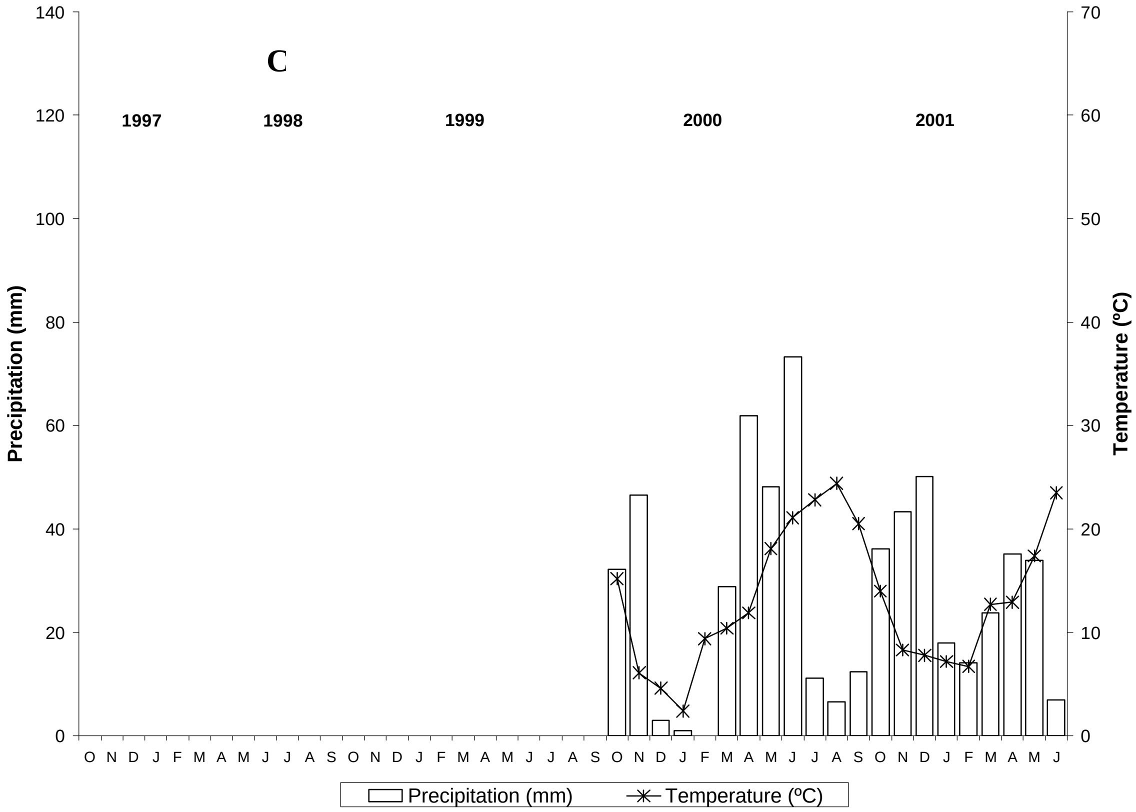


Figure 4.2. c. Climatic data of Nalec (Anoia, Catalonia). Data from the nearby observatory in Tàrrega located at 41.668º latitude, 1.164º longitude and 420 m altitude.

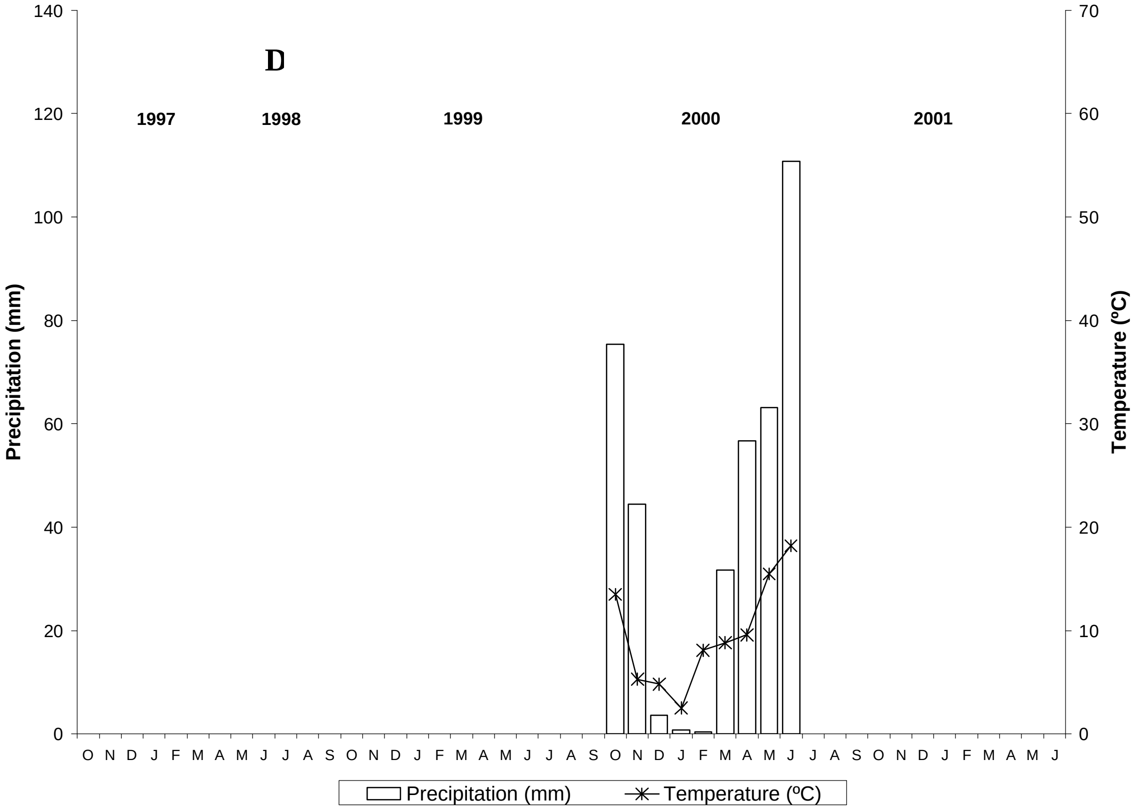


Figure 4.2. d. Climatic data of Bellprat (Anoia, Catalonia). Data from the nearby observatory in Santa Coloma de Queralt located at 41.530º latitude, 1.368º longitude and 718 m altitude.

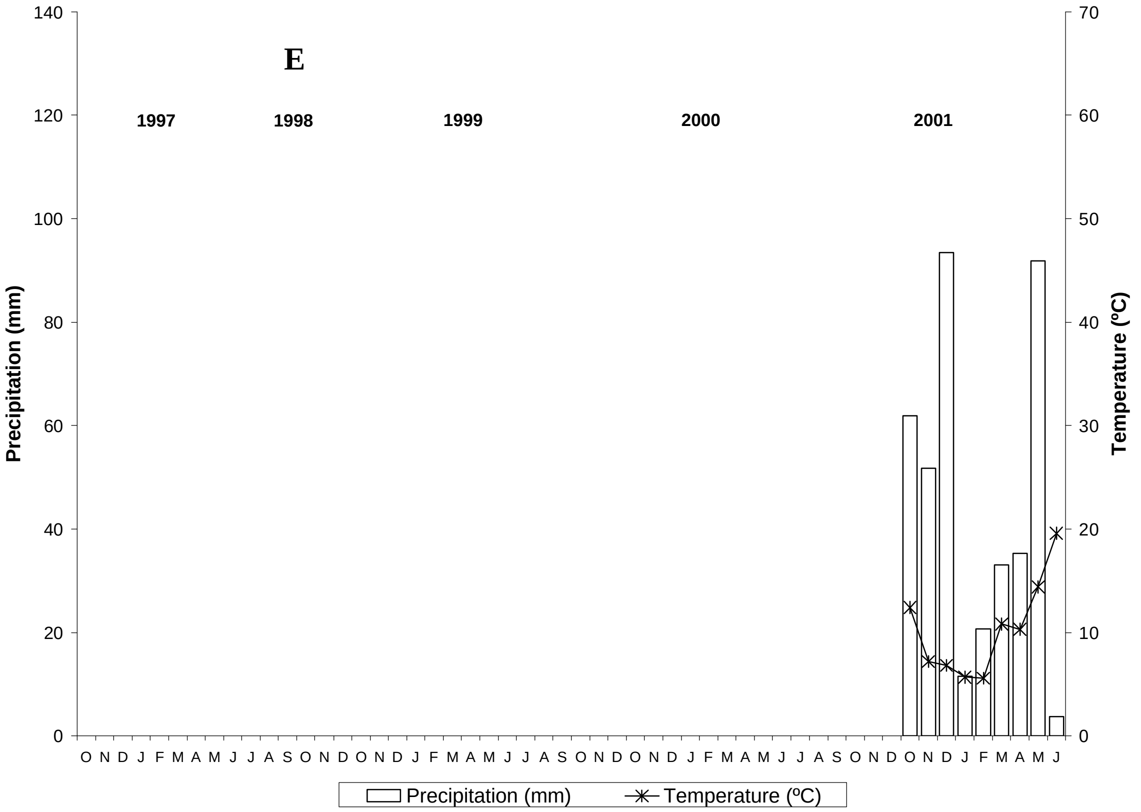


Figure 4.2. e. Climatic data of Santa Coloma de Queralt (Conca de Barberà, Catalonia). Data from the observatory in Santa Coloma de Queralt located at 41.530º latitude, 1.368º longitude and 718 m altitude.

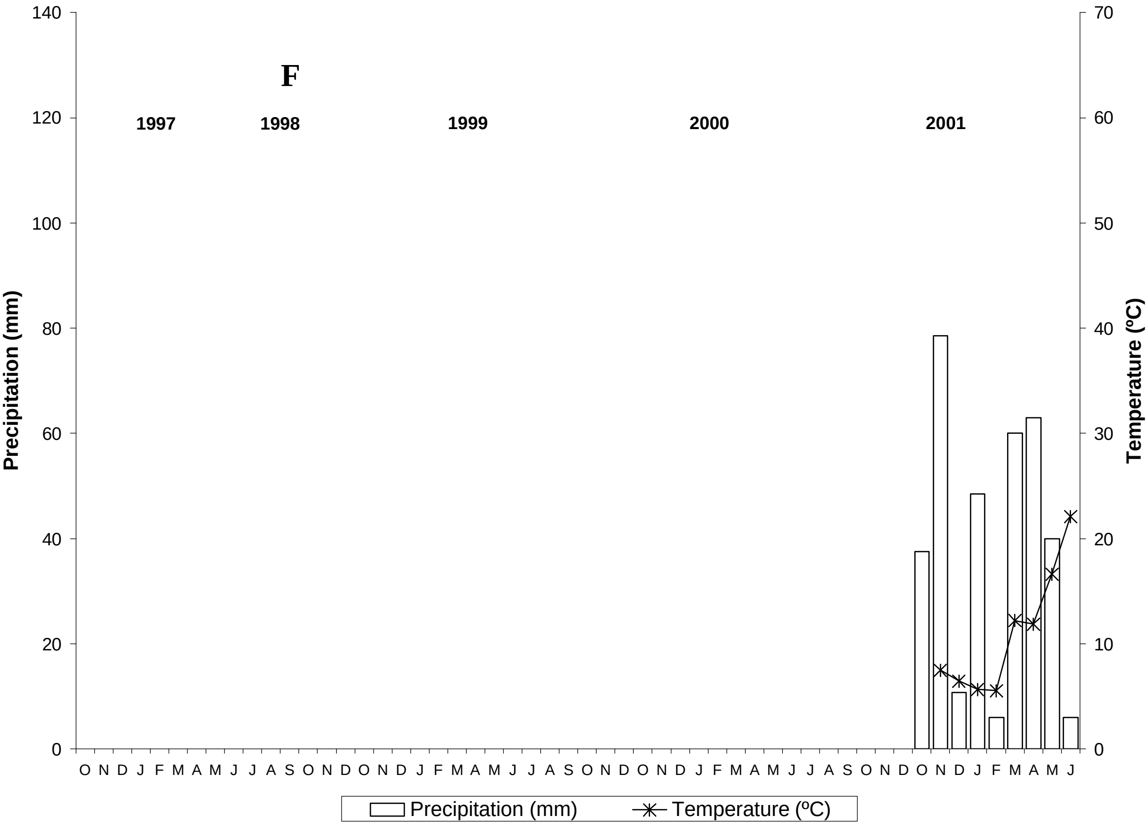


Figure 4.2. f. Climatic data of Sanaüja (Segarra, Catalonia). Data from the nearby observatory in Artesa de Segre located at 41.896º latitude, 1.047º longitude and 315 m altitude.

The sites, where most precipitation was recorded were Baldomar (season 1997-98) and Santa Coloma de Queralt (2000-01). Nalec and Torrelameu recorded the least rainfall (Table 4.3.).

Table 4.3.: Precipitation (mm) registered during the different cropping seasons comprising from October to June in the different experimental locations.

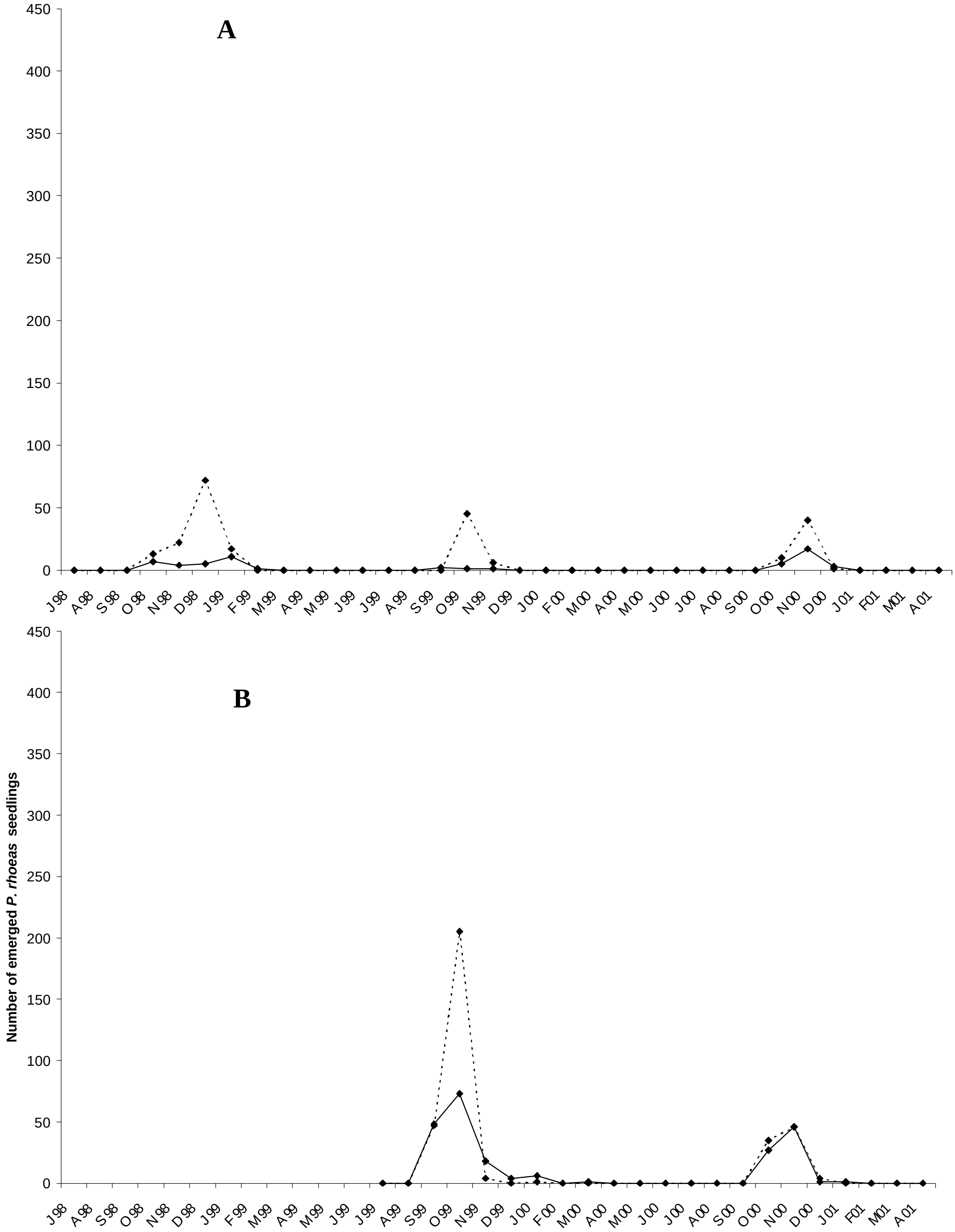
Location	Cropping season	Precipitation (mm) between October and June
Baldomar	1997-98	413.8
	1998-99	386.0
	1999-00	338.0
	2000-01	372.2
Torrelameu	1998-99	298.2
	1999-00	272.5
Nalec	1999-00	294.8
	2000-01	262.0
Bellprat	1999-00	387.0
Santa Coloma de Queralt	2000-01	403.4
Sanaüja	2000-01	350.3

Results and Discussion

Germination habits

First experiments
Algayón

The overall germination period of *P. rhoeas* in Algayón of the first experiment was between September and March (Figures 4.3. and 4.4.).



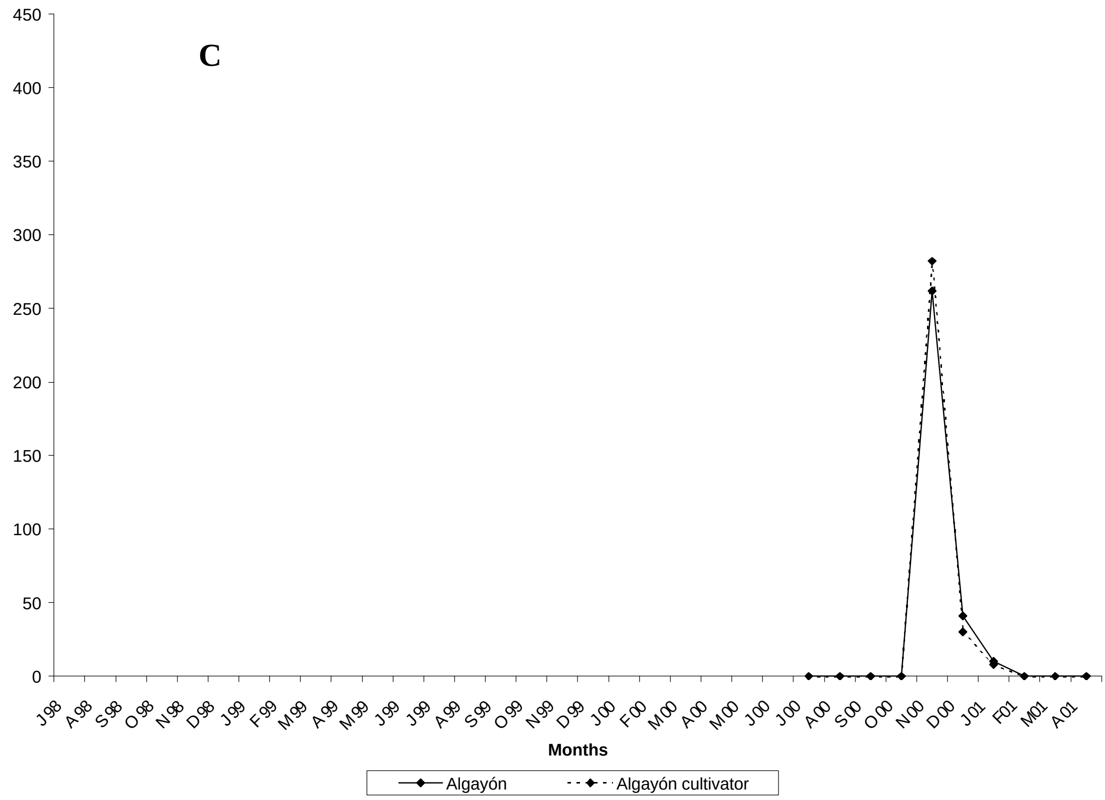
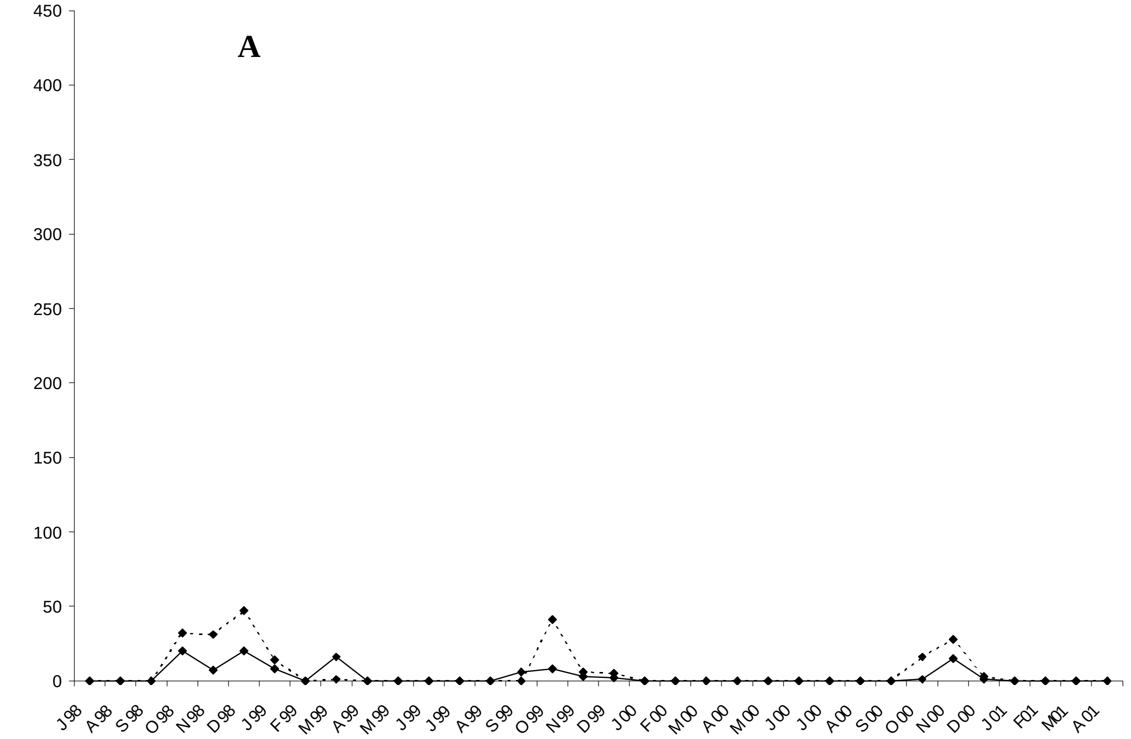


Figure 4.3. Germination of *Papaver rhoeas* seeds coming from Algayón sown in Algayón. This population was susceptible to herbicides. 1000 seeds were sown in each pot containing weed-free soil. In half of the pots cultivation was imitated in winter of each year. Three replicates of each treatment were placed on the field. A) Pots established in summer 1998 B) in summer 1999 and C) in summer 2000.

In 1998, emergence was maximum in December in all cases (Figures 4.3. and 4.4.). In 1999, main germination was recorded in October, also for all the treatments and seeds origins. In 2000, maximum germination was recorded in November, again independently of the different factors.

Germination occurred between October and January in the cropping season 1998-99 and slight germination was recorded also in March in the non-cultivated Baldomar population. In the season 1999-00 germination occurred between September and January. In the season 2000-01 germination was observed between October and January.

The cultivation treatment influenced the germination period in some cases so that the germination period was longer for the non-cultivated seed lots (Figures 4.3. b, 4.4. a and 4.4. b). This was observed in one occasion for seeds coming from Algayón and in three occasions for seeds from Baldomar. The sowing year had little influence on the germination period in Algayón. In most cases, the population of Baldomar had a bit wider germination period than the population of Algayón germinating earlier and also later (Figures 4.3.a, b and 4.4. a, b).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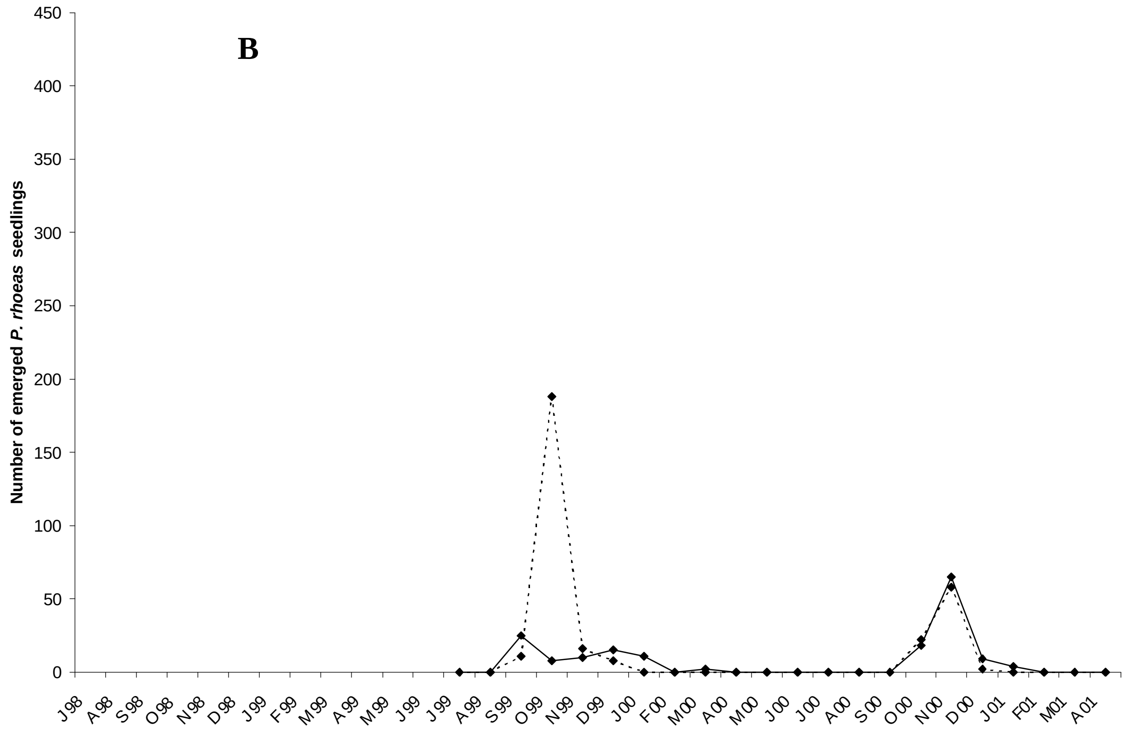
0
J98 A98 S98 O98 N98 D98 J99 F99 M99 A99 M99 J99 J99 A99 S99 O99 N99 D99 J00 F00 M00 A00 M00 J00 J00 A00 S00 O00 N00 D00 J01 F01 M01 A01


B
Number of emerged P. rhoeas seedlings
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J98 A98 S98 O98 N98 D98 J99 F99 M99 A99 M99 J99 J99 A99 S99 O99 N99 D99 J00 F00 M00 A00 M00 J00 J00 A00 S00 O00 N00 D00 J01 F01 M01 A01

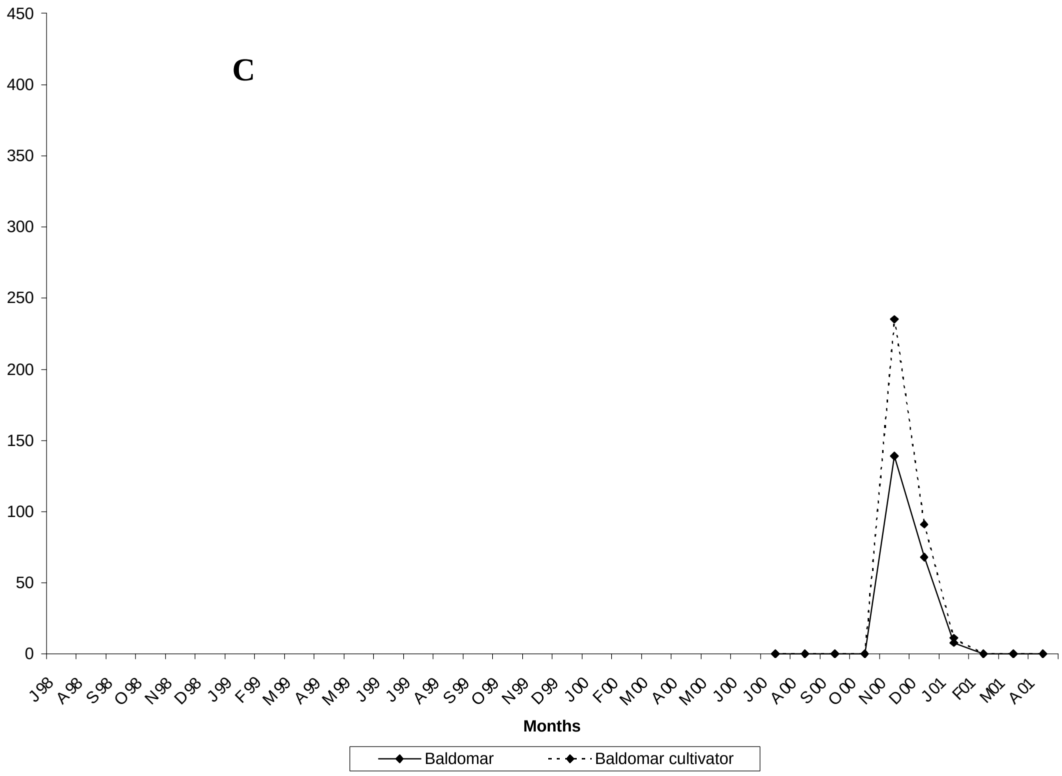
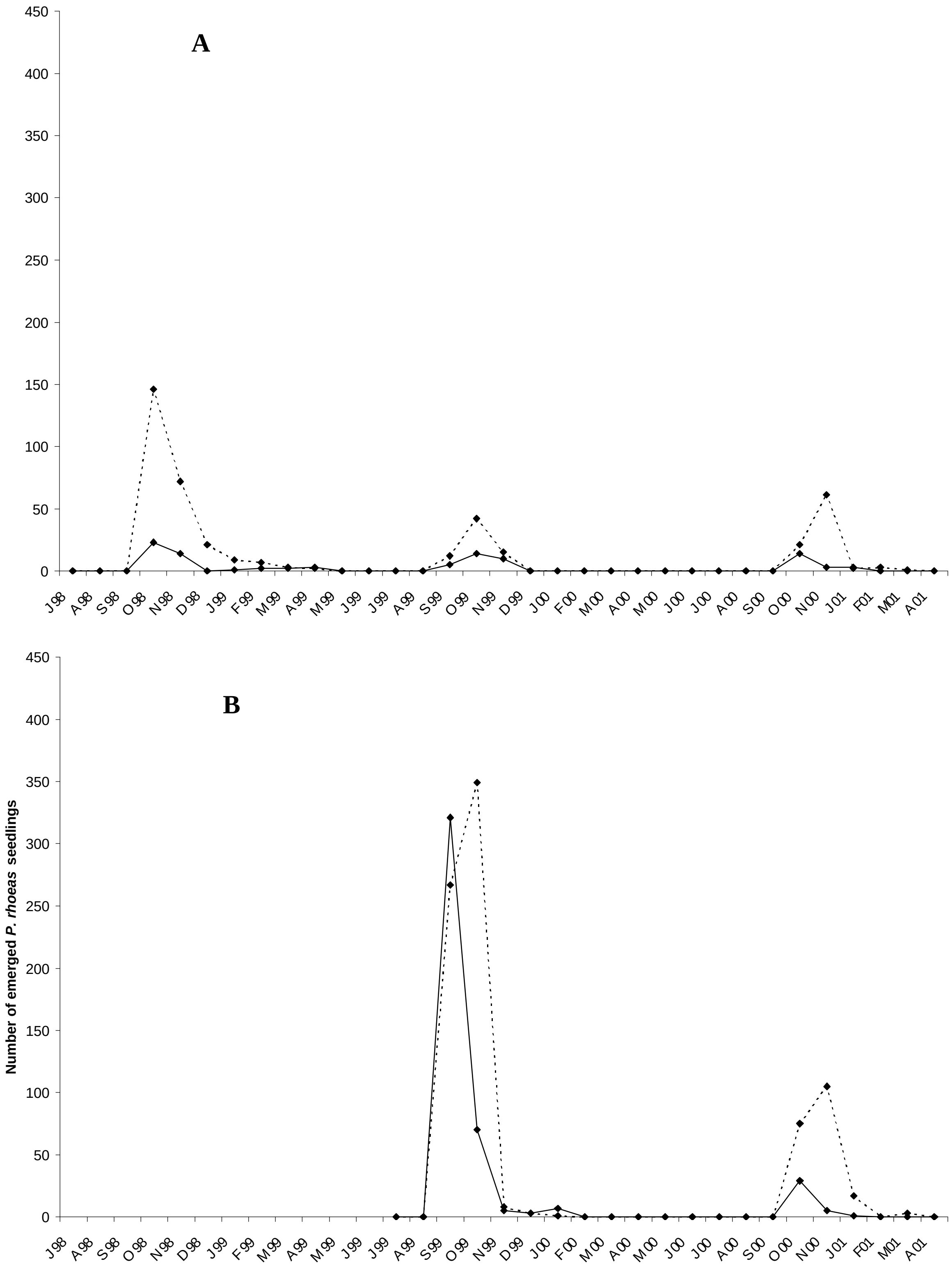


Figure 4.4. Germination of *Papaver rhoeas* seeds coming from Baldomar sown in Algayón. This population was resistant to tribenuron-methyl and to 2.4-D. 1000 seeds were sown in each pot containing weed-free soil. In half of the pots cultivation was imitated in winter of each year. Three replicates of each treatment were placed on the field. A) Pots established in summer 1998 B) in summer 1999 and C) in summer 2000.

Baldomar

The overall germination period of *P. rhoeas* in Baldomar of the first experiment was a bit longer in spring than in Algayón namely between September and April (Figures 4.5. and 4.6.).



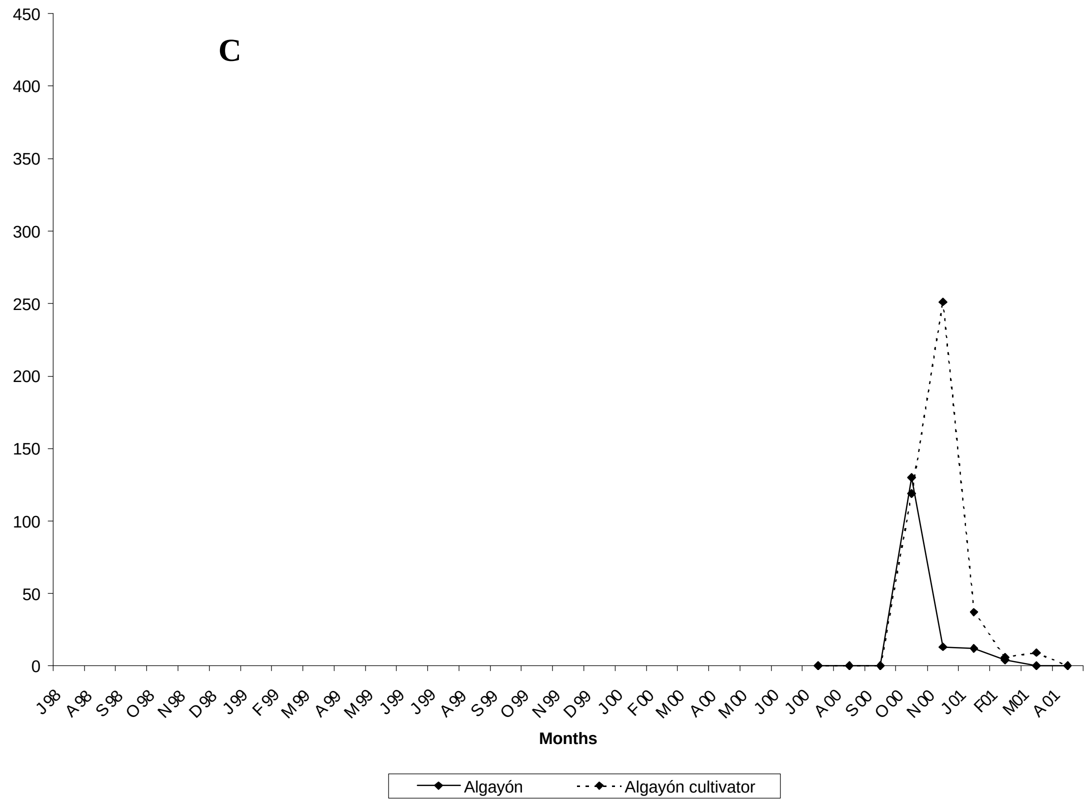
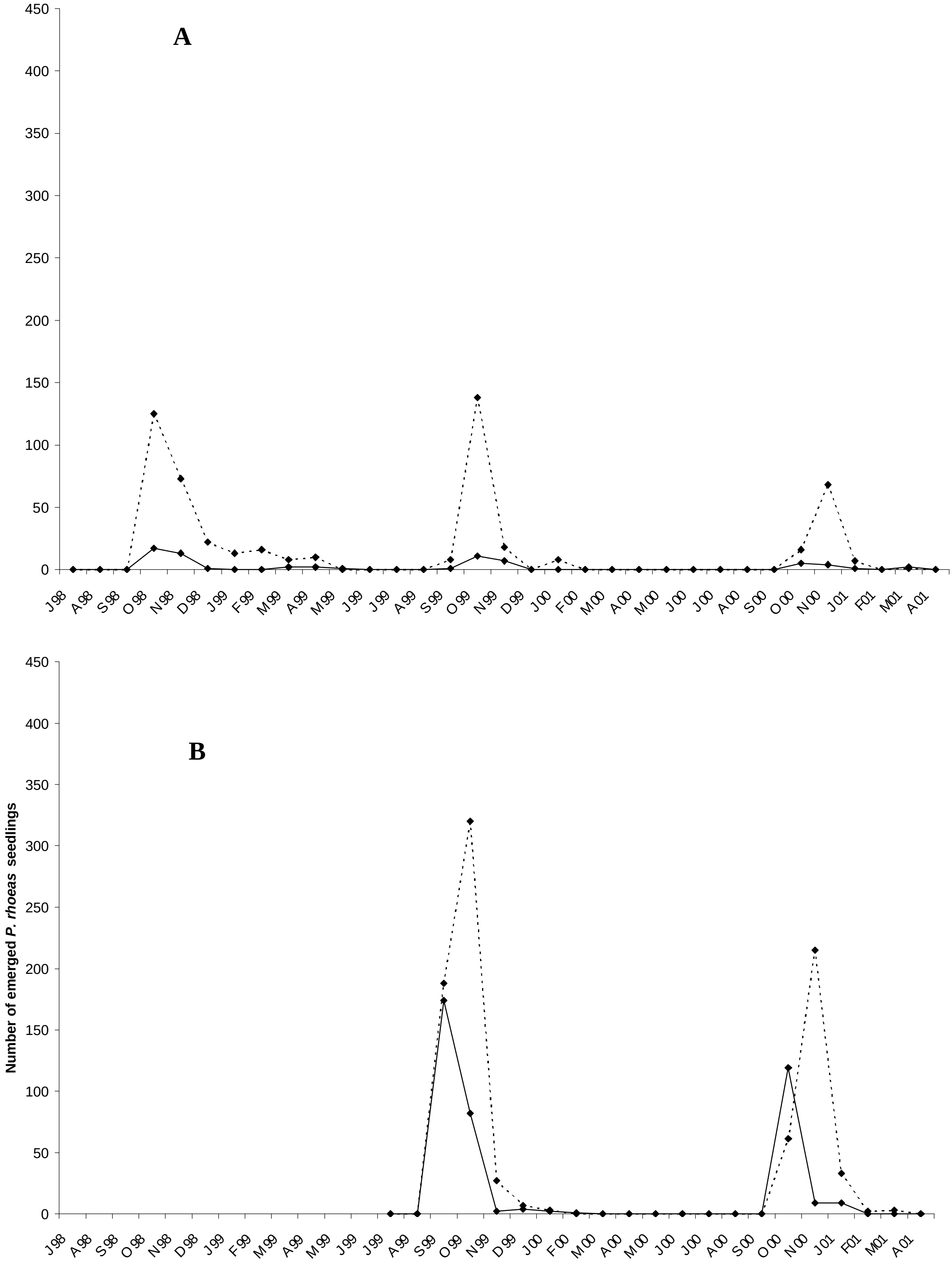


Figure 4.5. Germination of *Papaver rhoeas* seeds coming from Algayón sown in Baldomar. This population was susceptible to herbicides. 1000 seeds were sown in each pot containing weed-free soil. In half of the pots cultivation was imitated in winter of each year. Three replicates of each treatment were placed on the field. A) Pots established in summer 1998 B) in summer 1999 and C) in summer 2000.

In 1998, emergence was maximum in October in all cases. In 1999, main germination was recorded in October for older seeds and between September and October for freshly placed seeds depending on the cultivation treatments (Figures 4.5. b and 4.6. b). In 2000, maximum germination was recorded in October for the non-cultivated seed lots and in November for the cultivated pots (Figures 4.5. and 4.6.).



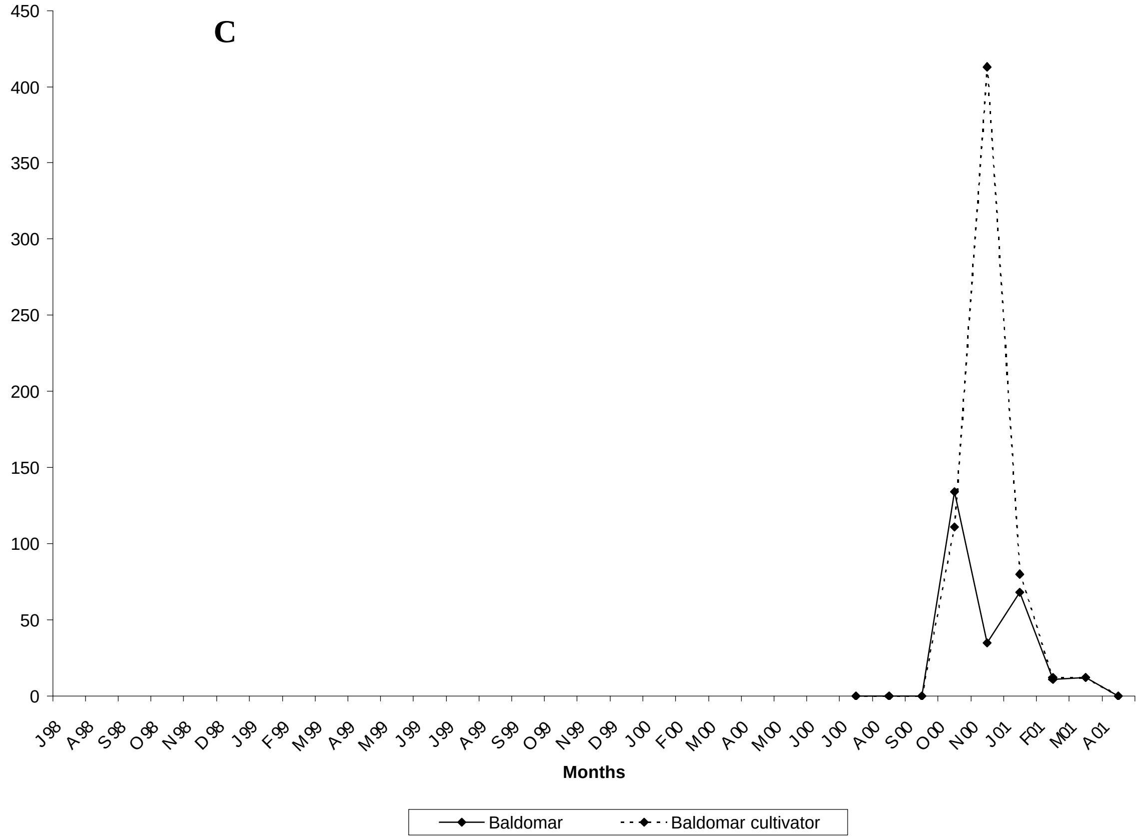


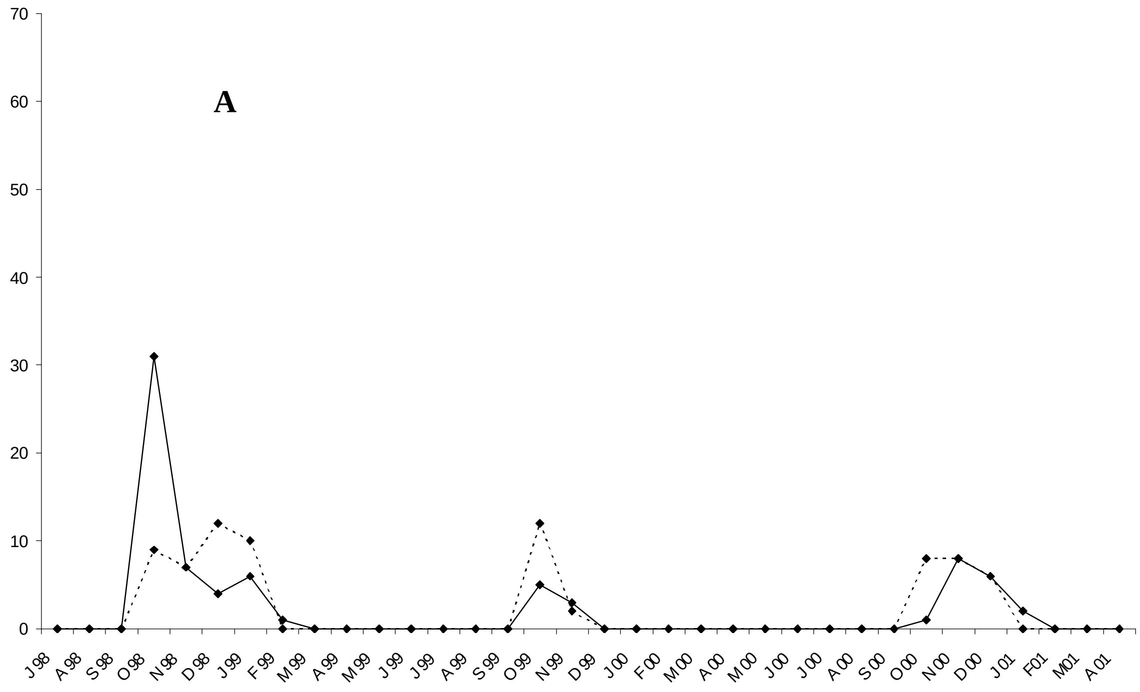
Figure 4.6. Germination of *Papaver rhoeas* seeds coming from Baldomar sown in Baldomar. This population was resistant to tribenuron-methyl and to 2.4-D. 1000 seeds were sown in each pot containing weed-free soil. In half of the pots cultivation was imitated in winter of each year. Three replicates of each treatment were placed on the field. A) Pots established in summer 1998 B) in summer 1999 and C) in summer 2000.

Germination occurred between October and April in the cropping season 1998-99, between September and January in 1999-00 and from October to March in 2000-01. Opposite to the behaviour of some samples in Algayón, in some cases the germination period was longer for the tilled seed lots (Figure 4.5. a, 4.5. b and 4.6. a). This could be observed for seeds of Algayón in one occasion and of seeds from Baldomar in two occasions. The population of Baldomar germinated in a wider range than Algayón in two cases only (Figure 4.5. a and 4.6. a).

Slight differences in germination periods of *P. rhoeas* were thus found depending on the sowing year, the tillage treatment and the population's origin. More important differences were caused by the cropping season and by the location (Figure 4.1. a, b), which refers also to climatic conditions as the soil used was the same in both locations. The main difference was a longer germination period in Baldomar than in Algayón in two out of the three cropping seasons.

Second experiment with seeds collected from the field soil

In Algayón, the germination period was a bit shorter in this second experiment, in which soil with different aged seeds was placed in the pots. It ranged between October and February, only (Figures 4.7. a, b, c). Main germination peaks were recorded in October or December for the non-cultivated and cultivated pots established in 1998, respectively. In 1999, main germination was recorded in October regardless if the seeds were one year old or freshly sown. In 2000 main germination occurred in October and November for the cultivated and non-cultivated pots established in 1998, respectively, and in November for all the other cases (Figures 4.7. a, b, c).



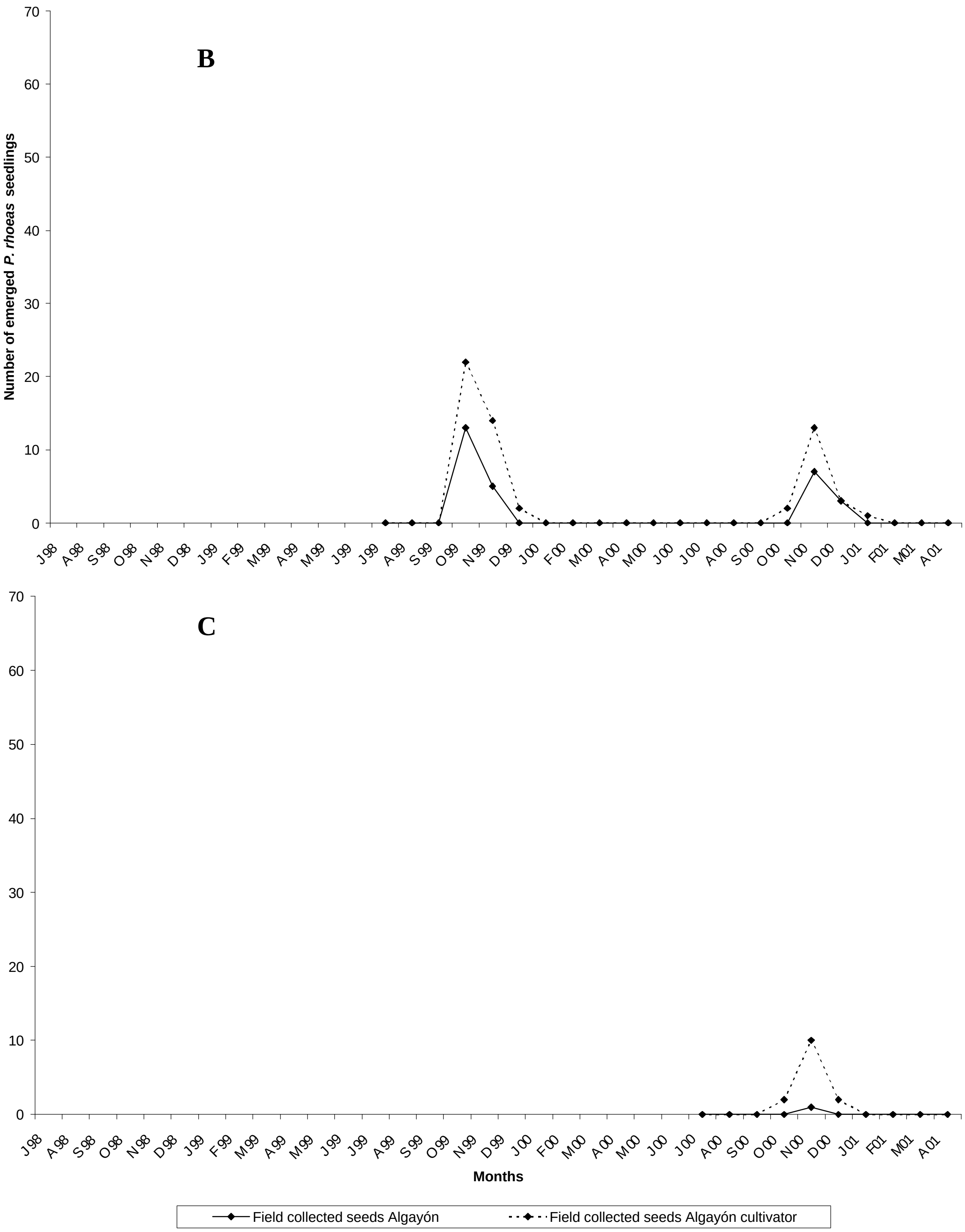
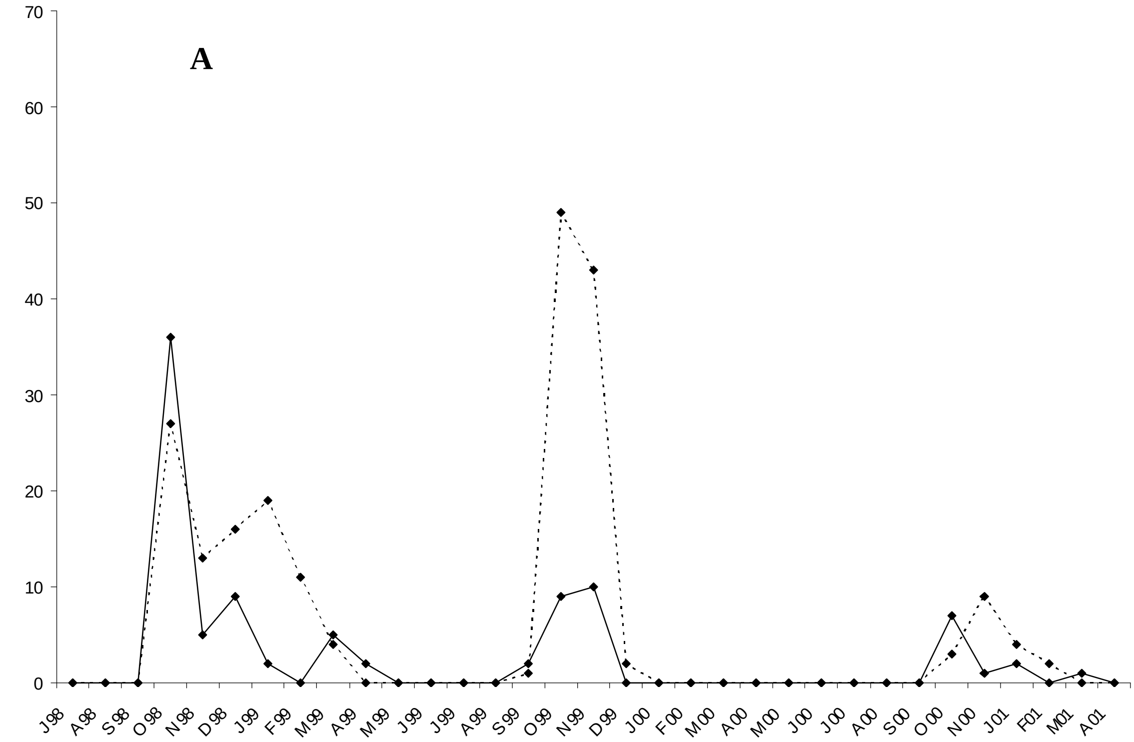
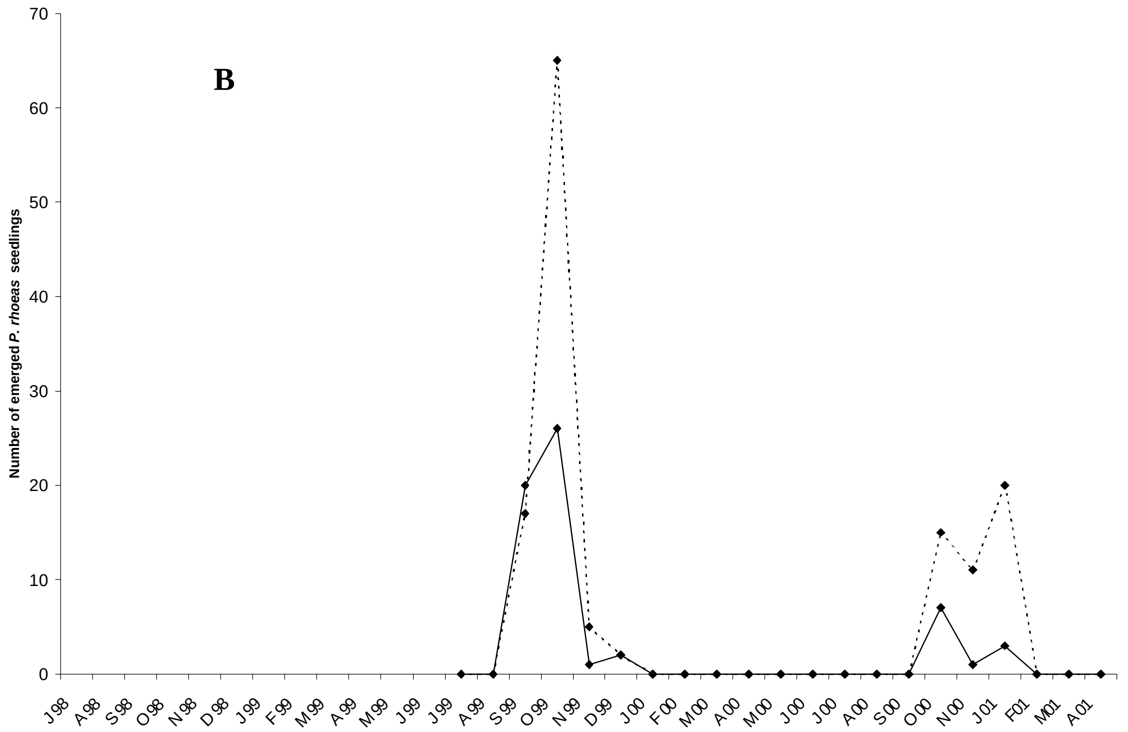


Figure 4.7. Germination of *Papaver rhoeas* seeds coming from Algayón sown in Algayón. Pots were filled with superficial soil of the field containing an unknown number of seeds. This population was susceptible to herbicides. In half of the pots cultivation was imitated in winter of each year. Three replicates of each treatment were placed on the field. A) Pots established in summer 1998 B) in summer 1999 and C) in summer 2000.

In Baldomar, the germination period was the same in the second experiment than in the first one (Figures 4.8. a, b, c). The behaviour of a mixture of different aged seeds was the same than of one-, two- and three-year old seed lots. In 1998, main germination peaks were observed in October. With the exception of the maximum for the non-cultivated pots established in 1998 in November, main germination found in 1999 was also in October. In winter 2000-01 highest germination peaks were recorded in October for the non-cultivated pots regardless of the seed age. In the cultivated pots maximum values was found two times in November and one time in January depending on the seed age (Figure 4.8. a, b, c).





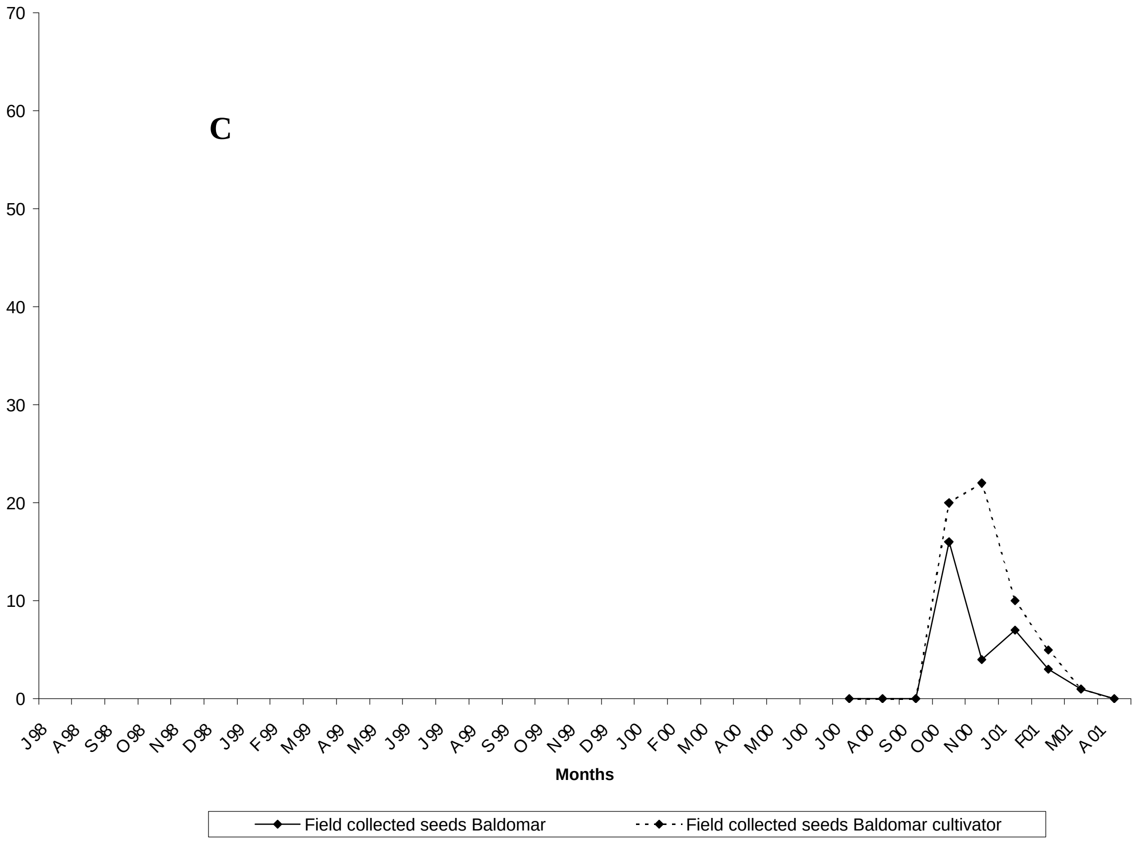


Figure 4.8. Germination of *Papaver rhoeas* seeds coming from Baldomar sown in Baldomar. Pots were filled with superficial soil of the field containing an unknown number of seeds. This population was susceptible to herbicides. In half of the pots cultivation was imitated in winter of each year. Three replicates of each treatment were placed on the field. A) Pots established in summer 1998 B) in summer 1999 and C) in summer 2000.

These data show that *P. rhoeas* has an autumn and winter germination pattern. Only slight differences were found depending on the population's origin, the experimental location, the cultivation treatment and the age of the seeds.

The different sites, annual climatic conditions, cultivation practices and other parameters may influence the exact monthly distribution of germination. Izquierdo and Recasens (1992) found germination of *P. rhoeas* in North-eastern Spain between September and December, only.

Holm *et al*. (1997) quote Roberts & Boddrell (1984) who describe that germination of *P. rhoeas* in British conditions occur mainly from February to April with a secondary flush in August to October. The differences with the behaviour observed in the present experiments in the first period are probably due to the lower temperatures in Britain, delaying germination compared to Spanish conditions.

Only a single flush was observed in the North-eastern Spanish conditions. As *P. rhoeas* occurs mainly in cereal fields (McNaughton and Harper, 1964), this is maybe due to the different cropping systems in both countries, as almost no spring-sown cereal is cultivated in Spain in contrast to Britain.

Quantification of the germination

Algayón

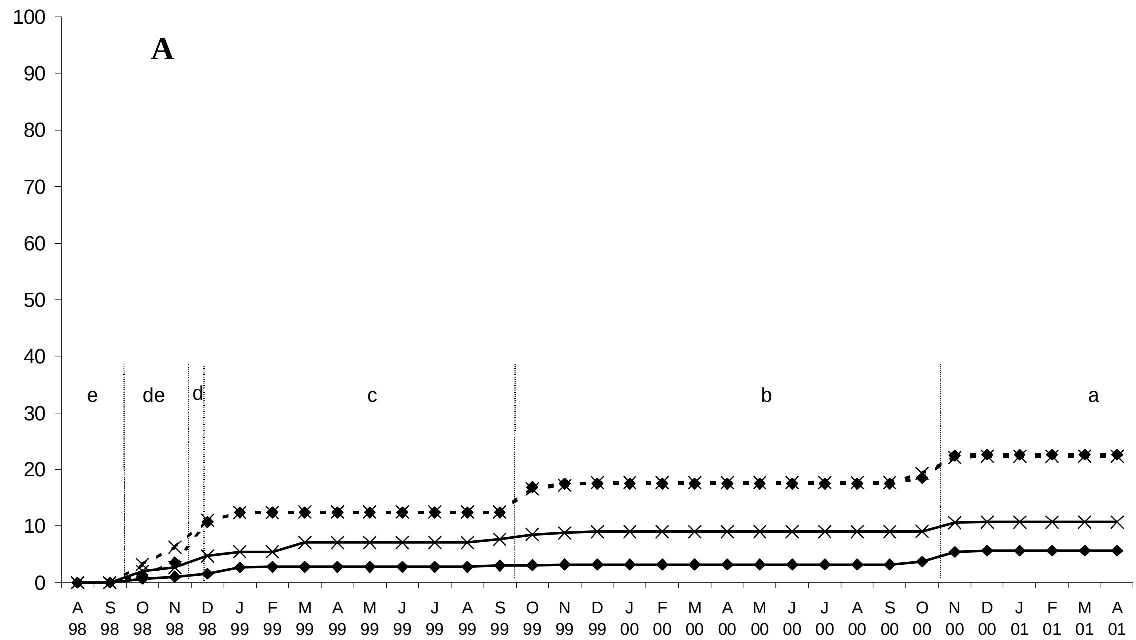
Differences in the percentage of germination were detected between the different years. In general, germination was higher in the pots established in 2000 than in 1999 and much inferior in the 1998 pots (Figures 4.7. a, b, c). After the first germination flush in the first winter of the seeds sown in 1998, the maximum germination reached about 12% for the cultivated pots of both populations (Figure 4.7. a). In the 1999 established pots this value was between 21 and 26% depending on the population's origin (Figure 4.7. b) and between 32 and 33% for the seeds sown out in 2000 also depending on the population's origin (Figure 4.7. c). In the pots established in 1998 germination continued sparse in the following years in spite of the high initial seed viability.

Final germination 33 months after establishing the pots ranged between 6 and 22% depending on the cultivation treatment and on the population's origin (Figure 4.7. a). In the pots established in 1999 final germination in April 2001 reached between 16 and 34% 21 months after sowing (Figure 4.7. b). In the pots placed in 2000 the germination reached between 21 and 33% already 9 months after burial (Figure 4.7. c). As initial seed viability was high for all cases and the soil was also the same between the years, these differences were probably caused by the climatic conditions.

No big germination response, however, was found in winter 1999 and 2000 in the pots established in 1998. Thus, the combination of fresh seeds and good climatic conditions seemed to favour germination. Older seeds may have started a secondary dormancy period, which could be more difficult to break than fresh seeds in primary or even without dormancy. Apparently, if the climatic conditions didn't favour emergence like in year 1998 immediately after sowing, germination was very low and gradual even in the following seasons in spite of good climatic conditions.

Seed age

Figures 4.9., 4.10, 4.11. show the amount of germination during the different months.



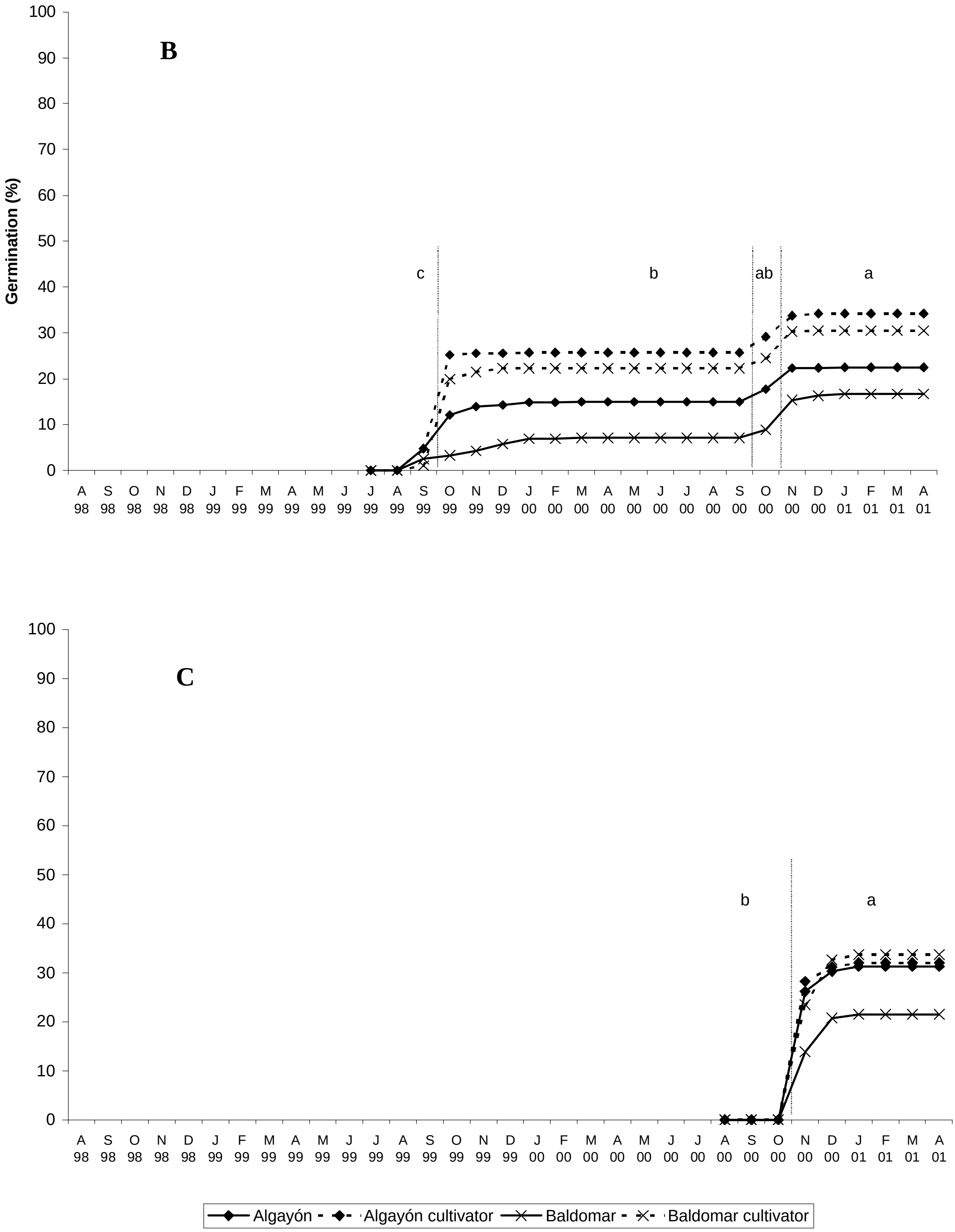


Figure 4.9. Accumulated germination of two *Papaver rhoeas* populations from Algayón and from Baldomar in Algayón. 1000 seeds were sown in each pot containing weed-free soil. Seeds from Algayón were susceptible to herbicides. The population from Baldomar was resistant to tribenuron-methyl and to 2.4-D. In half of the pots the soil was stirred in winter imitating cultivation. A) Seed lots were sown out in summer 1998 B) in summer 1999 C) in summer 2000. Different letters mean statistical significant differences between months following the Duncan mean separation test with $P<0.05$.

In the two cases in which germination evolution was assessed during more than one year it was observed that germination increases in the second winter were less than in the first winter (Figures 4.9. a, b). Probably this was due to second dormancy processes, impeding older seeds to germinate. This would assure a gradual weed emergence by providing the soil seed bank always with some dormant seeds. Also possible depredation or rotting could have affected the remaining seeds.

Cultivation

Cultivation clearly stimulated germination. Statistically significant differences with $P<0.05$ were obtained for both populations. No significant differences were detected in the months with few accumulated germination i.e. August since November 1998 for the pots established in 1998, and August and September 1999 for the pots established in 1999. In the pots established in summer 2000, tillage stimulated germination only in the Baldomar population but didn't increase germination in the Algayón seeds. Exactly these pots were the ones, which had to be re-sown in September. The environmental influence on the seeds on the field was therefor shorter and different in these pots, influencing probably the results.

Population's origin

Referring to the population's origin, statistically significant differences in germination were found in all three sequences. In the pots established in 1998, the Baldomar population germinated more than the Algayón population but only when the non-cultivated pots were compared. Under cultivation the germination rates were not different. In the pots established in 1999 the Algayón population germinated more. In the pots placed in 2000 also the Algayón population germinated more but comparing again only the non-cultivated pots. The seed weights of the Algayón and Baldomar populations over the year were probably not related with these responses as they exactly weighed more when they germinated less (Table 4.1.).

The months in which more germination was recorded each year were October, November and December. If means of emergence were separated from month to month with the Duncan means separation test at $P<0.05$, very clear groups resulted (Figures 4.9. a, b, c). These divisions clearly reflected the emergence periods.

Baldomar

Also in Baldomar, in general less germination was recorded in the pots established in 1998 than in the pots placed in 1999 and in 2000 (Figures 4.10. a, b, c). In this case, this affected mainly the non-cultivated seeds, which germinated much more in the pots established in 1999 and in 2000 than in the 1998 established pots.

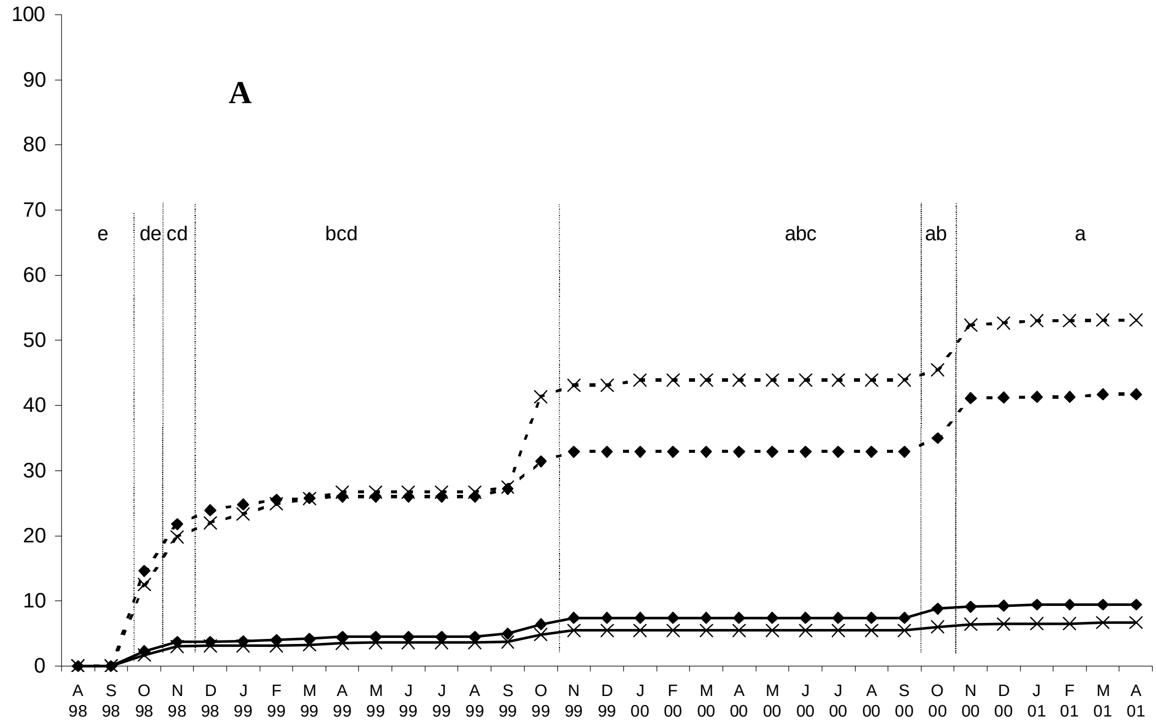
In the cultivated pots a quite high germination rate was observed even for the pots placed in 1998. After the first winter maximum germination was 26% for the pots sown in 1998 (in the cultivated treatment of both populations), 54-62% for the cultivated pots established in 1999 depending on the population's origin and 42-63% for the cultivated pots placed in 2000, also depending on the population's origin (Figures 4.10. a, b, c). In the pots established in 1998, 33 months after burial the germination in April 2001 was between 7 and 53%, only (Figure 4.10. a). In the pots established in 1999 final germination 21 months after sowing was between 40 and 86%. And in the seed lots sown out in 2000, germination between 16 and 63% was recorded 9 months after establishment.

As in Algayón, an important factor for stimulating germination was probably the combination of environmental conditions and the seed age. So, germination was very high in autumn 1999 only especially for the new-sown seeds and not so much for the older seeds, which had already been in the soil for one year.

Apparently, if germination was favoured in one first year, soil seed bank depletion of *P. rhoeas* can be very fast, as observed with the up to 86% germination of a cultivated population 21 months after placement.

Seed age

As in Algayón, seed emergence was generally less in the second or third year compared to the germination in the first winter both in the cultivated and in the undisturbed pots (Figures 4.10. a, b).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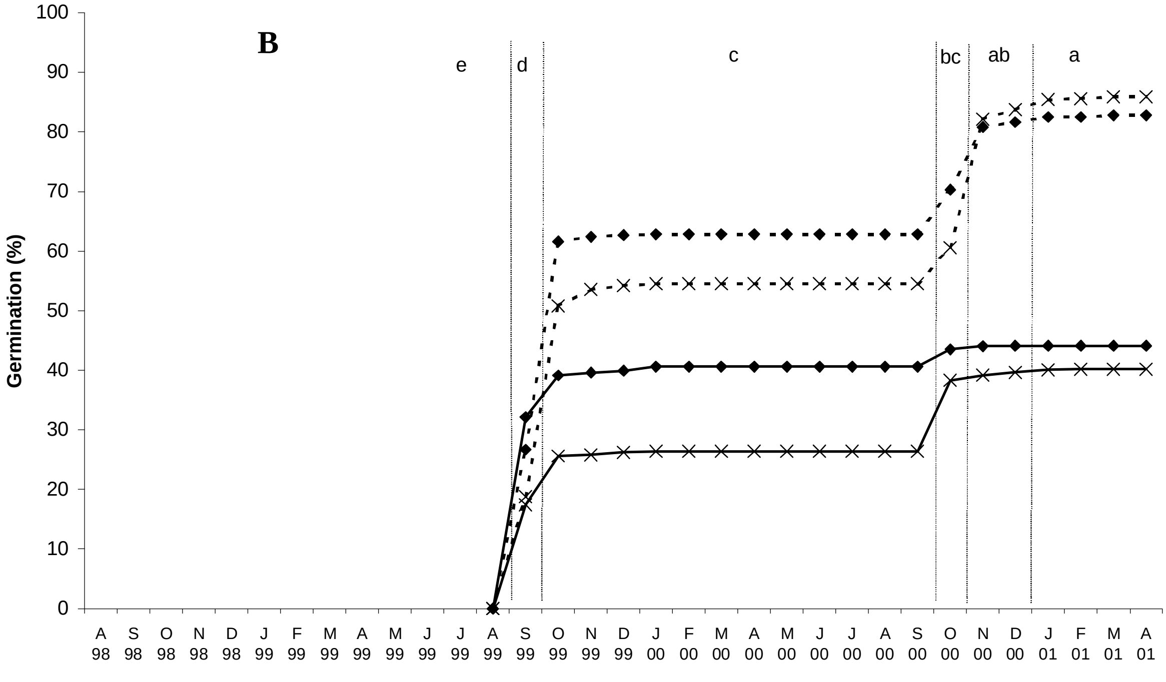
B
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d
c
bc
ab
a
Germination (%)
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90
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70
60
50
40
30
20
10
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A 98
S 98
O 98
N 98
D 98
J 99
F 99
M 99
A 99
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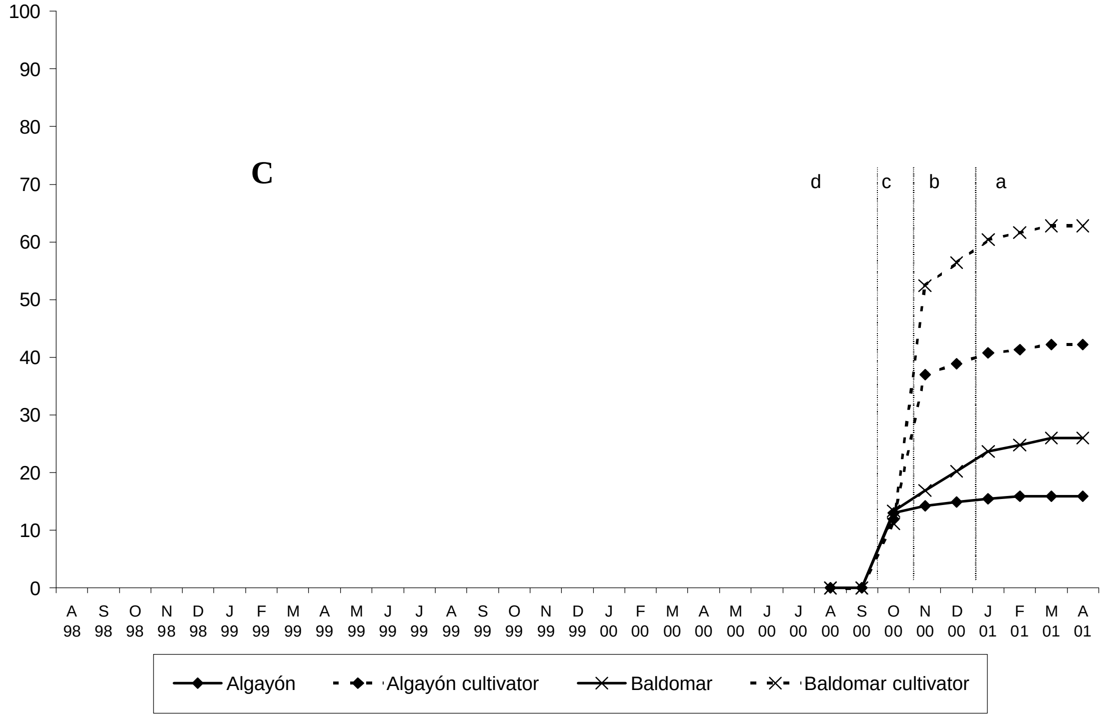


Figure 4.10. Accumulated germination of two *Papaver rhoeas* populations from Algayón and from Baldomar in Baldomar. 1000 seeds were sown in each pot containing weed-free soil. Seeds from Algayón were susceptible to herbicides. The population from Baldomar was resistant to tribenuron-methyl and to 2.4-D. In half of the pots the soil was stirred in winter imitating cultivation. A) Seed lots were sown out in summer 1998 B) in summer 1999 C) in summer 2000. Different letters mean statistical significant differences between months following the Duncan mean separation test with P<0.05.

Cultivation

Apart from the first months after establishing the experiment, cultivation clearly stimulated *P. rhoeas* germination in the other months causing statistically significant differences. The exceptions were August since October 1998 for the first lots, August and September 1999 for the second lots and August since October 2000 for the pots established in 2000.

In the undisturbed pots sown in 1998 *P. rhoeas* germinated very little in all three years as it happened in Algayón. This observation is consistent with the findings of Roberts & Feast (1972) who recorded only a fifth of germination for *P. rhoeas* in undisturbed soil compared to cultivated soil. The seeds in undisturbed pots sown in 1999, however, germinated very much exceeding even 40% after two cropping seasons (Figure 4.10. b). Germination in the pots established in 2000 was in between the other two years for the undisturbed pots. Differences between cultivated and undisturbed samples were, thus, very high in the 1998 sown seeds but much less in the 1999 sown seeds due to high germination in the undisturbed samples.

Population's origin

Independently of the cultivation treatments, germination of the seeds collected in Baldomar germinated more than the seeds collected in Algayón in the pots established in 1998 and in 2000. On the other hand, the seeds from Algayón germinated more in the pots placed in 1999.

Monthly divisions for the 1998 established pots following the means separation of the Duncan test (P<0.05) gave less clear categories than in the case of Algayón (Figures 4.5. a, b, c). This was probably due to a smaller germination increase in the second and third winter than in Algayón (Figure 4.5. a). Nevertheless, still the main divisions reflect clearly the *P. rhoeas* emergence periods.

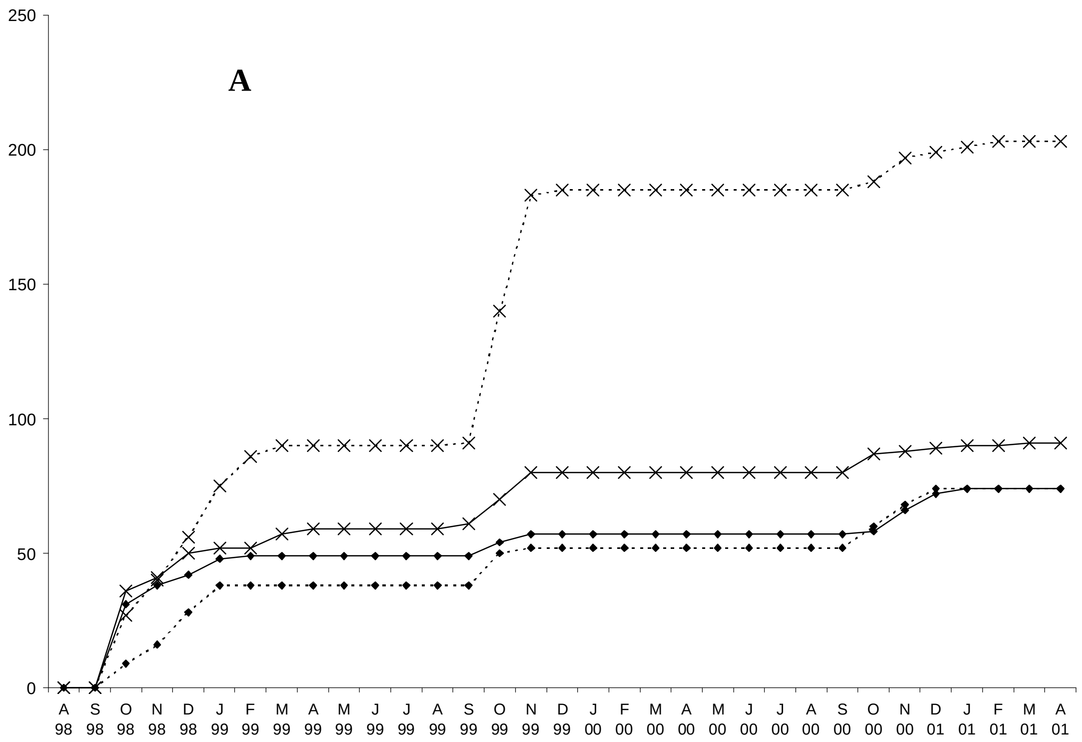
Comparison between locations

As the soil placed into the pots and the sown seed samples were the same, the only possible factors causing differences in germination were of climatic origin. Much higher germination rates were generally recorded in Baldomar than in Algayón (Figures 4.9. and 4.10.). The exception was the experiment placed in Algayón in 2000 which probably is not comparable to the others due to the re-sowing done in September. These differences between locations correlate with the fact that in the Baldomar area the *P. rhoeas* problems are generalised and enhanced even more by the growing herbicide resistance. In Algayón, *P. rhoeas* emergence is much more irregular depending on the year.

In each location, the two populations behaved very similar. Nevertheless, some differences were detected depending on the sowing year. These differences, however, were less than the differences caused by the location. Thus, their germination behaviour depended more on the location conditions than on the seed origin.

Second experiment with seeds collected from the field soil

As the initial seed number was unknown in the pots containing the field collected seeds, accumulative germination was analysed (Figure 4.11. a, b, c). In opposition to the observations made in the previous experiment, in Algayón generally more germination was observed in the pots established in 1998 and 2000 than in 1999 after the first winter. In Baldomar, almost the same amount of seeds germinated in all three years in the non-cultivated pots but more seeds germinated in the cultivated pots established in 2000 (Figure 4.11. a, b, c).



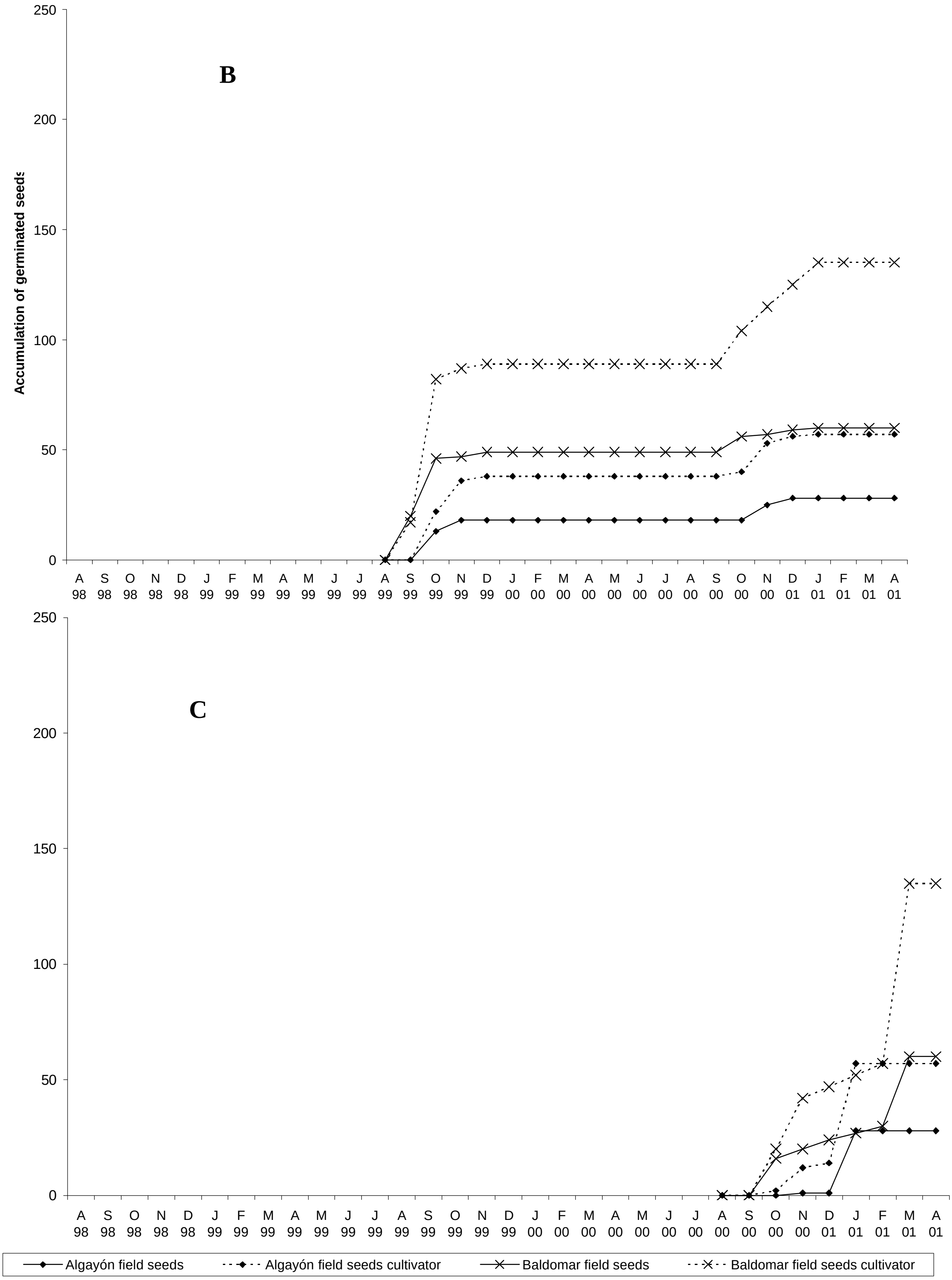


Figure 4.11. Accumulated germination of two *Papaver rhoeas* populations from Algayón and from Baldomar placed in the respective location, only. Pots were filled with superficial soil of the field containing an unknown number of seeds. Seeds from Algayón were susceptible to herbicides. The population from Baldomar was resistant to tribenuron-methyl and to 2.4-D. In half of the pots the soil was stirred in winter imitating cultivation. A) Seed lots were sown out in summer 1998 B) in summer 1999 C) in summer 2000.

As in the previous experiment, tillage favoured germination in Algayón and in Baldomar causing statistically significant differences with the exception of the pots placed in Algayón in 1998, in which more seeds germinated in the non-cultivated samples.

Germination was generally higher in the first winter than in the following ones (Figure 4.11. a, b). In the pots placed in Baldomar in 1998, however, germination in the cultivated pots in the second winter was almost as high as in the first one, but decreasing again in the third winter. All these differences in germination quantity, however, could also be caused by an possibly irregular amount of *P. rhoeas* seeds in each soil sample.

As the results indicate, mainly depending on the environmental conditions in the first year after being incorporated into the soil, *P. rhoeas* seeds can have very different emergence responses in the subsequent years. Germination in the first year seems to be the key for exhausting the seed bank. Seeds can germinate very quickly already in the first winter causing big infestations and reducing the seed bank very fast if seed rain is prevented. If conditions are not very favourable the first season, the germination can be much more staggered and the seed bank reduced very slowly, guaranteeing a certain yearly emergence. This second case of germination habit is consistent with the observation made by McNaughton and Harper (1964) who describe that *P. rhoeas* seeds show an intermittent and long drawn out sequence of seedling emergence. Holm *et al.* (1997) quote the results of Roberts & Boddrell (1984) who found 21% of the seedling emergence of all the *P. rhoeas* seeds sown the first year in United Kingdom. Only 1 to 7% of the remaining seeds emerged over the next 3 years accounting for 38% of the seeds. This indicates a slow depletion of the seed bank starting with similar germination percentage the first year as found in not very favourable years in the present study. In an experiment conducted by Roberts and Feast (1972) it was observed that although germination of *P. rhoeas* was the greatest during the first year, there was still an appreciable emergence in the second year. In our case, this behaviour was observed in Baldomar in the 1999 and 2000 established pots. In the 1998 established pots and in the Algayón location *P. rhoeas* emergence was more gradual and similar between the first, second and third year after burial.

No important differences in the germination behaviour were recorded between the two populations when sown out in the same site. Apparently neither the different origin nor their different susceptibility towards tribenuron-methyl and 2.4-D herbicides caused big differences. Their behaviour seemed to depend more on the location's characteristics than on their origin.

Following the review done by Saari et al., (1994) in some cases faster germination in ALS-resistant *Lactuca serriola* seeds was observed, although the general trend observed in ALS-resistant weeds was no fitness penalty nor differences in biological behaviour. Boutsalis and Powles (1998) compared the germination pattern of ALS-herbicide-resistant *Sisymbrium orientale* towards a susceptible biotype. No differences during the 3-years period were observed between these two populations either. These data are, thus, consistent with the present findings.

Coupland (1994) reviewed the resistance to 2.4-D quoting fitness penalty on herbicide resistance on 2.4-D only for *Sinapis arvensis* but not for *Daucus carota, Carduus nutans, Stellaria media, Ranaunculus acris, Convolvulus arvensis* nor for *Matricaria perforata*. It is thus probably that a possible difference in any characteristics between the two studied *P. rhoeas* populations is not related to their distinct susceptibility towards herbicides.

The observations of germination stimulation by cultivation is also consistent with the observations of other scientists. Mohler and Galford (1997) conducted experiments burying seeds of *Amaranthus retroflexus, Chenopodium album* and *Abutilon theophrasti* in different depths simulating in some cases soil tillage. In most of the cases, germination was higher in the tilled than in the undisturbed treatments. Also Boutsalis and Powles (1998) found that cultivation stimulated seedling emergence in ALS-herbicide resistant and susceptible *Sisymbrium orientale* populations. Germination was increased three- to six-fold in *Setaria faberii, Amaranthus retroflexus* and *Chenopodium album* in a no-tillage cropping system when enough rainfall was recorded (Mulugeta & Stoltenberg, 1997).

Roberts & Feast (1972) studied survival and emergence of different weed species depending on the burial depth. At the upper layer of 2.5 cm which corresponds to the present experimental design 4% of the initially buried *P. rhoeas* seeds were recovered in cultivated and 8% in undisturbed conditions after five years of burial. Compared to other weed species, the authors found these values of recovered seeds for *P. rhoeas* very low. Provided that the initial germination capacity in that experiment was high, the losses were due to rotting or pathogen attack on the seeds or due to a germination failure. Probably, this reduction of the viable *P. rhoeas* seeds affected also the present pot experiment and may partially explain the reduction in germination of the seeds during the second and third year.

Plant survival in field conditions

Baldomar

The same field named as 1 was assessed during the cropping seasons 1997-98, 1999-00 and 2000-2001. During 1998-99, plant evolution in the neighbour field called 2 was analysed (Table 4.2.). The initial *P. rhoeas* seed bank was very high in both fields, after several years of lack of control of the herbicide resistant weed population. Comparing *P. rhoeas* plant number in the different years in field 1, the density was statistically higher in 1997-98 during the whole cropping season than in any other year (Figure 4.12.). Also more precipitation was recorded in that cropping season (Table 4.3.), probably favouring emergence. Density in field 2 was much more similar to the values observed in field 1 during 1999-00 and 2000-01. Slight differences were detected when two experiments were conducted in the same field demonstrating little spatial variation of *P. rhoeas* density inside the field (Figure 4.12.).

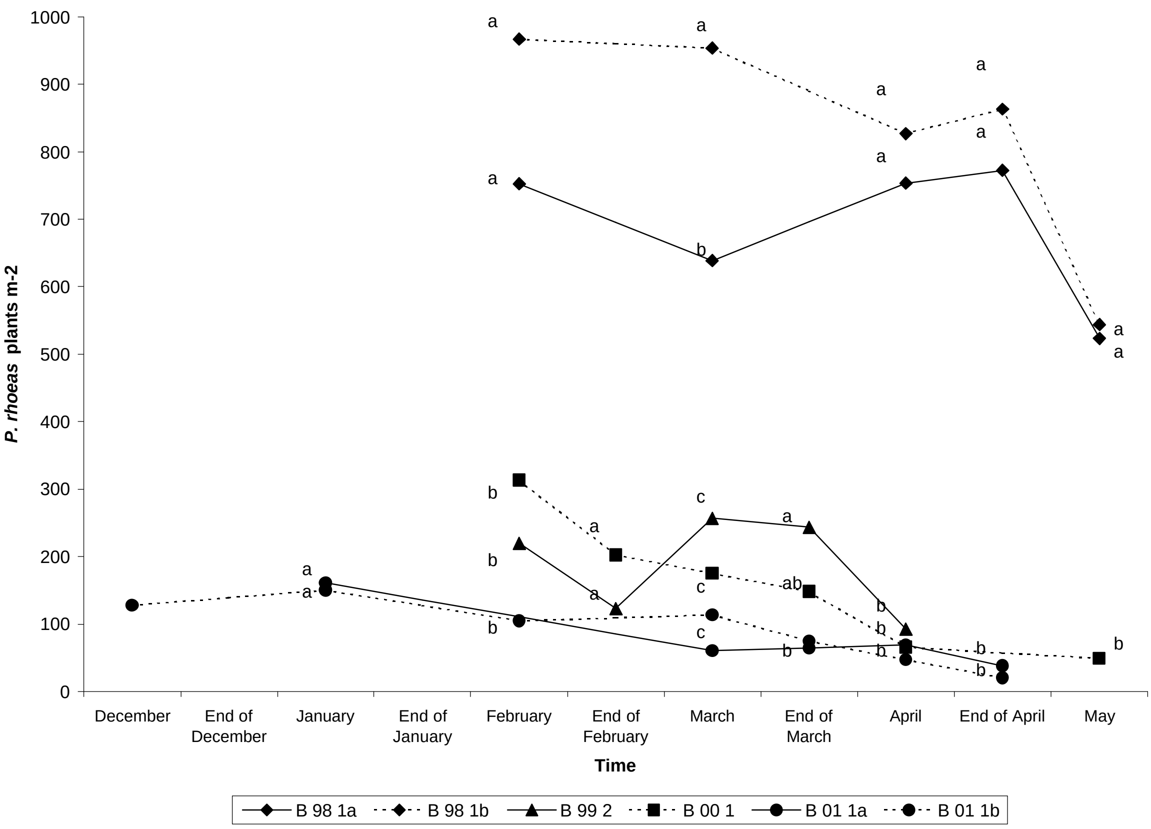


Figure 4.12. Evolution of *Papaver rhoeas* plant number per m^2 in Baldomar during the cropping seasons 1997-98, 1998-99, 1999-00 and 2000-01 in untreated plots. Three counts in three plots were conducted in 0.1 m^2 frames at each assessment date. Different letters indicate statistical significant differences with P<0.05.

Plant germination generally stopped in February. The only statistically significant increase in plant number afterwards was recorded in 1998-99 between end of February and March. Other slight increases e.g. between March and end of March in 1997-98 were not statistically significant.

In 1997-98 a first decrease in plant number occurred in February and continued after end of April. In 1998-99 the plant mortality started to be clear in March. In 1999-00 the decrease in *P. rhoeas* population started already in February. In 2000-01 there was also a gradual decrease since end of January. In all cases, the most important plant number decrease was recorded before April. No clear relationship between the beginning of mortality and the precipitation could be found.

Final weed density was observed to vary between 20 and 860 plants m^{-2} even if in most cases the level was under 70 plants m^{-2}.

Plant number decrease caused by natural plant mortality throughout the cropping season was found to be statistically different in some cases only: in 1998-99 plant decrease in February and between end of March and April were statistically significant. The decrease in plant number in 1999-00, was gradual so that significant differences were observed only when density was compared skipping one count. In 2000-01 at field number 2, the only statistically different value was the first assessment towards the rest. In the other three experiments the decreases were not statistically relevant in any case.

Torrelameu

In this location, two different parts of one field was studied during two cropping seasons (Table 4.2.). Plant density was very similar between replicates and also comparing the two years (Figure 4.13.). Statistically significant differences were found at the beginning of the cropping season, only. These differences diminished in time due to plant mortality. The plant number decrease continued throughout the year since the last assessments in April. No effective weed control was achieved in 1999-00 in the field so that the seed bank was not reduced. Nevertheless, significantly less germination occurred in 1999-00 compared to 1998-99 but finishing with a very similar weed density. *P. rhoeas* density gradually decreased since February, with the exception of the statistically non-significant increase in field trial a in 1999-00 between February and March. Plant number reduction due to mortality was statistically important in 1998-99, comparing the density between February and April in field b and comparing counts between March and April or later or comparing density at end of March with end of April in field a.

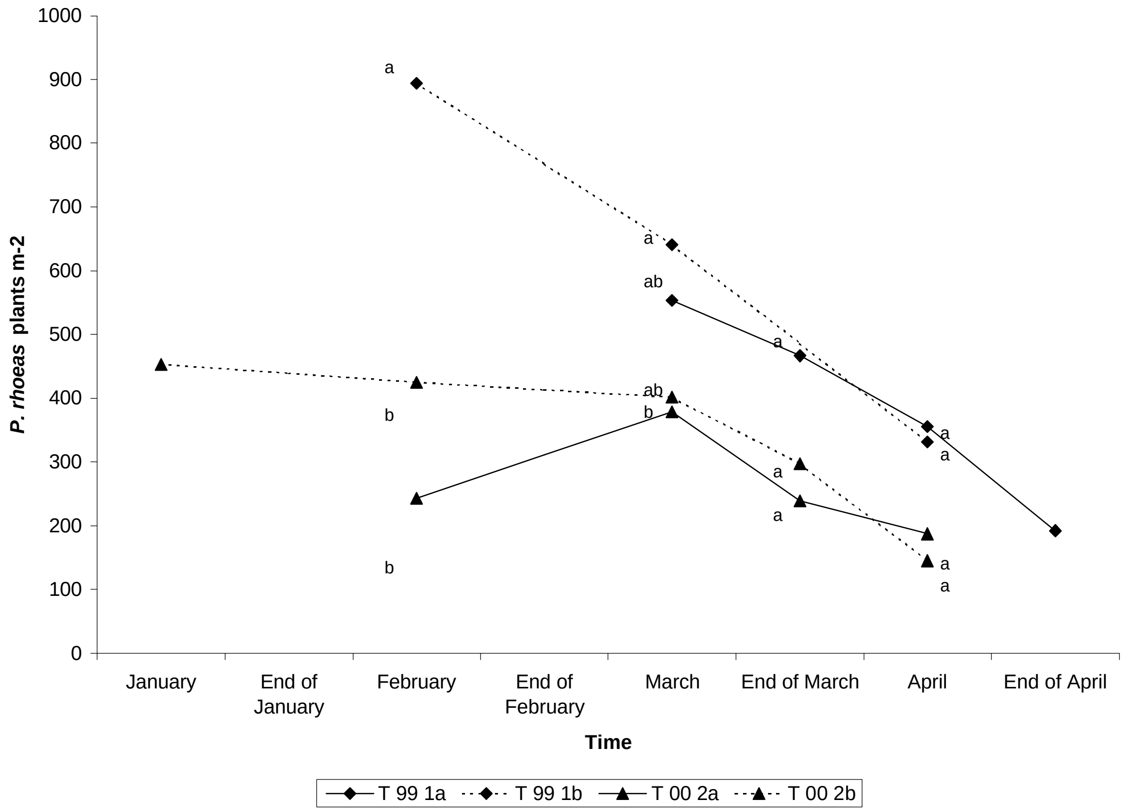


Figure 4.13. Evolution of *Papaver rhoeas* plant number per m^2 in Torrelameu during the cropping seasons 1998-99 and 1999-00 in untreated plots. Three counts in three plots were conducted in 0.1 m^2 frames at each assessment date. Different letters indicate statistical significant differences with P<0.05.

Nalec

Initial plant number was lower compared to some other locations in some years (Figure 4.14.) even if also in this field control problems due to herbicide resistance had been severe during previous years. Statistical significant differences of density between years were found in March only (Figure 4.14.). The tendency of plant number evolution was a continuous decrease since February until the end of the assessments in April. Neither the plant number decreases nor the increases were statistically significant in none of the field trials. This was probably due to the relatively low initial plant number.

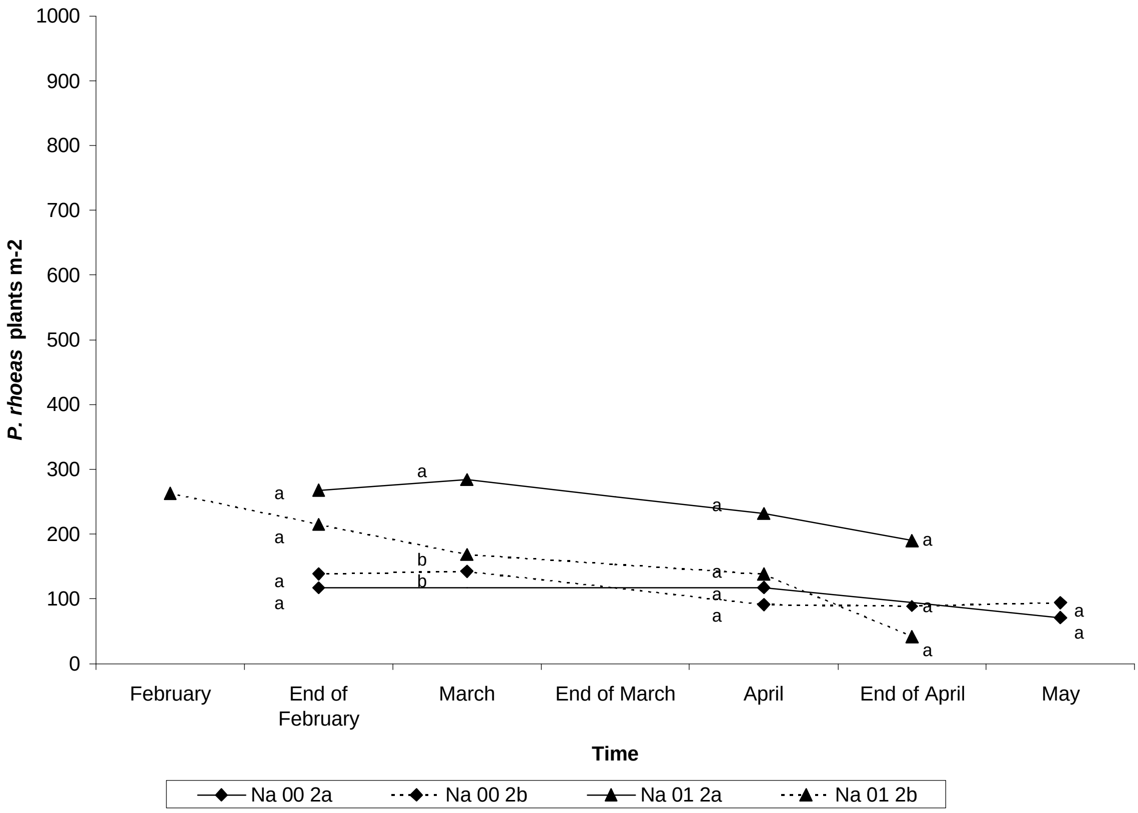


Figure 4.14. Evolution of *Papaver rhoeas* plant number per m^2 in Nalec during the cropping seasons 1999-00 and 2000-01 in untreated plots. Three counts in three plots were conducted in 0.1 m^2 frames at each assessment date. Different letters indicate statistical significant differences with $P<0.05$.

Other locations

A big variability, especially in initial *P. rhoeas* plant number was found (Figure 4.15.). The biggest initial plant density was recorded in Santa Coloma de Queralt. In the other three cases, very similar densities around 200 plants m^{-2} were described. The decreases in time were statistically significant for the Santa Coloma location between February and end of March and comparing data of end of March with April. At the end of the cropping season, however, natural plant mortality approached the values of the other populations drastically so that in fields with initial low or high plant density the final values were almost the same.

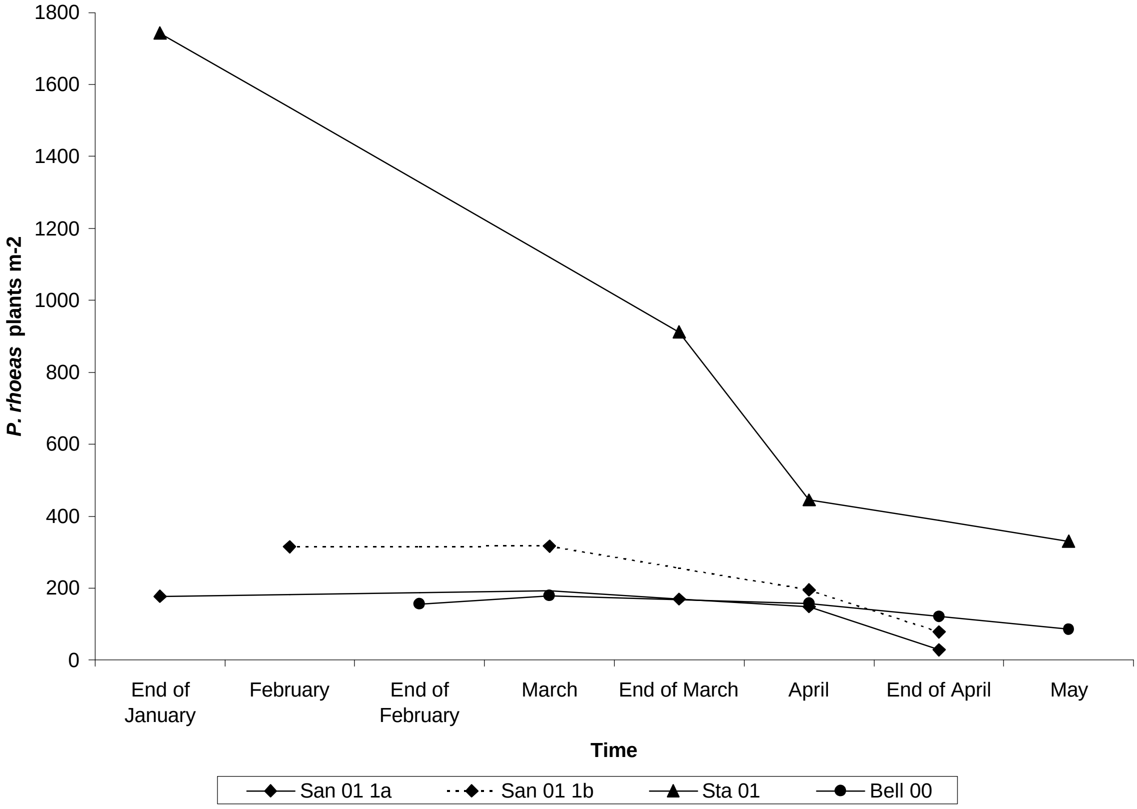


Figure 4.15. Evolution of *Papaver rhoeas* plant number per m^2 in Sanaüja, Santa Coloma de Queralt in the cropping season 2000-01 and in Bellprat during 1999-00. Three counts in three plots were conducted in 0.1 m^2 frames at each assessment date.

Table 4.4. summarises the plant mortality in the different fields calculating the percentage of plant number reduction between the first and the last assessment date.

Plant mortality is probably influenced by numerous factors. Some are the speed of crop establishment and of the crop density in and between rows, which was very fast in Sanaüja 2000-01; unfavourable moisture conditions can also drastically reduce weed density (as observed in Torrelameu in 1999-00); late freezing in spring as observed in 2000-01 in numerous locations can also affect final *P. rhoeas* plant number.

Table 4.4.: Natural mortality (%) in the untreated plots on *Papaver rhoeas* over the cropping period.

Location	Year	Field	Mortality
Baldomar	1998	1a	30.5
	1998	1b	43.8
	1999	2	58.2
	2000	1	84.3
	2001	1a	76.4
	2001	1b	84.4
Torrelameu	1999	1a	65.3
	1999	1b	63.0
	2000	1a	22.6
	2000	1b	70.0
Nalec	2000	1a	45.8
	2000	1b	32.4
	2001	1a	29.1
	2001	1b	84.0
Bellprat	2000	1	44.9
Sta. Coloma de Queralt	2001	1	74.4
Sanaüja	2001	1a	84.3
	2001	1b	77.6

General observations

In most of the field trials initial *P. rhoeas* density of 100 to 300 plants m^{-2} was found. Some exceptions were Baldomar in 1997-88, Sta. Coloma de Queralt in 2000-01 and Torrelameu 1998-99 with much higher initial plant density. In the first two cases this was coincident with higher rainfall records (Table 4.3.). In all cases natural mortality was observed during the cropping season caused probably by inter- and intraspecific competition. Germination generally stopped in February, although in some cases slight increases in plant populations were detected since end of March (B 98 1a, B 99 2, Na 00 2b, Na 01 2a and Bell 00). Final plant density at crop earing ranged between 49 plants m^{-2} and 543 plants m^{-2}.

All kind of behaviour was observed. Some initially very high populations reduced up to quite low *P. rhoeas* density (e.g. in field San 01 1b). Other initially very high populations e.g. B 98 1a and B 98 1b did not decrease so much. Some initially relatively low-level populations as e.g. Na 00 2a and Na 00 2b maintained their population density almost the same while others as San 01 1a decreased even more. Thus, regardless of the initial weed population size bigger and smaller plant density reductions can be expected.

In some of the fields, very high initial and final weed populations were recorded. In these cases, weed control can only be targeted by a combination of several control techniques. The natural mortality could boost the control effect in some cases as it happened in Torrelameu in 1999-00 due to the drought and in Sanaüja in 2000-01 due to the crop competition. Due to all these factors, no prediction towards a possible *P. rhoeas* plant density in time can be made in North-eastern Spain.

Possible practical use of the results for a cultural Papaver rhoeas *management*

Tillage clearly favoured *P. rhoeas* germination. In a fallow condition, probably harrowing would enhance germination favouring the population's reduction. Main germination took place from September to December continuing since April but in a much more irregular and lower extent. As cereal can be sown in North-eastern Spain since the end of winter, delaying sowing since end of January or since February could probably an effective reduction strategy. Delaying sowing two weeks or one month only since late November or early December could probably not be enough as many seeds could still germinate. In these cases, lack of moisture in early winter and early frost could also make crop establishment too difficult with the consequent yield reductions found for this area by Planes *et al.* (1999).

Preparing the seedbed earlier in autumn could also enhance the *P. rhoeas* emergence but without assuring no new germination flushes. Conducting as many tillage operations as possible before sowing probably would promote more *P. rhoeas* germination. In a fallow situation harrowing would also enhance germination. As no important differences in behaviour were detected for a herbicide resistant population compared to a susceptible population, the same control strategy can be chosen for both situations.

On the other hand, if soil is not disturbed any more after sowing the crop, no emergence is probably to be expected in spring in the tested area. Control methods should therefor be developed previously. Natural plant mortality was moderate and even high in many cases. This can have an additional positive influence on the efficacy of any control method.

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