Effects of Maize Development and Phenology on the Field Infestation Dynamics of the European Corn Borer

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Summary:

1 Plant-pest interactions are governed by phenology, i.e. the timing of stage transitions in interacting species. In response to herbivory, plants have evolved defense mechanisms that vary over the course of their development, meaning pests could face varying levels of plant defenses depending on infestation onset. Here, we investigated how the phenological match/mismatch between pests and plants could impact the success of pests in the field.

2 A two-year field experiment was carried out on a panel of 23 early to late flowering maize inbred lines. Inbreds were sown at three different dates in order to confront European corn borers to different maize developmental stages. Plant development and pest infestation dynamics were monitored over the entire cropping season.

3 Contrasted climatic conditions between the two years of the experiment led to different plant and pest phenologies and allowed us to study the relationship between plant development and the timing of pest attacks. Results showed that pest attack dynamics were affected by plant development and that this relationship was not straightforward.

4 For both years, the significant effect of the interaction between inbred line and sowing date on the success of pest infestation resulted in a complex relationship between infestation success and plant development. The shape and intensity of the relationship depended on the timing of the onset of the pest infestation.

5 When infestation occurred when plants were in a vegetative stage, a non-linear relationship between development and pest incidence was observed with the least and most developed plants being the most infested. When infestation occurred when all plants were in the mature phase, the most developed plants were the least infested.

6 *Synthesis:* Our results highlight the variability of pest infestation dynamics and underline the effect of plant-pest phenological match/mismatch on pest success.

Keywords : field experiment, infestation dynamics , match/mismatch, phenology, plant-pest interaction

Introduction

The interactions between species are stage dependent and vary throughout the respective life cycles of these species (Yang and Rudolf, 2010). Hence, the occurrence of these interactions is governed by the timing of life cycle stage transitions in both species, i.e their respective phenology. This is notably the case for herbivorous insects, where host seeking and feeding behaviours are specific to certain life stages (Hagstrum and Subramanyam, 2010). In the European corn borer *Ostrinia nubilalis* Hübner (Lepidoptera: Pyralidae), a pest of maize (*Zea mays* L.), neonates have a pre-feeding dispersal phase while late larval instars mainly bore maize stems in order to develop and survive winter (Ross and Ostlie, 1990). The timing of the phenological events in both species is influenced by genetic, abiotic (Ausin et al., 2005) and biotic factors (Hanley and Fegan, 2007; Rasmussen and Yang, 2022). For maize crop, the timing of events is also determined by agricultural practices, in particular sowing dates.

The impact of phenology on species interactions has been studied in the context of climate change, as rising temperatures and the increase in frequency of extreme meteorological events can shift the phenology of species. This shift may differ from one species to the next (Parmesan, 2007) and thus could desynchronize interacting species and consequently impact their interactions. When resource availability is limited in time, the demand by the consumer must be synchronized with the provider (Bewick et al., 2016; Cushing, 1990; Ferreira et al., 2023). This is the case in herbivory interactions, where individuals requering a resource for their development must be able to feed when this resource is available. For example, it has been demonstrated that an early post-winter resurgence of the herbivorous *Operophtera brumata* Linnaeus (Lepidoptera: Geometridae) prior to the vegetative recovery of its host can lead to the starvation of the neonates and thus a decrease in fitness (Tikkanen and Julkunen-Tiitto, 2003).

Plant-pest interactions are also impacted by the defense strategies developed by plants in response to predation during plant-pest co-evolution. There is a large body of work investigating plant defense mechanisms from a range of perspectives from molecular genetics to physiology and

ecosystem ecology, and many hypotheses have been formulated regarding the mechanisms for reducing herbivory and its impact (Gong and Zhang, 2014; Stamp, 2003). Plant defense mechanisms have been classified into three groups (Painter, 1951): (i) tolerance mechanisms that minimise the impact of herbivore damage (ii) antixenosis that reduces the probability of contact between pests and plants, and (iii) antibiosis, i.e. the ability of a plant to reduce or stop the growth and/or development of the pest. Plant defense traits include both physical structures (e.g. spine, leaf toughness) and chemical compounds such as synthesised toxins or volatile organic compounds (Mithöfer and Boland, 2012) that can be either constitutive and/or inducible, i.e produced in response to pest infestation. In maize, the model plant considered here, lignin content is a constitutive morphological trait whose concentration can change in response to insect feeding (Meihls et al., 2012; Rodríguez et al., 2012). Defense traits can also be related to dispersal, plant size or phenology (Boege and Marquis, 2005). Plant defense mechanisms may vary during plant development, between individuals due to genetic variation, and with environmental conditions due to phenotypic plasticity (Ballhorn et al., 2011). Hence, genetic variation and temporal variation are two different components of plant defenses that may interact with the match/mismatch of pest and plant phenologies, with contradictory consequences in terms of pest attack dynamics.

Plant defenses tend to increase with physiological development until maturity and can later either decrease or be maintained depending on the reproduction strategy (Boege and Marquis, 2005). However, meta-analyses aiming to characterise general patterns of plant defense acquisition during development showed that this can vary between species, within species, and with the type of defense (physical or chemical) (Barton and Koricheva, 2010; Quintero et al., 2013, Cao et al. 2019). Temporal changes in physical and chemical defenses may result in non-linear dynamics of defense acquisition. For example, the concentration of DIMBOA (benzoxazinoid), one of the main constitutive chemical defenses in maize leaves, decreases as the plant matures while physical defenses such as lignin and silica concentration (cell wall components) tend to increase over time (Meihls et al., 2012).

The production of these defenses requires resources. However, plants must simultaneously ensure at least three key functions to maximise their fitness: growth, reproduction and defense, potentially leading to a trade-off between defense and growth (Herms and Mattson, 1992; Züst and Agrawal, 2017). This implies that, within a species, plant defenses could be higher in slow-growing genotypes. This growth-defense trade-off has been the subject of several studies with contrasted results depending on the species considered (Bode and Kessler, 2012; Santiago et al., 2016; Stowe and Marquis, 2011; Todesco et al., 2010). This trade-off could interfere with plant defense acquisition during development and affect plant-pest interactions. The consequence on pest success of the interplay between (i) the match/mismatch of plant and pest phenologies, (ii) plant defense acquisition, and (iii) the trade-off between growth and defense in the host plant, may vary over the course of the season depending on the timing of the presence of the pest and the developmental stage of the host. Within the framework of crop pest control, understanding how the interplay between pest phenology and host plant development impacts pest dynamics in the field could lead to the development of alternative control strategies.

Here, we focus on the interaction between maize crop and corn borers in an area where the main corn borer is the European Corn Borer (ECB; *Ostrinia nubilalis)*. ECB is a major arthropod pest of maize in Europe and can infest up to 60% of fields and cause losses of 5% to 30% (Meissle et al., 2009). Studies of the impact of maize earliness and/or phenology on corn borer infestation success have produced contradictory results. A study by Ordas et al. (2013) showed that, within a panel of early and late inbred lines derived from a common ancestral line, early inbred lines suffered less damage from borers (in terms of tunnel length, ear damage) . This result can be explained by the more advanced developmental stage and greater tissue maturity of early lines at the time of infestation. By contrast, another experimental study showed that late maturing inbred maize lines displayed better pest resistance (Schulz et al., 1997). This could be explained by a growth-defense trade-off or other genetic correlations between resistance and developmental traits. Other studies explored the role of plant development by shifting the sowing date in order to synchronize or

desynchronize the most susceptible stage of crop development and the peak of pest attack, and found that late-sown plants suffered higher yield loss (Malvar et al 2002) and higher ear damage (Blandino et al, 2008).

These studies have quantified the impact of pests on plants through harvest observations providing integrated traits of plant resistance or tolerance. However, their contrasted results could be due to variations in the dynamics of pest attacks over the season. Hence, it may be interesting to monitor infestation dynamics in parallel with plant development in order to dissect the trajectories leading to the variable success of the pest. This could help us understand how the interplay between the dynamics of defense acquisition in plants and the phenological match/mismatch between plants and pests can affect pest success.

Using a panel of inbred maize lines that differ in their phenology, we investigated how natural field infestation dynamics were driven by plant development, earliness and their interplay with the timing of the onset of the pest infestation.

Materials and methods

Plant material

Plant material consisted of a panel of 23 maize inbred lines with contrasting values for several traits including flowering time (Table S1). Thirteen inbred lines, for which leaf secondary metabolites and physiological traits at two developmental stages were previously characterised (Cañas et al., 2017), were selected from a core collection representing European and North American maize genetic diversity (Camus-Kulandaivelu et al., 2006). Three additional inbred lines (Cm484, F271, F66) were chosen for their differences in cell wall digestibility (lignin, hemicellulose or p-coumaric acid content) (El Hage et al., 2018; Zhang et al., 2019) and three other inbred lines were chosen for their tolerance (F618) or susceptibility (Mo17, B37) to *O. nubilalis* (Panouillé et al., 1998; Willmot et al., 2004). Finally, two pairs of sister-inbred lines derived from two ongoing divergent selection experiments (DSEs) for flowering time were included (Durand et al., 2010). Each DSE was initiated

from an initial inbred line (F252 or MBS). Cycles of selection for earliness or lateness were carried out from each ancestral inbred line by applying divergent selection. One inbred line derived from the 18th selection cycle for earliness and one derived from the 18th selection cycle for lateness from each initial inbred line were included in the panel (early FP036 and late FT318 from F252, and early MP052 and late MT040 from MBS). The two pairs of DSE-derived lines are therefore genetically close but differ in their days to flowering.

Field experiment

A field experiment was carried out in 2021 and 2022 in Gif-sur-Yvette, France (N 48°717401', E 2°153018'). In both years, inbred lines were sown at three sowing dates (blocks) (24 April, 12 May and 15 May 2021; 28 April, 11 May and 13 May 2022) in order to confront pests to different developmental stages. Each block was divided into thirteen sub-blocks (0.80m apart) of sixteen plots (0.80m apart) (Figure S1). Each inbred line was assigned to a different plot consisting of a single row. Twenty-five seeds were sown in each plot. The first and last plot of each sub-block, as well as the first and last sub-block of each block, were used as borders and sown with MBS847. At each sowing date, each inbred line was sown in six plots (replicates). The six replicates for each inbred line at each sowing date were randomized in the blocks with the condition that the same inbred line must not be sown more than twice in one sub-block. Due to a lack of seeds, inbred lines ND283 and ND36 could not be sown in 2021 and were replaced by G18-M52 from the DSE. Experiments were performed under natural infestation.

Data collection

Plant development and phenology

To assess plant phenology, the dates of female flowering (silking) and male flowering (tasseling) were recorded. The date of silk/tassel emergence corresponded to when 50% of plants in a plot showed silks/tassels.

Pest infestation dynamics and pest development

In order to assess the natural infestation dynamics of *O. nubilalis* (European corn borer (ECB)) in the field, weekly plant monitoring was carried out between mid-June and mid-September. A plant was considered to be infested when it had at least one of the following signs: one or more series of rectilinear holes on the leaves made by the larvae (Figure S2a), holes and larval frass on the tassels (Figure S2b), stems (Figure S2c), and/or ears, and/or a broken stem (Figure S2d). A plant recorded as being infested on a given date was not longer monitored after that date. In order to determine ECB voltinism, two sources of data were used. First, five to ten plants from the MBS847 inbred line sown at the first sowing date were dissected each week and examined for the presence of pupae. Second, data collected in the Ile-de-France region by a national pest surveillance system (Bulletin de Santé du Végétal n°32, 28/09/21 and 20/09/22) were used to assess the number of pest generations and the approximate flight dates.

Statistical analyses

In order to study the effects of the dynamics of plant development on the dynamics of pest infestation, parameters describing both dynamics were defined for each plot in the field. We describe below how we characterised plant phenology, earliness and pest infestation dynamics. Data from each year of the experiment were analysed separately due to contrasting climatic conditions (cold spells and excessive rainfall in 2021, heat waves and drought in 2022), which led to different developmental dynamics for both plant and pest.

Characterising maize phenology

For each plot, phenology was quantified as the flowering date (FD) in days after 1 July. Earliness was quantified by computing the thermal time between sowing and flowering (TTF), i.e a standardised measure of the number of days of development at 20°C required to flower (Parent et

al. 2010). Tasseling and silking were characterized separately.

Characterising infestation dynamics

To characterise the dynamics of pest infestation on each inbred line for each sowing date, we first estimated the key dynamics parameters at the plot level.

Over the two years of the experiment, we observed contrasted infestation dynamics, reflecting the differences in the climatic conditions of 2021 and 2022. In particular, from the survey of MBS plants, we observed a single ECB generation in 2021 compared to two generations in 2022. Bivoltinism in 2022 resulted in two periods of infestation (referred to here as the first and second pest generation), compared to univoltism that resulted in a single period of infestation in 2021 that was more spread out over the season than each of the two infestations of 2022. Thus, for a given plot, the ECB infestation dynamics were quantified by the incidence at time *t (t* being the calendar date), noted I_t, and defined as the proportion of infested plants within a plot at time *t*. Incidence data were fitted to a single logistic growth equation for the 2021 data and a double logistic growth equation for the 2022 data (Fernandes et al., 2017) using a non-linear regression:

$$I_t = \frac{i_1}{1+e^{-r_1(t-t_1)}} + \frac{i_2 - i_1}{1+e^{-r_2(t-t_2)}} + \epsilon_t \qquad \text{eq. 1}$$

To obtain a single logistic growth equation, the second term of eq. 1 is omitted. Parameter i_1 corresponds to the maximum incidence at the end of the first wave of infestation; parameter i_2 corresponds to the maximum incidence at the end of the second wave. Thus, the quantity $i_2 - i_1$ corresponds to the incidence that is exclusively due to the second pest generation. Parameters r_1 and r_2 are the slope parameters of the first and second waves, respectively. Parameters t_1 and t_2 are the inflection points of the incidence during the first and second waves, respectively. Each of these points corresponds to the date at which the incidence reaches half of i_1 (half of i_2, respectively). ε_t are Gaussian homoscedastic residuals (Figure S3). These parameters (i_1, $i_2 - i_1$, r_1, r_2, t_1 and t_2) characterise the infestation dynamics and were estimated for each plot. Both single and double logistic growth equations (corresponding to one or two infestation waves, respectively) were fitted

for each plot in 2022. The best fit between single and double logistic growth was selected both with the deviance information criterion (DIC) and by visual inspection. For both years, the date of the first attack on the plot t_0 was also recorded.

Identifying the sources of variation

All traits related to maize phenology and infestation dynamics were fitted to a Gaussian conditional autoregressive (CAR) model that includes inbred line and sowing date factors and accounts for spatial autocorrelation. Model parameters were estimated in a Bayesian framework (Lee, 2013):

$$F_{ijk} = \alpha_i + \beta_j + \theta_{ij} + \phi_{z(ijk)} + \varepsilon_{ijk} \text{with } \varepsilon_{ijk} \sim N\left(0, \sigma^2\right) \qquad \text{eq. 2}$$

where F_{ijk} is the dependent quantitative variable (either a plant or an infestation-related trait) of the kth plot sown with inbred line i at sowing date j, α_i is the ith inbred line effect, β_j is the jth sowing date effect, θ_{ij} is the term of the interaction between α_i and β_j , $\phi_{z(ijk)}$ is the spatial effect defined by equation 3 in (Leroux et al., 2000), and ϵ_{ijk} are the residuals. Independent Gaussian priors were defined for α_i , β_j, and θ_{ij}, and uniform priors for ρ and τ^2.

$$\phi_{z(ijk)} \sim N\left(\frac{\rho \sum_{i=1}^{K} w_{ki} \phi_i}{\rho \sum_{i=1}^{K} w_{ki} + 1 - \rho}, \frac{\tau^2}{\rho \sum_{i=1}^{K} w_{ki} + 1 - \rho}\right) \qquad \text{eq. 3}$$

All analyses were performed using R software and the CARBayes package (Lee, 2013). Five independent chains were run; the Geweke coefficient was used to assess the convergence of the different simulations. Each chain was run for at least 3,500,000 iterations with a burnin period of 500,000 iterations and a thinning of 200 in order to avoid any correlation in the final sample. All chains were then concatenated for each parameter to form the final distributions. To provide an estimate of each parameter, the median and the 95% credible interval defined by the 2.5% and 97.5% quantiles were calculated. An effect was considered to be significant when the 95% High Density Interval (HDI) did not contain zero. The median of the posterior distribution predicted by the fitted models was used to characterise each trait for each inbred line-sowing date combination.

Linking infestation dynamics and plant phenology

In order to investigate the effect of plant development and phenology on the dynamics of ECB infestation, we studied the relationship between each infestation parameter (i_1, $i_2 - i_1$, r_1, r_2, t_0, t_1, and t_2) and the plant development and phenology traits FD and TTF. Plant development at different dates was characterised by computing the ratio between the thermal time at that date and the TTF. This ratio was computed at the beginning of the first pest generation (DEVSTAGE1) and the second pest generation (DEVSTAGE2). To estimate the start date of the first and second waves, we aggregated the raw data over the three sowing dates and over all inbred lines and chose the week where the exponential phase of the first and second generation began. Both linear and non-linear relationships were investigated with the two following models:

$$I^{ij} = (c_0 + c_j) + (d_0 + d_j) * trait_{ij} + G_i + \varepsilon_{ij} \text{with } e_{ij} \sim N(0, \sigma^2) \qquad \text{eq. 5}$$

$$I^{ij} = c_0 + d_0 * trait_{ij} + d_0^2 * trait_{ij}^2 + G_i + \varepsilon_{ij} \text{with } e_{ij} \sim N(0, \sigma^2) \qquad \text{eq. 6}$$

I_{ij} is the value of an infestation parameter (i_1, $i_2 - i_1$, r_1, r_2, t_1, or t_2) for inbred line i sown at sowing date j estimated with eq. 2; $trait_{ij}$ is the value of a biological trait (FD, TTF, DEVSTAGE1, DEVSTAGE2) for on inbred line i sown at sowing date j estimated with eq. 2. The parameters of these two models are: c_0 the intercept, c_j the effect of sowing date j on the intercept, d_0 the slope of the relation, d_j the effect of sowing date j on the slope, d_0^2 the quadratic component, and G_i the individual effect of inbred line i on the intercept modeled as a Gaussian random effect of variance σ^2_B. Traits were included in separate models to avoid highly correlated co-variables. When the biological trait considered was DEVSTAGE1 and DEVSTAGE2, we omitted the effect of sowing date on the slope and the quadratic component, as DEVSTAGE is highly dependent on the sowing date (early sown plants tend to be more developed than late sown plants). The Akaike information criterion (AIC) was used to select between the linear (eq. 5) and non-linear (eq. 6) model. When the linear model was selected, statistical tests were carried out to assess the significance of the model parameters. First, we tested the null hypothesis $d_1 = d_2 = d_3 = 0$, then $c_1 = c_2 = c_3 = 0$. When the non-

linear model was selected, similar statistical tests were carried out on d_0 and d_0^2.

Results

To decipher the interplay between plant and pest phenologies, a two-year field experiment was carried out on 23 maize inbred lines sown at three different calendar dates. Both plant phenology and the natural infestation dynamics of the European corn borer O. *nubilalis* were monitored and characterised from different traits. The results below describe these traits and their relationship.

Plant and pest phenologies

The lines in our maize diversity panel displayed a wide range of flowering times. For the sake of simplicity, this section describes only tasseling dates as qualitatively similar results were observed for silking dates. The median FD, TFF and DEVSTAGE obtained with the Bayesian model described in eq. 2 are summarised in Table S2 for each sowing date for each year. The median TTF ranged from 56.1 (95% credible interval = [54.9, 57.3]) to 89.5 thermal days [88.4, 90.6]. For both years, the sowing date effect, the inbred line effect, and their interaction were significant for both TTF and FD (Figure S4 and S5).

Spatial autocorrelation within sub-blocks was detected for both TTF and FD ($\rho = 0.27$) in 2022 but not in 2021. Figure 1 shows the distribution of the median tasseling date of for each sowing in 2021 and 2022. Flowering occurred on average earlier in 2022 than in 2021, and there was more variation between sowings in 2022 than in 2021. The average difference between two successive sowings was ~ 1 to 5 days in 2021 and ~ 8 days in 2022. In 2021, the second sowing flowered approximately at the same date as the third sowing. This can be related to the cold temperatures between the two sowing dates, which slowed down the development of plants from the second sowing. The effect of sowing date on the rate of plant development varied between years. Later sowing corresponded to a faster development in 2021 and a slower development in 2022 (Table S2). These results illustrate the extent of plant phenotypic plasticity.

The survey of pest incidence allowed us to estimate the date of the onset of pest infestation t_0 on each plot and revealed the existence of a second generation of pests in 2022 (Figure 1). These data were compared with data collected in the Ile-de-France region by the national pest surveillance system (number of captured imagos). As shown in Figure 1, both data sources are congruent. Despite clear environmental differences between the two years, the onset of infestation occurred a few weeks before maize flowering in both years. However, results show a clear difference between the two years, with one pest generation in 2021 and two generations in 2022. The trait DEVSTAGE1 quantifies the average plant developmental stage at the onset of the first wave of infestation. As shown in Table S2, DEVSTAGE1 was dependent on the sowing date and the year. Plants from the first sowing were more advanced in their development. The most striking difference concerns the third sowing in 2022; on average, plants from those plots were at an earlier developmental stage (DEVSTAGE1 = 25%) than plants from the other sowings (DEVSTAGE1 ranging from 40% to 55%). As previously mentioned, these average values can mask significant variations between inbred lines due to inbred line-sowing date interactions.

During the two years of experimentation, a global synchrony between the onset of infestation and plant flowering time was observed. However, this synchrony is affected by the contrasted phenologies of the inbred lines from the panel and the effect of sowing date. The design of this experiment allowed us to explore a new facet of the genetic diversity of this maize panel.

Infestation dynamics

For each plot, the infestation dynamics were characterised with eq. 1 using several parameters summarising the timing and intensity of the infestation by each pest generation. Each parameter was then estimated with the model described by eq. 2 to characterise each inbred line from each sowing. Estimates of each parameter for each sowing are presented in Table 1. Figure 1 shows the dynamics of the mean pest incidence (the proportion of attacked plants) for the three sowings in 2021 and 2022.

We observed contrasted patterns between years and between sowings in a given year. There were two major differences between 2021 and 2022. First, two generations were observed in 2022 as opposed to one in 2021. Second, the infestation dynamics differed between sowings. In 2021, both t_0, t_1 and r_1 were higher in the first sowing meaning that this sowing was infested later in the season; however incidence increased more rapidly in the first sowing than in the later sowings (Table 1 and Figure S6). The final incidence of the single pest generation i_1 was slightly higher in the first sowing (Figure 1a, Table 1 and Figure S6). In 2022, the infestation of the second sowing occurred significantly later (t_0) and was less intense than that of the first and third sowings (i_1) (Table 1 and Figure S7). No significant difference was found between the growth rates (r_1) of the different sowings (Table 1 and Figure S7). For the second pest generation of 2022, the first sowing was less infested than the other two sowings ($i_2 - i_1$), and the last sowing was more rapidly infested (r_2) and twice as highly infested than the first two sowings (Figure 1b, Table 1, and Figure S7). We were able to classify each plot in 2022 as being infected by larvae from the first generation, the second generation, or both generations by using the fit of the incidence at the plot level. Results are presented in Figure 2a and show a clear-cut difference between different sowings The distribution of these different infestation dynamics depended on the sowing date (Chi-squared test pvalue = 1.83e-7) with 74% of plots sown at the third sowing date being mainly infested by both pest generations as opposed to 40% of plots sown at the first and second sowing dates. This can be explained by the fact that plants from the third sowing were less developed at the onset of the second pest generation (Table S2).

We also observed contrasted patterns between inbred lines and an interaction between lines and sowing dates. First, from the different infestation dynamics identified in 2022, we found that early inbred lines seemed more sensitive to first-generation larvae, while late inbred lines seemed more sensitive to larvae from both generations (Figure 2b). This pattern is consistent with the one observed for sowing dates: more developed plants tended to be less sensitive to the second generation. Second, in both years, the inbred line effect and its interaction with sowing date was

significant with respect to the final incidence of the first (i_1) and the second ($i_2 - i_1$) generation (Figure S8 and S9). The estimated median of i_1 and $i_2 - i_1$ for each inbred line and sowing date for each year was significantly different between inbred lines (Figure 3) with the final incidence of the first generation (i_1) ranging from 36% to 91% in 2021 (Figure 3a) and 2% to 41% in 2022 (Figure 3b). The incidence due to the second generation ($i_2 - i_1$) ranged from 2% to 52% (Figure 3c). The effect of the inbred line-sowing date interaction was particularly noticeable for i_1. For example, in 2021, although a positive additive effect of the first sowing has been estimated, the most infested sowing was the first only for 47% of lines, the second for 24%, and the third for 29%. For $i_2 - i_1$, the interaction between inbred line and sowing date was weaker: the last sowing, which was significantly more infested, was also the most infested for 91% of the inbred lines. For both years, the inbred line effect was significant for t_1 and r_1; however the interaction between inbred line and sowing date was significant only for t_1 (Figure S8 and S9). No pattern was detected within inbred lines in either year for r_1 and t_1 in 2022. However, t_1 tended to be higher (i.e. later in the season) irrespective of the sowing date for early inbred lines in 2021.

In 2022, although all effects were significant for t_2 and r_2, only small differences were observed between inbred lines. This is consistent with the fact that each of the two infestation periods were shorter than the extended flight of the single pest generation of 2021, thus attenuating the potential variability between lines in 2022.

Overall, we observed an effect of both sowing date, inbred line and their interaction on the dynamics of pest attack. To explain this pattern further, we explore below whether the incidence after the first and second generation can be explained by plant phenology and development at the onset of the attack.

Relationship between plant development and phenology and infestation dynamics

Results highlight the effect of a match-mismatch between plant and pest phenologies on the success of pest infestation. The effect of plant development on the final incidence was consistent over the

two years of the experiment but differed between the two waves of infestation in 2022.

In 2021, the timing (t_1) and incidence (i_1) of the pest attacks varied linearly with phenology (FD), earliness (TTF) and developmental stage (the fraction of development DEVSTAGE1) at the onset of the infestation (Figures S10 and S11, Table S3). The most mature plants at the time of arrival of the pest were infested later and to a lesser extent than less developed plants. However, this relationship is blurred by the significant inbred line effect, suggesting differences in genetically determined resistance factors between inbred lines.

In 2022, the level of infestation after the first pest generation i_1 was non-linearly related (eq. 6) to FD (Figure S12a), TTF (Figure S12b), and DEVSTAGE1 (Figure 4b). For the first sowing, more developed plants (i.e early inbred lines) tended to be more infested by the first pest generation than less developed plants (i.e late inbred lines) (correlation coefficient = 33.2 %, p-value = 0.1217). By contrast, for the third sowing, less developed plants tended to be more infested than more developed plants (correlation coefficient = -18.6%, pvalue = 0.39). For the second sowing, there was no evidence of a relationship between i_1 and DEVSTAGE1. The level of infestation after the second pest generation $i_2 - i_1$ was linearly related (Table S4) to FD (Figure S13a) and TTF (Figure S13b), and non-linearly related (Table S3) to DEVSTAGE2 (Figure 4c). The linear equation (eq. 5) used for FD and TFF enabled us to take into account the effect of sowing date, which impacts FD and TFF. These results show that the most developed plants at the start of the second pest generation tended to be less infested than the least developed plants. This pattern seems better explained by the fraction of development (DEVSTAGE2) than by earliness (TTF). Indeed, when modeling the relationship between $i_2 - i_1$ and TTF, the strength of this relationship increased with sowing date with a correlation coefficient of 57% (p-value = 0.004) for the first sowing, and 74% (p-value = 5.164e-5) and 80% (p-value = 4.401e-6) for the second and third sowings, respectively. We observed that late inbred lines from the first sowing and early inbred lines from the third sowing had reached the same level of development and experienced comparable levels of infestation. These results show that the stage of plant development affects the success of the pest, but the non-linearity

of this relationship highlights the importance of the match-mismatch between plant and pest phenologies.

Discussion

We set up a field experiment with shifted sowing dates to investigate the drivers of pest success on a panel of maize inbred lines that differ in earliness. The objective was to understand how the dynamics of plant development and plant-pest phenological match/mismatch could impact the dynamics of pest infestation . Whereas most field experiment studies on maize have quantified pest resistance by measuring borer-induced damage at the end of the season without monitoring the infestation dynamics over the season (Beres and Gorski, 2012; Blandino et al., 2008; Malvar et al., 2002; Ordas et al., 2013; Schulz et al., 1997), our results highlight the fact that pest success can be affected by the dynamics of maize development and plant-pest synchrony. Indeed, maize defenses can vary over time and consequently the host plant that is most favourable to the pest may change during the season. Thus, monitoring infestation dynamics over the season contributes to understanding the components that determine pest success. In this study, pests were confronted to a panel of maize inbred lines at different stages of plant development. Infestation dynamics parameters were estimated and related to parameters characterising plant development, earliness and phenology. We found that infestation dynamics varied between inbred lines, sowing dates, and years. Moreover, we found that this variability was related to maize earliness and development at the onset of the pest attacks.

First, differences in infestation dynamics were observed between inbred lines. Pest tolerance, resistance, or cell wall components of some of the inbred lines included in the panel have been previously studied using different approaches. For instance, inbred line F618 was found to be resistant, with low damage scores at maturity (Panouillé et al., 1998) and low leaf palatability (Sanane et al. 2023). In this study, we found that F618 was one of the lines that was the least infested by the first pest generation in 2022, which seems coherent as leaf palatability can be linked

to the level of damage made by early instars that feed on young leaves (Balachowsky, 1972). The three inbred lines Cm484, F66, and F271 are known to have stems with a high lignin content (El Hage et al., 2018), which is a known defense trait. This is consistent with our findings as these three inbred lines were one of the least infested in both seasons. B73 and Mo17 are known to be sensitive to ECB attacks, as shown by the length of the tunnels made by borers in several environments (Willmot et al., 2004). Mo17 displayed a variable level of resistance to tunneling depending on the environment, whereas B73 was among the most susceptible genotypes in all environments. In our study, the susceptibility of these inbred lines depended on the year and the pest generation. In 2021, Mo17 and B73 were not among the most infested lines, and in 2022, they were among the least infested by the first pest generation but were the most infested by the second generation. These discrepancies between years and previous studies may be due to various reasons, including the nature of the trait used to quantify resistance and the panel of lines to which these lines are compared, but also to the pest population and the plant/pest phenology match/mismatch. Moreover, our results provide new data on the plasticity of the inbred lines in this panel concerning their susceptibility to ECB attack.

Second, we found that infestation dynamics depended on the sowing date. The effect of sowing date on pest infestation has been the subject of previous studies focusing mainly on second generation and late-season damage. We found that in 2022, ECB was bivoltine in 2022; our results are congruent with previous studies showing that second generation ECBs are more abundant in late sowings (Blandino et al., 2008; Malvar et al., 2002) and prefer to lay their eggs on and attack late sowings (Beard, 1943; Spangler and Calvin, 2000). Infestation dynamics have rarely been studied over the entire season, so first generation damage is seldom recorded when ECB is bivoltine. One exception is the study by Balachowsky (1972), which describes the effect of sowing date on ECB infestation for both univoltine and bivoltine populations. For univoltine ECBs, early sowings were found to suffer more from the infestation. In our study, the most infested sowing in the year where ECB was univoltine (2021), was the first sowing. However, little difference was found between the

two other sowings, which could be explained by the fact that plants from these two sowings were at similar developmental stages. Moreover, the peak of the first generation in 2021occurred when most of the inbred lines were in a vegetative stage or when developmental differences were irrelevant or irrelevant to ECB preferences. For bivoltine ECBs, April sowings are described as being more sensitive to first generation ECBs whereas late May sowings are described as being less infested than early May sowings (Balachowsky, 1972). Such results are only partially consistent with the experiment described here. In 2022, the first sowing (in April) was indeed more susceptible to the first pest generation than the second sowing (early May) but the third sowing (late May) was more infested than the second. As for second generation infestations, later sowings are described as being more sensitive to second generation ECBs than earlier sowings (Balachowsky, 1972), which is consistent with our results.

These studies interpret their results by linking maize development to pest sensitivity (Balachowsky, 1972; Malvar et al., 2002; Ordas et al., 2013). Here, two factors influenced the development of maize plants: the earliness of each inbred line and the sowing date. In the literature, a link between earliness and resistance traits, as well as leaf consumption, has been found. (Ordas et al., 2013, Sanane et al., 2023). Tunnel length and ear damage were shown to be less important in early flowering inbred lines than in late flowering lines (Ordas et al. 2013). Furthermore, one of the experiments carried out in (Ordas et al. 2013) mimicked an infestation by second generation ECBs, and their results are consistent with ours. A study of the feeding behaviour of ECB larvae in relation to leaf palatability in the same panel of maize lines of our study (Sanane et al. 2023), using leaf consumption tests in inbred lines at the same developmental stage, revealed that earliness was linked to leaf palatability, with late flowering inbred lines being consumed more and more quickly than early lines. Moreover, analyses of the maize genome have shown that QTLs for resistance to ECB were co-localized with QTLs for days to flowering (Jiménez-Galindo et al., 2019), although the genetic correlation between both characters was low. However, a significant positive genetic correlation between days to flowering and tunnel length was found in flint material (Ordas et al.,

2010) and in a flint x dent population (Samayoa et al., 2014). Our results partly support a link between earliness and susceptibility, although this link is not straightforward and depends on pest phenology. Indeed, in 2021, the correlation between final incidence and earliness was significant only for the first sowing and was weaker for the two later sowings. In the first sowing, early inbred lines were less infested than late lines. One explanation could be that the adult oviposition peak occurred when all inbred lines of the second and third sowings were in the vegetative stage, with comparable defense levels, while for the first sowing, a fraction of the inbred lines were still in the vegetative stage and others had flowered leading to contrasted levels of defense in these plants. In 2022, the relationship between the incidence of the first pest generation and earliness was not linear, and convexity was observed. Incidence was maximal in the earliest and latest inbred lines suggesting that the effect of earliness is not straightforward. A similar relationship was observed between incidence and the fraction of development, with the least and most developed inbred lines being the most infested. This could be explained by the non-linearity of defense acquisition in the early stages of development (Cambier et al., 2000). The first pest generation feeds mainly on leaves and growing tassels. In early stages of development, the main defense of maize is DIMBOA, a chemical component whose concentration in leaves decreases with development (Meihls et al., 2012). At the onset of the infestation, the least developed lines may not produce other complementary defenses, while the most developed lines might not produce DIMBOA anymore. Inbred lines at an intermediate stage of development may combine DIMBOA with other complementary mechanisms, which may explain why they were less infested, and underlines the role of a phenological match between plant and pest in the success of the pest. For the second pest generation, the correlation between earliness and incidence was significant for each sowing, with early inbred lines being less infested than late inbred lines. However, the correlation was weak for the first sowing and stronger for the second and third sowings. All inbred lines had already flowered and were no longer in the vegetative stage when the second pest generation arrived in the field. Cell wall composition changes with development, particularly lignin composition, which limits pest

success (Sanane et al. 2023). In the first sowing, late inbred lines were sufficiently lignified to prevent young larvae from feeding and not suffer from pest infestation; indeed they showed similar infestation levels as early lines. This explains why there is only a weak relationship between the incidence of the second pest generation and earliness in the first sowing. In the delayed (second and third) sowings, late inbred lines are less developed and thus could be preferred by the pest compared to early inbred lines. This could explain the higher correlation between incidence and earliness in later sowings.

In this study, we have highlighted a variability in the success of the pest infestation, which is influenced essentially by plant development. The early flowering lines, developing faster than the late flowering lines, were less infested at the end of the season. However, the susceptibility of late lines can be limited by sowing them early so that they are at an advanced stage of development when the pest arrives. This relationship nevertheless depended on the match between plant and pest phenologies as the relationship was non-linear, especially when all plants were in the vegetative stage. These results highlight the importance of studying phenology in plant-pest interactions. This study of the complex dynamics of infestation and its relationship with plant development complements studies on temporal variations in plant defenses, and underlines the importance of understanding these mechanisms.

Author contribution

C.D, J.L, N.G, and S.R conducted the experiment. S.R analysed the data. S.R wrote the manuscript. A.B, C.D, F.R, J.L, R.A.M, conceived and designed the research. All authors contributed to the article and approved the submitted version.

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Conflict of Interest statement

The authors declare no conflicts of interest.

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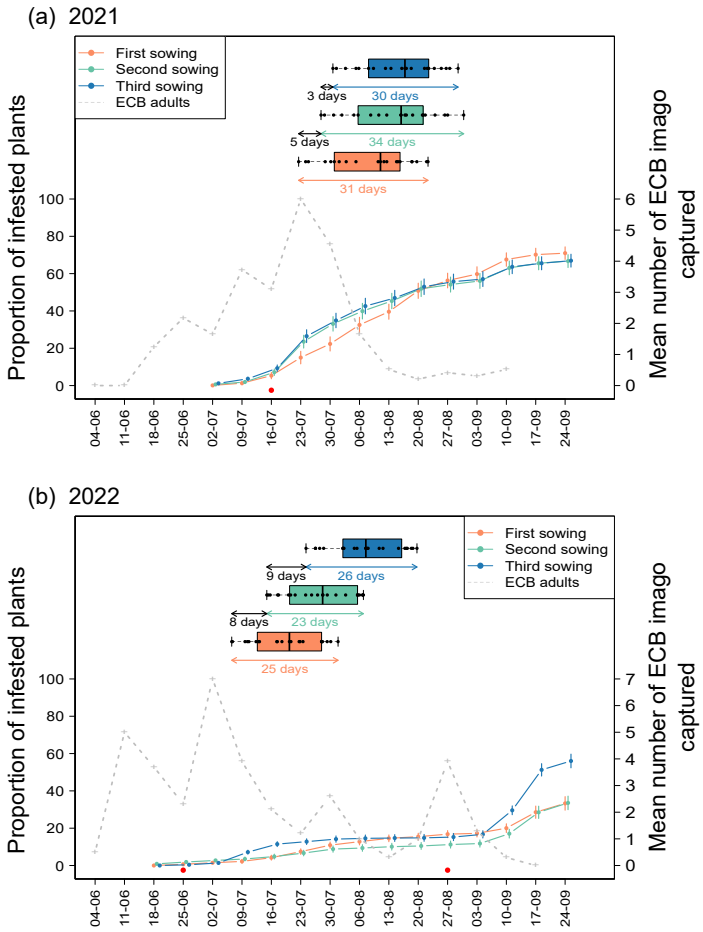

Figure 1: ECB infestation dynamics and plant phenology (FD) in 2021 (a) and 2022 (b). The x-axis represents the week of observation. The left y-axis represents the proportion of infested plants and the right y-axis the number of ECB imagos captured. The mean number of infested plants from each sowing and their 95% confidence interval are shown in orange, green and blue corresponding to the first, second and third sowing, respectively. The mean number of ECB imagos captured in the Ile-de-France region (BSV n°32, 28/09/21 and 20/09/22) are shown in grey. Median male flowering date distributions for each sowing are shown in orange, green and blue corresponding to the first, second and third sowing, respectively. Each point within the boxplots represents the estimated median flowering date, estimated with equation 2, of each inbred line sown at a given date. The red dots represent the date at which the ratios of development (DEVSTAGE1 and DEVSTAGE2) were computed. Error bars represent the 95% credible interval for each estimate.

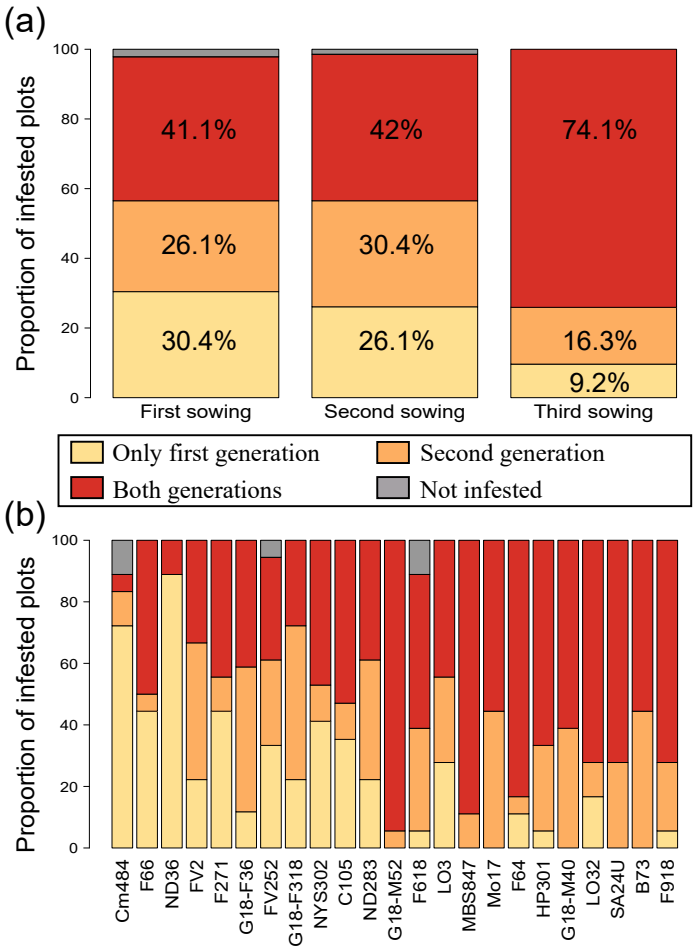

Figure 2: Proportion of plots infested by the first generation, the second generation, both generations and not infested in 2022. The proportion of plots in each category is represented by sowing date in (a), and by inbred line in (b). Inbred lines are ordered by precocity (from early to late) as observed in the field in plants from the first sowing.

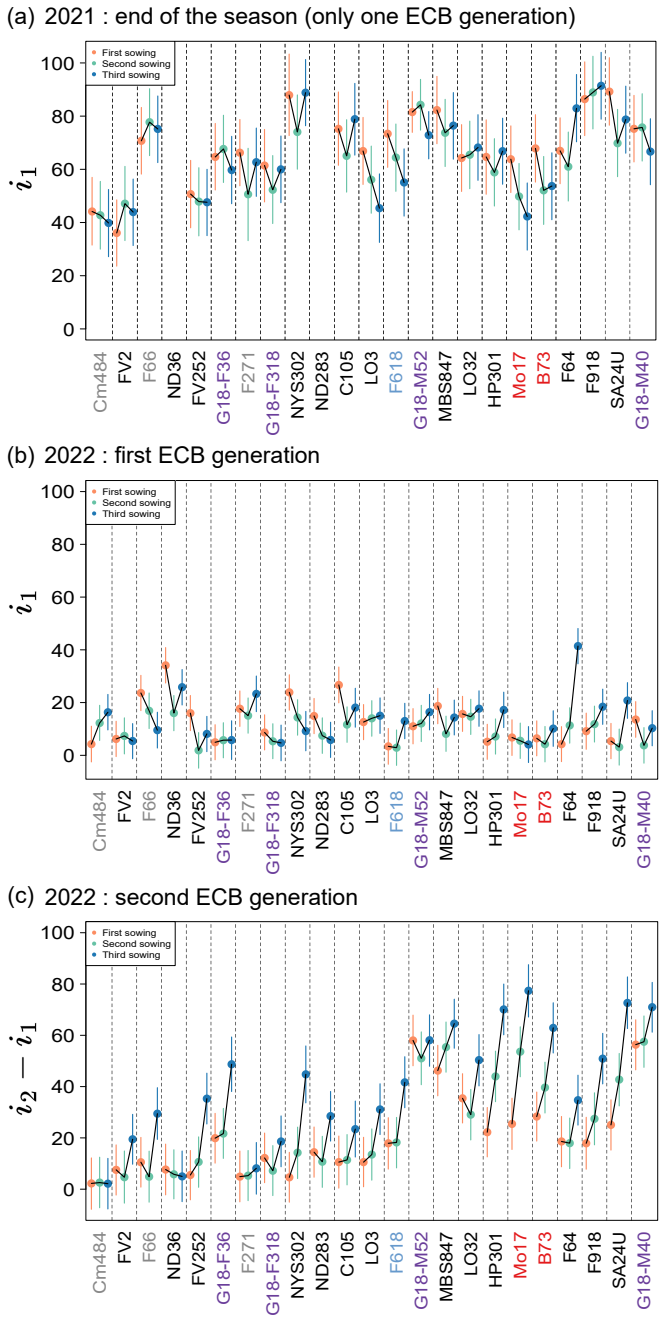

Figure 3: Median proportion of infested plants by inbred line and sowing date in 2021 and 2022. Inbred lines are ordered by earliness (from early to late) as observed in the field in plants from the first sowing in 2021. Each point represents the estimated median proportion of infested plants for each inbred line sown at different dates (for both years, the first, second and third sowings are in orange, green and blue, respectively). Inbred lines in grey (Cm484, F66, and F271) have been studied for their cell wall digestibility (El Hage et al., 2018). Inbred lines in purple (G18-F36, G18-F318, G18-M52, and G18-M40) are issued from a divergent selection experiment on flowering time (Durand et al., 2010). The inbred line in blue (F618) is known for its tolerance to ECB (Panouillé et al. 1998). Inbred lines red (Mo17, B37) are known for their susceptibility to ECB (Willmot et al. 2004). (a) Proportion of plants infested by the single pest generation in 2021. (b) Proportion of plants infested by the first generation of borers in 2022. (c) Proportion of plants infested by the second generation of borers in 2022.

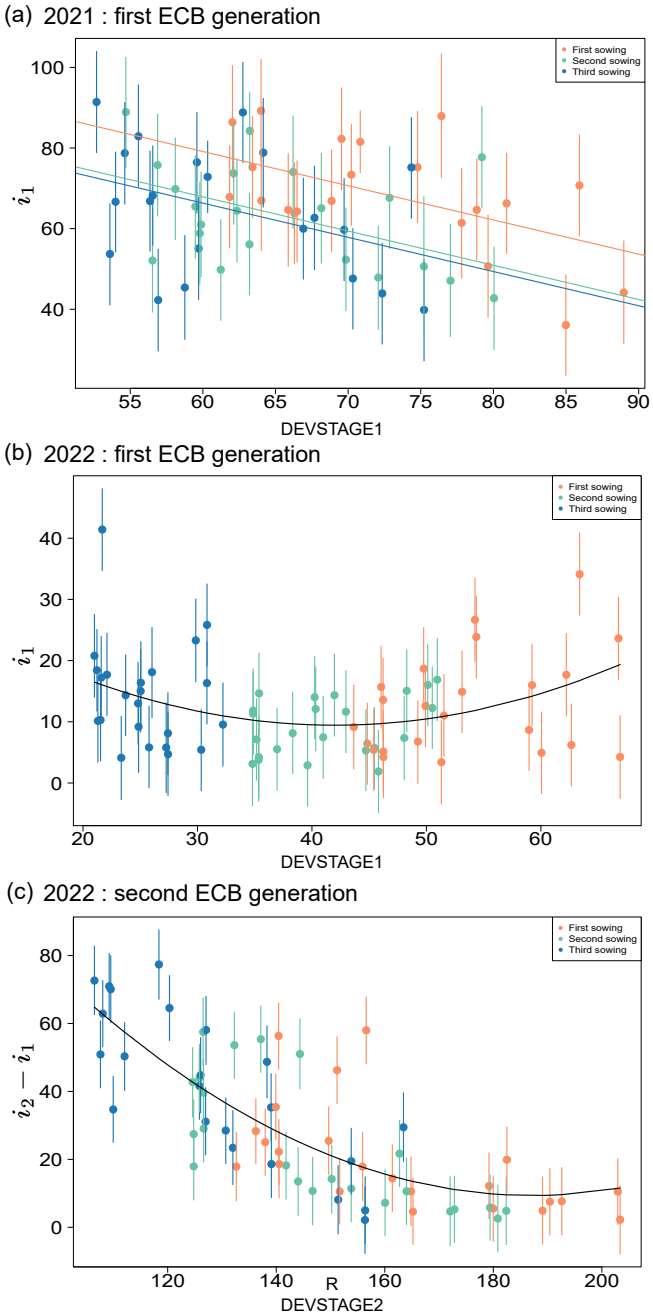

Figure 4: Relationship between the median proportion of infested plants and plant development in 2021 and 2022. Each point represents the estimated median proportion of infested plants for each inbred line sown at different dates (for both years, the first, second and third sowings are in orange, green and blue, respectively). The x-axis represents the fraction between the thermal time to flowering of each inbred line and the thermal time associated with the date at which plant development was studied. (a) Proportion of plants infested by the single pest generation in relation to plant development at the onset of the 2021 infestation. (b) Proportion of plants infested by the first generation of borers in 2022 in relation to plant development at the onset of the first infestation wave. (c) The proportion of plants infested by the second generation of borers in 2022 in relation to plant development at the onset of the second infestation wave. Error bars represent the 95% credible interval for each estimate.

Year	Infestation parameter	1st sowing date	2nd sowing date	3rd sowing date
2021	t_0	28.8 days [27.2,30.5]	23.7 days [22.1,25.3]	19.5 days [17.9,21]
2021	t_1	47.7 days [45.7,49.8]	42.6 days [40.6, 44.7]	40.9 days [38.9,42.9]
2021	r_1	3.0 [2.0, 4.0]	2.6 [1.6, 3.6]	1.2 [0.23, 2.8]
2021	i_1	68.5% [64.4, 72.6]	63.1% [58.9, 67.4]	64.6% [60.6, 68.7]
2022	t_0	23.9 days [20.3, 27.5]	28.5 days [25, 32.2]	24.1 days [20.3, 27.7]
2022	t_1	26.2 days [24.1, 28.2]	19.3 days [17.2, 21,4]	16.8 days [14.9, 18.7]
2022	r_1	3.1 [2.5, 3.7]	3.0 [2.4,3.7]	3.5 [2.9, 3.5]
2022	i_1	12.7% [11.2, 14.2]	9.2% [7.7, 10.7]	14.3% [12.8, 15.9]
2022	t_2	67.8 days [64.7, 71.11]	78.3 days [75.5, 81]	78.5 days [76.1, 80.8]
2022	r_2	2.25 [1.5, 3.5]	3.3 [2.3, 4.2]	3.8 [3.0, 4.5]
2022	$i_2 - i_1$	20%[16.8, 23.3]	23.8% [20.1, 27.1]	41.2% [38, 44.5]

Table 1: Median and 95 % high density interval of each of the infestation parameters by sowing date for each year.