Junglerice (*Echinochloa colona* L.) seedling emergence model as a tool to optimize pre-emergent herbicide application

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Highlights

- Weed occurrence indirectly increases the number of herbicide applications in Argentina.
- Reducing the number and volume of herbicide applications contributes to mitigating environmental impact in the short term.
- There is a critical time during weed emergence in which chemical control via herbicide application is most effective.
- Seedling emergence models are useful management tools to predict critical timing for weed control.

Abstract

Junglerice (*Echinochloa colona*), one of the worst and most problematic weeds in the world, causes large economic losses as a result of yield loss and control cost increase. Taking into account that this weed emerges in approximately five months –from September to January–, and considering that reducing herbicide use is key in the current intensification of agricultural production systems, the present study was carried out under the hypothesis that there should be an optimal moment for pre-emergent herbicide application to achieve maximum weed control effectiveness and efficiency. Experiments were therefore carried out from August 2016 to January 2021 in Pergamino, Buenos Aires province, Argentina, using a double-logistic emergence model of junglerice seedlings. Bicyclopyrone plus s-metolachlor, clomazone, and pyroxasulfone plus saflufenacil were applied at different times between 92 and 478 growing degree days (GDD). Single applications between 348 and 399 GDD were observed to reduce junglerice seedling emergence by 85-99%, depending on the herbicide used. Such a seedling emergence reduction could, in combination with a competitive crop and within a sustainable production system, be a convenient strategy to provide significant weed suppression in the field. The overall results from the present study lead to the conclusion that using predictive models for pre-emergent herbicide applications ensures a more effective use of herbicides and reduces the amounts of herbicides used as well as the risks of selecting herbicide-resistant junglerice populations.
Introduction
Herbicides are failing worldwide to do the job for which they have been designed due to the fact that weeds have evolved resistance to them and this poses great challenges to weed management (Chauhan, 2020). They are nonetheless being increasingly used as the major solution to the numerous problems resulting from weed control, making herbicidal use be a less economically profitable practice and with a great negative impact on the environment (Stewart et al., 2011). The negative impacts caused by indiscriminate herbicide use are the major aggravating factors that drastically affect the environment, product safety, and human health (Zhu et al., 2020) on the one hand, and the loss of weed susceptibility to herbicide control (Beckie, 2020), on the other. In such a scenario, it urges that changes be implemented to rationalize weed management putting the focus of attention on sustainable herbicide use to reduce not only their use but also the negative impacts they have on the community and the environment. The weed biology would be a key tool to reducing herbicide use by determining critical timing for weed control (Nazarko et al., 2005).

Compared to multiple herbicide applications, timely applications of pre-emergent herbicides not only reduce environmental impact, but also improve the profitability and efficacy of weed control (Wiles, 2004). Seedling emergence models can be used as tools to optimize herbicide use within a decision support system (DSS) (González-Andújar et al., 2011). Despite numerous investigations on the development of seedling emergence models (Forcella et al., 2000; Grundy, 2003; González-Andújar et al., 2016), they have not yet been adopted as weed control tools due to the complexity of their adjustment and the nature of data required for their prediction (Grundy, 2003). There are nonetheless simple and easy-to-use air temperature-based seedling emergence models which have acceptable goodness of fit and prediction accuracy (Myers et al., 2004; De Corby et al., 2007; Izquierdo et al., 2009).

Among weeds, junglerice (*Echinochloa colona* L. Link) is considered to be one of the worst weeds in the world (Rao et al., 2007; Peerzada et al., 2016). In Argentina, it has been found to be the most
predominant weed in soybean monoculture fields, which requires from three to five herbicide applications per growing cycle including glyphosate and acetyl CoA carboxylase (ACCase)-inhibiting herbicides (Principiano and Acciaresi 2017). Junglerice emergence cycle occurs in approximately five months—from September to January. Therefore, considering that reducing herbicide use is mandatory in the current intensification of agricultural production systems, the present study was carried out under the hypothesis that there should be an optimal moment for pre-emergent herbicide application to achieve maximum herbicidal effectiveness and efficiency in junglerice control.

Timely herbicide application could greatly help to i) optimize herbicide application schedules, ii) attain the highest use efficiency and effectiveness, and iii) reduce the number of interventions during crop growing cycle. A seedling emergence model for junglerice was tested in the present study to evaluate its potential as a tool to optimize pre-emergent herbicide application.

**Materials and methods**

Experiments were carried out between September 2016 and January 2021 at the Instituto Nacional de Tecnología Agropecuaria (INTA) (Pergamino, Buenos Aires province, Argentina, 33.95°S, 60.57°W, 56m asl). Due to crop rotation, experiments were conducted in the 2016/17 and 2017/18 corn growing seasons and in the 2018/19 and 2020/21 soybean growing seasons. Herbicide treatments were therefore repeated in each crop twice.

One pre-emergent treatment in corn and two in soybean were applied at different times during junglerice seedling emergence period. Seedling emergence was calculated using the following predictive model (Eq. 1):

\[
\text{Eq. 1} \quad EM = 47.42 + \frac{52.04}{1 + 1.39 \times e^{(-0.04 \times (GDD - 123.3))}} - \frac{52.04}{1 + 7.8 \times e^{(0.007 \times (GDD - 186))}}
\]

fitted to the study area (Picapietra et al., 2020). In this non-linear model, the beginning of thermal
time sum was in late August, when mean air temperature was lowest, below the base temperature (10 °C).

Thermal time was calculated as growing degree days (GDD) using temperature forecasts (SMN, 2021) and herbicide applications were scheduled. GDD were subsequently calculated using real air temperature data provided by INTA Agrometeorology station in Pergamino. GDD sum started on 8/21, 8/20, 8/31 and 9/2 in 2016, 2017, 2018 and 2020, respectively.

**Experimental design and herbicide application**

The experiment was conducted under a randomized complete block design with a split-plot arrangement. Application timing was the main plot and herbicides treatment the subplot. Pre-emergent herbicides (Table 1) were sprayed with a compressed air (1.7 bar) experimental sprayer in 15 m long by 2 m wide subplots. Spray volume of 115 L ha\(^{-1}\) and six 8001 flat fan nozzles spaced 33.3 cm apart were used. In order to control the emerged weeds at the time of herbicide application, glyphosate potassium salt 860 g ae ha\(^{-1}\) was added to the sprayer mixture. Herbicide applications began in late September when weather conditions allowed, in coincidence with the application time of pre-sowing herbicides in fields in the surrounding area. The first herbicide treatment for each season was applied on 9/21, 9/25, 9/30, and 9/28 in 2016, 2017, 2018, and 2020, respectively, and the last application timing was model-estimated around 90% of the seedling emergence (430 GDD). With the date of the first and last application defined, the remaining ones were distributed homogeneously in the period (Figure 1).

Although four application timings were scheduled for the first two seasons, the fourth application timing of the second season was not considered based on prediction falling after 500 GDD and therefore none of the corresponding emerged weeds were controlled. In view of this, five application timings before 500 GDD were scheduled for the last two seasons. The first application of saflufenacil plus pyroxasulfone in 2018 was not considered because replications were lost for reasons unrelated to the experiments.
**Crop planting**

During the experiments, glyphosate-resistant corn and soybean were cultivated under no-tillage system. Crop management was similar every year, i.e. a foliar herbicide in August was applied, treatments were subsequently applied at different times, and finally the crop was sown in early December.

To avoid the direct disturbance of seed drill on the seedling count, crop planting was performed in the back half of the plots. Corn and soybean were seeded at densities of 8.5 plant m$^{-2}$ and 32 plant m$^{-2}$, respectively, and at a row spacing of 0.70 m and 0.52 m, respectively.

**Seedling counts**

Emerged seedlings were counted in triplicate every 15 days in three 0.5 m x 0.35 m permanent sections located randomly within the front half of the plots. After each counting, plants were carefully removed with minimum soil disturbance. In the non-treated plots, counting began in early September, at the beginning of the emergence period (Picapietra et al., 2020), whereas in the herbicide-treated plots counting began after herbicide application.

**Cumulative relative emergence and effective control period**

Cumulative relative emergence (Er) was calculated with data resulting from the following equation (Eq. 2):

\[ Er_i = \frac{\sum (E_0 - E_i)}{E_n} \cdot 100 \]

where \( Er_i \) is the cumulative relative emergence (%) at time \( i \), \( E \) is the number of seedlings from the beginning \( (E_0) \) to time \( i \) \( (E_i) \), and \( E_n \) is the total number of emerged seedlings in the corresponding section of the untreated plot.
To determine the effective control period (EC) GDD were calculated from the beginning of the emergence cycle to the emergence of new seedlings after herbicide application (Figure 2). Foliar control and residual control were considered.

**Statistical analysis**

The data corresponding to the different application timings of each herbicide were analyzed. This was due to the fact that our interest was not focused on determining which herbicide was the best but on determining which timing was the best for herbicide application in junglerice seedlings. Thus, as different herbicides and crops were included in the present study, and taking into account a significant year-application timing interaction, the data collected were analyzed per herbicide and per year separately.

To assess the effect of application time on Er, a Generalized Linear Mixed Model (GLMM) was fitted through a repeated measures design. The application timing factor, time factor as GDD and the interaction between them were included as fixed effects, whereas replication and application timing were both considered as random crossover effects (Di Rienzo et al., 2017).

EC values were analyzed by one-way ANOVA as only application timing was considered. Means were separated by LSD test ($P < 0.05$). Normality and variance homogeneity assumptions were checked. Analyses were performed using the statistical software Infostat ver. 2020p (Di Rienzo et al., 2020).

**Results**

During the experimental period, December was wetter than the ten-year-average, with the exception of the last experimental cycle (2020/21) in which monthly rainfall was below the historical average. It is also worthy of note that whereas August was 3.0 °C warmer than the ten-year-average both in 2017/18 and 2020/21, November 2017/18 was 3.3 °C colder (Figure 3).

**Bicyclopyrone plus s-metolachlor**
In the 2016/17 season, maximum efficiency of the mixture bicyclopyrone plus s-metolachlor was achieved when applied at 348 GDD due to lower Er observed (2%) (Figure 4). This application timing was found to be different from the other application timings evaluated (p <0.05). In the following season, only 3% of seedling emergence was recorded in the plots treated at 399 GDD. Also, these application timings achieved the maximum EC values of 1282 and 993 GDD in the 2016/17 and 2017/18 seasons, respectively (Figure 5).

**Pyroxasulfone plus saflufenacil**

In the 2018/19 season, no differences between the applications of pyroxasulfone plus saflufenacil at 378 or 457 GDD were observed. These plots showed an average ranging between 4 and 5% of cumulative seedling emergence (Figure 6). These application timing were also showed the higher EC for the season (998 and 913 GDD, respectively) (Figure 7). At other application timings, Er was higher than 35% and EC was of 678 GDD. In the 2020/21 season, maximum seedling control was achieved in the treated plots at 396 GDD, seedling emergence was on average 4% and EC was of 1092 GDD. An Er higher than 18% and an EC lower than 405 GDD were also observed, thus indicating that the herbicide mixture application was not effective before 396 GDD (Figure 6, 7).

**Clomazone**

Junglerice control was maximum when clomazone was applied at 457 and 472 GDD in the 2018/2019 and 2020/2021 seasons, respectively. At these application timings, cumulative seedling emergence was 15% (Figure 8) and EC was of 913 and 770 GDD in the 2018/19 and 2020/21 seasons, respectively (Figure 9).

The remaining application timings evaluated were found not to be optimal for junglerice control. Er in the clomazone-treated plots before 457 and 472 GDD in the 2018/19 and 2020/21 seasons was higher than 22% and 24%, respectively.
Discussion

Herbicide use

As the herbicides bicyclopyrone and s-metolachlor have no foliar action (Lewis et al., 2016), glyphosate was the only herbicide in the mixture responsible for the control of emerged plants. In this sense, the application at 478 GDD in the first experimental season was not effective as a result of the deficient foliar control on the plants with great development which correspond to the first emerged cohorts.

The combined use of pyroxasulfone with saflufenacil was effective at up to 378 and 396 GDD in the two years evaluated. The application of this mixture at 472 GDD in 2020/21, despite being the latest, achieved good control of junglerice probably as a result of the joint foliar effect of glyphosate and saflufenacil. However, a lower residual control was observed probably because of herbicidal binding to larger plants (Congreve and Cameron, 2019).

As to the herbicide clomazone, previous research has recommended a rate ranging between 960 and 1200 g ai ha\(^{-1}\) to increase weed control percentage (Esqueda, 1999). While the herbicides evaluated in the present study yielded a similar EC, a high number of seedlings were observed in the clomazone-treated plots by the end of the study. This could indicate that herbicide soil persistence is limited in agreement with Lewis et al. (2016) who observed that the minimum loss time of 90% of the initial herbicide (dt90) was 65-day long. In this connection, it should be noted that the dt90 is also affected by microbial degradation in warm and humid soils (Santos et al., 2008).

Although clomazone application either before or immediately after weed emergence was observed not to yield efficient control over junglerice, the most effective applications were the last ones. This coincides with previous observations by Esqueda (2000) who reported a slightly lower control effect than that in the pre-emergence stage between the second and fourth unfolded leaves in clomazone-treated plots (Esqueda, 2000).

Emergence predictive model as a decision-support tool
The double-logistic model of junglerice emergence can predict emergence cycles across years (Picapietra et al. 2020) using air temperature values which can be estimated from weather forecast data. This tool not only improves chemical weed control efficiency but also reduces the number of herbicide applications in a crop sequence. In other words, adequate control can be achieved with a single herbicide application if precisely timed were implemented (Principiano and Acciaresi, 2017). Furthermore, this double-logistic model provides insight into the demographic process of junglerice and therefore makes it feasible to reduce the number of herbicide applications during crop growing cycles. It can thus be considered an effective and sustainable weed management tactics (Chauhan, 2020). Despite the high efficiency observed by a timely single application, this is only a strategy to improve the herbicide use and it must be integrated into a weed management program. An inadequate use of this knowledge could lead to the selection of herbicide-resistant weeds, favored by pre-emergent herbicides selection-pressure.

Conclusions

The results obtained here indicate that an emergence model based on thermal time permits adjust herbicide applications improving their efficiency and effectiveness. This, in turn, contributes to reducing not only the number of applications but also the volume of herbicides. Herbicide application efficiency was found to be highest shortly before reaching 400 GDD from the beginning of the emergence cycle of junglerice. Thus, with a mixture of residual herbicides and foliar herbicides 400 GDD could be set as the critical timing to junglerice control. Further research aiming at exploring herbicides other than those analyzed in the present study were needed and analyzing rotation of herbicides with different mechanisms of action will contribute to reducing the risk of selecting herbicide-resistant junglerice populations.

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Table 1. Herbicides used, active ingredients (herbicide) and dose applied (Dose, ai ha\(^{-1}\)) in each corn season (2016/17 and 2017/18) and soybean season (2018/19 and 2020/21).

| Crop    | Herbicide                  | Dose (ai ha\(^{-1}\)) |
|---------|----------------------------|------------------------|
| Corn    | bicyclopyrone              | 200 g                  |
|         | + s-metolachlor            | + 960 g                |
|         | + glyphosate               | + 860 g                |
| Soybean | clomazone                  | 720 g                  |
|         | + glyphosate               | + 860 g                |
| Soybean | pyroxasulfone              | 170 g                  |
|         | + saflufenacil             | + 31.5 g               |
|         | + glyphosate               | + 860 g                |

Figure 1. Application timing of different herbicide treatments (triangles, GDD) according to the air temperature-based double-logistic seedling emergence model (Picapietra et al. 2020). Bicyclopyrone plus s-metolachlor for each corn season (2016/17 and 2017/18) and clomazone and pyroxasulfone plus saflufenacil for each soybean season (2018/19 and 2020/21).
Figure 2. Diagram of effective control period (EC) calculated from the beginning of the GDD sum ($\theta$) until the emergence of new seedlings ($B$), including seedling emergence onset ($\lambda$) and herbicide application time ($A$).

Figure 3. Monthly mean air temperature values (lines, $^\circ$C) and monthly rainfall values (bars, mm) from August to January corresponding to the four years of this study (2016/2017, 2017/2018, 2018/2019, and 2020/2021) and the ten-year-average (2006/2007-2015/2016).
Figure 4. Cumulative relative emergence of junglerice seedlings analyzed independently in the 2016/17 (blue) and 2017/18 (green) seasons. Comparison of different bicyclopyrone plus s-metolachlor application timings (lines referenced in the figure).

Figure 5. Effective control period (EC, GDD) of bicyclopyrone plus s-metolachlor during different cycles of junglerice emergence analyzed independently in the 2016/17 (blue) and 2017/18 (green) seasons.
Figure 6. Cumulative relative emergence of junglerice seedlings analyzed independently in the 2018/19 (blue) and 2020/21 (green) seasons. Comparison of different application timings of pyroxasulfone plus saflufenacil.

Figure 7. Effective control period (EC, GDD) of pyroxasulfone plus saflufenacil during different cycles of junglerice emergence analyzed independently in the 2018/19 (blue) and 2020/21 (green) seasons.
Figure 8. Cumulative relative emergence of junglerice seedlings analyzed independently in the 2018/19 (blue) and 2020/21 (green) seasons. Comparison of different application timings of clomazone (lines referenced in the figure).

Figure 9. Effective control period (EC, GDD) of clomazone during different cycles of junglerice emergence analyzed independently in the 2018/19 (blue) and 2020/21 (green) seasons.