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Characterization of emergence of autumn and spring cohorts of *Galium* spp. in winter cereals

Royo-Esnaol, A., Torra, J., Conesa, J.A. & Recasens, J.

Dpt. Hortofruticultura, Botànica i Jardineria, ETSEA, Universitat de Lleida, 25198, Lleida, Spain

Summary: The emergence of three artificial cohorts of *Galium aparine*, *G. spurium* and *G. tricornutum* with different accessions was studied over two years under the climatic conditions of northeastern Spain. Seeds were sown in November (1st cohort), January (2nd cohort) and February (3rd cohort). Higher and lower emergence percentages were obtained in first and third cohorts respectively, when fresher winter and dry spring occurred, but this tendency changed when the spring became wet and results of third cohorts overcame first cohorts' levels. Results suggest that low temperatures in winter break the dormancy of these species, that soil moisture provokes the germination and emergence of these weeds and that emergence is highly influenced by dormancy level periods during winter and spring. Lack of rain does not allow spring germination, hence, application of herbicides to control these populations is not necessary.

Key words: cohorts, dormancy, *Galium aparine*, *Galium spurium*, *Galium tricornutum*, germination

Introduction

Weeds in winter crops are divided in two general types, namely, strict winter annuals that only germinate in autumn and nonstrict winter annuals that can germinate in spring as well as in autumn (Baskin & Baskin, 1985). *Galium* species that are important in Mediterranean winter cereals, such as *G. aparine* L., *G. spurium* (L.) Simonkai and *G. tricornutum* Dandy, all belong to the second group. Most seeds of these species possess an innate dormancy that is generally lost during summer (Masuda & Washinati, 1992). As a consequence, a large proportion of seeds germinates and emerges in autumn. Seeds that do not germinate in autumn remain in the soil seedbank over winter and may germinate in spring as a second flush (Cussans & Ingle, 1999; Mennan & Ngouajio, 2006). Germination of *G. aparine* in autumn is normally much higher than in spring and should be the focus of control (Cussans & Ingle, 1999). However, the second flush

can be important too as the seeds produced by these plants ensure the persistence of *Galium* populations (Cussans & Ingle, 1999). The weed populations resulting from the two flushes can be considered as cohorts of the same population, but because of biological differences between the two flushes (Froud-Williams, 1985; Van der Weide, 1992), perhaps they should be considered as subpopulations of the same species.

Differences between autumn and spring germinating populations may have a genetic or phenotypic basis. Different ecotypes living in the same place have been offered as an explanation (Masuda & Washinati, 1991), which explains differences in dormancy, need of vernalization or even phenological stages between hedgerow and within-field populations (Froud-Williams, 1985; Nieman, 1988; Van der Weide, 1992). Others found different genotypes and genetic variation within *Galium* species (Hübner *et al.*, 2003; Taylor, 1999, Mitchelson *et al.*, 1995; Froud-Williams, 1985). Maternal effects, understood as information that is passed to the offspring in a non-Mendelian fashion (Baskin & Baskin, 1998), can be a factor for intraspecific variation on seed behaviour (Baskin & Baskin, 1998; Mulugeta & Stoltenberg, 1998; Luzuriaga *et al.*, 2006).

Emergence is considered the most important event in the life of an annual species because the moment of emergence determines subsequent survival and success of the plant (Forcella *et al.*, 2000). Dormancy is the main process that influences seedling emergence (Leon *et al.*, 2004; Forcella *et al.*, 2000), and is therefore crucial for the survival of a plant. Germination and subsequent emergence are influenced by factors both extrinsic (environment, habitat) and intrinsic (genotype, maternal effects, age of seeds) (Leon *et al.*, 2004). Royo-Esnal *et al.* (2010) developed Weibull models that predict the emergence of autumn populations of these *Galium* species. They also observed that the inclusion of moisture, together with temperature (hydrothermal time), appears crucial among the extrinsic factors for emergence. Knowledge of how these extrinsic factors could help improve weed control, especially if population sizes are maintained by spring cohort fecundity. Furthermore, *G. aparine* is known to have a seed viability of 2-3 years if dry stored (Holm *et al.*, 1977), and viability is lost significantly earlier in the field due to temperature fluctuations (Mennan, 2003). So, the control of the spring cohorts may contribute to the control of the weed infestations in *Galium* species with short-lived seeds.

The objective of this study was to test whether exposure of *Galium* seeds to different environmental conditions resulted in distinctive emergence patterns. Moreover, *Galium*

accessions from different origins were used to distinguish between genetically and environmentally induced variability in emergence responses.

Materials and methods

Plant material

Seeds of *G. aparine*, *G. spurium*, and *G. tricornutum* were collected from fields and river banks in west Catalonia, Spain, from late June until early July of 2005 and 2006 when most seeds were mature. British populations of *G. aparine* and *G. spurium*, and a German population of *G. aparine* were provided by Herbiseed (Table 1). These had been harvested at the beginning of August of 2004 and 2005 and, after a drying process that lasted one week, seeds had been cleaned and stored at 15°C and 15% of RH in sealed containers on the 14th of August. Both British populations were considered as winter-germinating populations, while the German *G. aparine* was considered as a spring-germinating population.

Experimental field management

Field experiments were conducted during the winter seasons of 2005-2006 and 2006-2007 in a 10 ha commercial winter barley field near Balaguer, 20 and 40 km from where the Spanish populations had been collected. The soil was a medium loam (37.7% sand, 43.4% silt, 19.4% clay), 1.52% organic matter and pH of 8.3. The experimental field was chisel ploughed in October and afterwards was left bare. No herbicides were applied during the trial.

Daily rainfall and maximum and minimum air temperatures were obtained from a meteorology station 14 km away. Simulated soil temperatures and water potentials were used to calculate hydrothermal time (HTT) based on the equation described by Roman *et al.* (2000):

$$\text{HTT} = \sum (\text{HT} \times \text{TT})$$

where $\text{HT} = 1$ when $\psi > \psi_b$, otherwise $\text{HT} = 0$; and $\text{TT} = T - T_b$ when $T > T_b$, otherwise $\text{TT} = 0$. ψ is the daily average water potential in the soil layer from 0 to 5 cm; ψ_b is the base water potential for seedling emergence; T is the daily average soil temperature in the soil layer from 0 to 5 cm and T_b is the base temperature for seedling emergence (Martinson *et al.*, 2007; Ekeleme *et al.*, 2005). With this formula, growing degree days are accumulated only when the water potential and temperature conditions were higher than the base water potential and temperature. The HTT

was estimated using the STM^2 model (Spokas & Forcella, 2009). STM^2 requires as input daily maximum and minimum temperatures and daily precipitation, along with information on the geographical location and soil texture and organic matter. HTT were accumulated over days beginning on the sowing date in each artificial cohort. For *G. aparine*, *G. spurium* and *G. tricornutum*, -2.5MPa, -1.2MPa and -0.8MPa were used as ψ_b respectively, while T_b was established at 0°C for the three species (Royo-Esnal *et al.*, 2010).

Experimental design

The experiment was a randomised complete block design with three replicates. Each block was 50 m long by 3 m wide and was subdivided into 26 plots of 2 m by 1 m. In each plot 400 seeds m^{-2} were manually spread and covered with a thin soil layer (<1cm) to avoid dispersion by wind. Treatments within a block were 1) *Galium* species (five populations in 2005-2006 and seven populations in 2006-2007) and 2) sowing dates (three dates). In the winter of 2005-2006, seeds were sown on 21 November, 27 December and 15 February. In the winter of 2006-2007, seeds were sown on 6 November, 10 January and 14 February. The sowing date was varied to artificially create cohorts based on emergence date. *Galium* seedling emergence was followed by weekly assessments in three 0.1 m^2 permanent quadrats in each plot.

Statistical analysis

Emergence data (%) were transformed ($\sqrt{\arcsin(x+1)}$) prior to analysis to correct sample heterogeneity. The effect of the climatic characteristics of each year on the transformed emergence data was tested by a two-way ANOVA followed by Duncan's test. The effects of *Galium* species accession and cohort on transformed emergence data were analyzed via a two-way ANOVA. Contrast analyses were performed to seek for differences among and within *Galium* species and between cohorts, with Least Square Means test between parameter estimates, with $\alpha = 0.05$. The analyses were performed with the statistical software SAS 8.1 (SAS Institute, 2000).

Emergence patterns predicted by Royo-Esnal *et al.* (2010) were superimposed on plots of actual seedling emergence of the cohorts. Differences between predicted and observed seedling emergence values were assessed by RMSE:

$$RMSE = \sqrt{1/n \sum_{i=1}^n (x_i - y_i)^2}$$

where x_i represents observed cumulative seedling emergence percentage, y_i is predicted cumulative seedling emergence percentage, and n is the number of observations (Mayer and Butler 1993). RMSE provided a measurement of the typical difference between predicted and actual values in units of seedling emergence percentage. The lowest RMSE value indicated that emergence model fit had been optimized. Roman *et al.* (2000) consider that an RMSE of 14 is acceptable for a model. In our study evaluation of RMSE was as follows: <5, excellent prediction; 5<10, very good prediction; 10<15, good prediction; >15, insufficient prediction. Those accessions-cohorts which were not optimally described by these models (RMSE over 15) were submitted to a new sigmoid growth model (Weibull, 1951), contrasting cumulative emergence and cumulative hydrothermal time (HTT):

$$y = K [1 - \exp (- b(x - z)^a)]$$

where y is the percentage of emergence, x is time expressed as growing degree days and K , b and a are constants which are fitted by the model; K is the maximum percentage of emergence recorded, b is the rate of increase in percentage of emergences, z is the lag phase and a is a shape parameter. Fitting of the Weibull model to the emergence data was performed using the NLIN procedure of SAS software version 8.1 (SAS, 2000). Model parameters were estimated by non-linear least-squares regression. The goodness of fit was checked using the contrast of joint hypothesis ($P < 0.05$).

Results

Climatic characteristics of the 2005-2006 and 2006-2007 winters

The two winters differed considerably in terms of temperature, total rainfall and rainfall pattern (Figure 1). From sowing in November to June total rainfall was 68 mm in 2005-2006, and 208 mm in 2006-2007. The first winter (December-February) was relatively cold (average temperature 3.3 °C), with little rain that mainly fell in January (29 mm). Early spring (February – May) was extremely dry (30 mm). The second year was marked by warmer weather in winter

(average temperature 5.4 °C) and much more rain in spring (169 mm between February and May).

Galium species emergence

Figures 2 and 3 show the emergence of *Galium* species, accessions and cohorts in both seasons. In 2005-2006, emergence of the first and second cohorts began together in GAes2 (river bank population) on 1 February, after 16.7 mm of rain, and increased rapidly during the following weeks. The third cohort received little rainfall until mid May (10 mm in one week). In the other two accessions, British (GAuk) and German (GAde), the first cohort started emergence two weeks after January rains, and the emergence of the second cohort was delayed by 1-2 weeks with respect to the first one.

In 2006-2007 in every accession of *G. aparine* the first cohort started emergence on the same date, 5 December, despite only 0.4 mm rain having fallen during the previous two weeks. But after rains of the following week (6 to 12 December) increased numbers of seedlings could be observed in every accession, and emergence of GSes and GT started. Densities exponentially increased and reached their highest levels by February even though very little rain fell. The second cohorts started emergence during 22 and 28 February, except in GAuk. In the previous two weeks (6 to 21 February) almost 14 mm of rain fell. The percentage of emergence significantly increased after rains between 30 March and 11 April (81 mm). These were also the dates when British accessions showed their highest emergence rates. Despite this, the second cohort showed a kind of first flush from 21-28 February to 21 March and a second flush from 22 March to 25 April. The third cohort only emerged after April rains. The emergence patterns in GSuk were very different. The first cohort hardly emerged in the last week of January, even with 11 mm of rain the previous two weeks. It increased greatly after the late March-April rains (110 mm), when it coincided with the emergence patterns of the second and third cohorts.

Comparison of populations that were included in both winters indicated that emergence was higher in 2006-2007 than in the previous winter (Table 2). The level of significance for differences between years tended to increase with sowing date. The percentage emergence decreased from the first to the third sowing date in 2005-2006, but not in 2006-2007.

The ANOVA showed the presence of very significant differences between the accessions and the cohorts ($P < 0.01$). Significant differences were also found between blocks in 2006-2007 ($P < 0.05$), but not in 2005-2006. Interactions between accession and cohort resulted in statistical significance in 2005-2006 ($P < 0.05$) and high significance in 2006-2007 ($P < 0.01$).

There were significant differences in the percentage of emergence between each cohort in 2005-2006 and between the first and third cohort in 2006-2007 (Table 3). Furthermore, there were significant differences in emergence between cohorts in the German population of *G. aparine* (GAde) and in the dryland population of *G. spurius* (GSes) in 2005-2006, and in the dryland population of *G. aparine* (GAes1) and in the British population of *G. spurius* (GSuk) in 2006-2007 (Table 3).

Description of emergence by Weibull growth models

Figures 4-6 depict the accuracy of the models developed by Royo-Esnal *et al.* (2010) for the emergence of species, accessions and cohorts. Out of 36 simulations, four were excellent, 12 were very good, 11 were good and only six did not reach the minimum level of accuracy (i.e., $RMSE > 15$). In *G. aparine*, best descriptions of the emergence were obtained in 2005-06 in the third cohorts of GAes2 ($RMSE = 4.3$, Figure 4) and GAuk (Figure 4) and in the second cohort of GAde (Figure 5) and in 2006-07 in the first cohorts (except in GAde, Figure 5), all of them with less than 10% of variation. The rest of the cohorts of the populations varied between 10-15%, and only second sowing dates of GAes2 and first and third sowing dates of GAde varied by more than 15% of the predicted emergence and, therefore, were less accurate than in the other cases. In *G. spurius*, simulation of the Spanish population (GSes, Figure 6) was excellent or very good in both years and for the first and the second cohorts; the third cohort was optimally described in 2005-06 ($RMSE = 4.0$), but not so well in 2006-07 ($RMSE = 14.3$). The British population of *G. spurius* (GSuk, Figure 6) could only be very accurately described in the third cohort of 2005-06 and in the second cohort in 2006-07 ($RMSE = 4.9$ and 4.0); the first cohorts both years and the second cohort in 2005-06 were well described, but the third cohort could not be properly described. Finally, the first and second cohorts could be very well and well described in *G. tricornutum* (Figure 5).

When those populations that could not be accurately described were examined with a new Weibull model, only one could be fitted (GAes2 second cohort in 2005-06) and five could not (Table 4).

The curves depicted in Figures 4-6 suggest that, in general, emergence of seeds from the first and second cohorts was delayed compared to the third cohort. In other words, seeds sown in November or December and January needed a higher HTT sum than seeds sown in February in order to initiate emergence. Generally, the onset of emergence of seeds sown in February (third sowing date) coincided with that of the first cohort in 2006-2007, but not in 2005-2006; here it emerged with a lower HTT sum. In 2006-2007, all *Galium* emergence resulting from the first sowing date showed a small second flush of emergence at about 1500 HTT, which coincided with the April rains and with the emergence of seedlings from the third cohort.

Discussion

Emergence in most weed species depends on the breaking of seed dormancy and the induction of germination. For *Galium* general thought is that these two events are governed by a) sufficiently low temperatures to break dormancy, b) sufficient soil moisture to induce germination and c) interactions between temperature and soil moisture to maintain germination and emergence throughout winter and spring (Royo-Esnal *et al.*, 2010). In general, emergence will be high when relatively cold winters (December-January) are followed by springs without droughts.

The degree to which the above described conditions for emergence were met differed between sowing dates and years. In 2005-2006, lower winter temperatures may have broken seed dormancy, but the dry periods in late winter and spring prevented many seeds from germinating, and thus, from emerging. In this year the highest percentages of emergence occurred in the first cohort followed by the second cohort. Both cohorts received the small January rainfall (29 mm), so the low temperatures needed to break the dormancy of *G. aparine* and *G. spurium* seeds (Grime *et al.*, 1988; Masuda & Washinati, 1992) would explain these results, as the first cohort was exposed for a longer period in the field. The third cohort was not exposed to low temperatures and received only an average of 7.5 mm rain per month and so, its emergence values were the lowest. The lack of spring rains also may have provoked the extremely low emergences of British *G. aparine* (GAuk) and the third cohort of German *G. aparine* (GAde) and prevented them from fitting to the Weibull function. In 2006-2007 winter was warmer, but high

emergence levels showed that temperatures were low enough to break dormancy; high rainfalls in spring provoked flushes of emergence during April, which was reflected in higher emergence levels than in the previous year. It also changed the tendency from the first to the third cohorts for decreased emergence. This year the first cohort continued showing high percentages of emergence, but the third cohort obtained higher (GAes1 and GSuk) or almost equal values (GAes2, GAuk, GAde, and GT). The sudden flushes of emergence of the third cohorts in spring did not allow their adjustment to the Weibull model in the British population of *G. spurium* (GSuk) and in *G. tricornutum* (GT). The second cohort resulted in the lowest percentages of emergence except in GAde and GSuk. These variations might be described as adaption of populations to unpredictable environments (Anderson & Milberg, 1998).

The degree to which the above described conditions for emergence were met corresponded to the emergence patterns. These emergence patterns, with a second and weaker flush of emergences in late winter or spring, also happen in other parts of the world, like in Turkey (Mennan & Ngouajio, 2006), in the UK (Cussans & Ingle, 1999) and in Canada (Malik & Vanden Born, 1988). The dormancy/non dormancy periods together with each season's climatic conditions might be the reason for the results: The first and the second cohorts correspond to the October-December flush, whereas the third cohort would correspond to a dormancy period. Cold stratification at 5°C is known to break dormancy in *G. aparine* (Slade & Causton, 1979) and in *G. tricornutum* (Chauhan *et al.*, 2006), suggesting that cold winters are necessary to achieve higher percentage of emergence in these species. But the great percentages of emergence of the third cohorts in season 2006-07 confirms the observations from Van der Weide (1992), who saw that some populations did not need vernalization for germination, and who suggests the importance of humidity for emergence. The lack of differences obtained in the percentage of emergence between sowing dates in 2006-07 in most accessions supports the high degree of plasticity in the timing of seed germination for *Galium* species (Defelice, 2002).

The distinctive responses obtained in the various species and accessions of *Galium* may have been caused by a combination of genetic differences and maternal effects (Anderson & Milberg, 1998). However, the two cannot be separated. The Spanish dryland populations of *G. aparine* and *G. spurium* (GAes1 and Gses) obtained higher percentages of emergence than the other populations. Among the foreign accessions, the one provided as a spring population (GAde) showed lower emergence, but increased when temperatures became milder and rainfall increased,

similar to the British accessions, also adapted to wetter climates than the Mediterranean. *G. tricornutum*, which is a species that grows in colder regions, obtained values similar to the spring population.

Observed emergence patterns are relevant to understanding natural populations of *Galium* species. Models developed by Royo-Esnal *et al.* (2010) described properly the emergence of most accessions and cohorts of this work. Despite this, some could not be accurately described. Among these, only one (second cohort in GAes2 in 2005-06) could be fitted to a new Weibull function, which would mean that other factors than HTT might have affected its emergence.

Our results contribute to a better description of *Galium* species emergence, namely, there will be potentially high emergence of these weeds when there are relatively cold winters (mean temperature $<5^{\circ}\text{C}$) accompanied by at least a minimum rainfall of 30 mm per month. If such a winter is followed by a wet spring a second flush of emergence can happen. On the other hand, less emergence will occur with warmer winters and droughts, which are very common in a Mediterranean climate. Such conditions would make herbicide applications unnecessary in some years as *Galium* spp. emergence would likely to be insignificant. Recently Royo-Esnal *et al.* (2010) noted that *G. spurium* and *G. tricornutum* are more sensitive to drought than *G. aparine*, which tolerates a wider range of humidity. Thus, correctly identifying *G. aparine* and *G. spurium* is critical before making any control decision.

Knowledge of emergence timing is essential to improve the efficiency of control of *Galium* spp. Faced with the difficulty of separating effects of intrinsic factors (genotype, maternal effect, etc.), knowledge of the external factors that govern emergence of *Galium* spp. is a useful tool and may assist in the control of these weeds

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