Seed Science Research

cambridge.org/ssr

Differences in seed germination response of two populations of *Phelipanche ramosa* (L.) Pomel to a set of GR24 concentrations and durations of stimulation

Stéphanie Gibot-Leclerc[1], Manon Connault[1], Rémi Perronne[2] and Fabrice Dessaint[1]

[1]Agroécologie, AgroSup Dijon, INRAE, Univ. Bourgogne, Univ. Bourgogne-Franche-Comté, Dijon F-21000, France and [2]IGEPP, INRAE, Institut Agro, Univ. de Rennes, Le Rheu F-35653, France

Short Communication

Cite this article: Gibot-Leclerc S, Connault M, Perronne R, Dessaint F (2021). Differences in seed germination response of two populations of *Phelipanche ramosa* (L.) Pomel to a set of GR24 concentrations and durations of stimulation. *Seed Science Research* **31**, 243–248. https://doi.org/10.1017/S0960258521000143

Received: 21 December 2020
Revised: 21 April 2021
Accepted: 30 April 2021
First published online: 15 September 2021

Key words:
arable weed; dose-response; oilseed rape; parasitic plant; strigolactone; time-to-event approach; tobacco

Author for Correspondence:
Stéphanie Gibot-Leclerc,
E-mail: stephanie.gibot-leclerc@inrae.fr

Abstract

Phelipanche ramosa is a major weed holoparasite characterized by a broad host range with a suboptimal development on numerous hosts, suggesting inter- or intra-species specificities. Seeds of *P. ramosa* germinate after exposure to exogenous chemicals exuded by surrounding host roots such as strigolactones, the concentrations of these germination stimulants varying between hosts. In France, *P. ramosa* is characterized by genetically differentiated populations presenting varying germination rates and a host specificity. The objective of our study was to investigate the sensitivity of seeds of two *P. ramosa* populations harvested on tobacco and oilseed rape, to a set of GR24 concentrations, a synthetic strigol analogue. The assessment of the germination rate was based on *in vitro* experiments. Seeds of *P. ramosa* were placed in Petri dishes with various concentrations of GR24. The cumulative number of germinated seeds of *P. ramosa* was counted several times after application of the treatment. Cumulative germination curves were analysed using a three-parameter log-logistic model and a time-to-event approach. The results show that the germination rate of *P. ramosa* seeds depends on the GR24 concentration and the duration of stimulation, but also that the response to these two factors varies greatly according to the origin of the *P. ramosa* seeds. The difference in germination speed between *P. ramosa* populations further shows distinct responses at the intra-specific level, thus suggesting that the specialization of *P. ramosa* probably occurs at least from the first stage of the holoparasite cycle.

Introduction

Broomrapes form a large group of root holoparasites, that is obligate parasitic plants adapted to acquire resources from the vascular system of autotrophic host plants. Among these holoparasites, a few *Orobanche* and *Phelipanche* species have become major arable weeds, damaging crop production, thereby causing significant economic damage worldwide (Parker, 2009). Some of these problematic broomrapes are generalist parasitic weeds characterized by a broad host range, particularly *Phelipanche ramosa* (L.) Pomel (syn. *Orobanche ramosa* L.; Joel, 2009) known to parasitize many species from different plant families (Parker, 2009; Gibot-Leclerc et al., 2016; Perronne et al., 2017).

However, suboptimal developments of *P. ramosa* are observed on numerous host species, suggesting host specificities. These host specificities seem to depend on the compatibility between *P. ramosa* and the host due to a specific recognition mechanism based on chemical signalling. Indeed, from the first phenological stage of seed germination, host recognition appears particularly critical because the haustorium, that is the organ invading the host and forming a physical bridge between plants, must be fixed within a few days after germination to allow the survival and growth of the parasitic plant (Yoshida et al., 2006; Kokla and Melnyk, 2018; Clarke et al., 2019). This crucial stage of seed germination is a two-step process, first requiring a conditioning period initiated by seed imbibition under suitable environmental conditions during which seeds will not germinate in response to favourable stimuli, and then a period during which seeds become sensitive to germination stimulants, that is exogenous chemicals exuded by surrounding host roots (Auger et al., 2012; Brun et al., 2018). Concerning *P. ramosa*, two main classes of germination stimulants, strigolactones and isothiocyanates, can induce the seed germination (Fernández-Aparicio et al., 2011; Auger et al., 2012; Wang and Bouwmeester, 2018; Brun et al., 2019). Moreover, the rate of induction of seed germination of *P. ramosa* is known to greatly differ between host species of different families, different genera, within different species in a same genus as well as between varieties within a

CAMBRIDGE UNIVERSITY PRESS

crop (Zehhar et al., 2003; Fernández-Aparicio et al., 2009; Gauthier et al., 2012; Gibot-Leclerc et al., 2016; Perronne et al., 2017).

Moreover, these host specificities can also be related to genetic differences between *P. ramosa* populations, also named pathovars (Le Corre et al., 2014; Stojanova et al., 2019). Thus, the biological life cycle of *P. ramosa*, the seasonal variation in the dormancy and mortality of the seeds, the aggressiveness of this parasitic plant as well as its germination success can greatly differ between populations of *P. ramosa* harvested on different host crops (Brault et al., 2007; Gibot-Leclerc et al., 2013; Pointurier et al., 2019; Stojanova et al., 2019).

However, to our knowledge, no study has investigated whether differences in germination rates of *P. ramosa* seeds may depend on seed origin (particularly the host species infested), that is that different pathovars of *P. ramosa* may appear more or less sensitive to different concentrations of the same germination stimulant.

To test this hypothesis, we used two *P. ramosa* populations harvested on two major host crops: tobacco, a long-known host of *P. ramosa* (Brault et al., 2007), and oilseed rape, a new preferred host with a massive expansion in France since the beginning of the 1990s (Gibot-Leclerc et al., 2012). For each population, we stimulated seed germination with different concentrations of the synthetic germination stimulant GR24 (Mangnus et al., 1992a). These *in vitro* experiments are widely considered as a standard in parasitic research to assess the optimal germination rate of several root holoparasite species, including *P. ramosa* (Mangnus et al., 1992b; Gibot-Leclerc et al., 2004). Based on the rational above, we addressed a set of questions:

(1) What is the sensitivity of the two *P. ramosa* populations to the GR24 germination stimulant concentrations and does this sensitivity to the germination stimulant differ between these populations?
(2) How the germination speed differs between *P. ramosa* populations for the different GR24 concentrations tested?
(3) How the maximum germination rate varies between *P. ramosa* populations?

Material and methods

Seed material

Seeds of *P. ramosa* were collected from natural populations within highly infested arable fields of winter oilseed rape (population R) in 2012 (45°56′41.363″N, 0°31′3.947″W; Saint-Jean-d'Angély) and of tobacco (population T) in 2017 (45°53′38.908″N, 0°0′36.803″E; Aigre) located in the Nouvelle-Aquitaine region. These populations can be genetically differentiated, with seeds from one oilseed rape field belong to the genetic group named 'Genetic group 1' comprising populations able to infest winter oilseed rape (Stojanova et al., 2019), and seeds from one tobacco field belong to a distinct genetic group named 'Genetic clust 05' comprising populations able to infest tobacco. All seeds were kept in watertight glass containers at approximately 20°C until the beginning of the experiments.

Seed conditioning treatments and seed viability determination

Before any *in vitro* experiment, *P. ramosa* seeds were surface disinfected under a laminar flow hood by a 5-min immersion in 70% ethanol, followed by a 5-min immersion in a solution of $Ca(OCl)_2$ at 3% (p/v) and Tween 20 (0.1%) to limit fungal spread (Gibot-Leclerc et al., 2012). They were then rinsed five times with twice-distilled water. After disinfection, seeds of *P. ramosa* were placed on a Whatman® GF/A paper sheet (Ø 90 mm) at the bottom of a Petri dish (Ø 90 mm) and hydrated with 3 ml sterile distilled water. Petri dishes were sealed with Parafilm® (American Can Company), wrapped in aluminium foil and placed in a dark growth chamber, at 20°C during 14 d, to condition *P. ramosa* seeds and thus make them susceptible to germination stimulants (Gibot-Leclerc et al., 2004).

Moreover, tests performed prior to the seed germination assay using 2,3,5-triphenyl tetrazolium chloride (TTC) were made to ensure the viability of seed lots of *P. ramosa* following the procedure described in Gibot-Leclerc et al. (2004). The viability of seed lots was high, ranging from 87 to 97%.

Seed germination assay

The germination experiment started on February 2018. After conditioning, *P. ramosa* seeds were moistened with 2 ml of the germination-triggering strigol analogue GR24 (provided by Dr. Binne Zwanenburg, Radboud University, Nijmegen, the Netherlands) at various concentrations. A preliminary analysis indicated that the sensitivity of the two *P. ramosa* populations to GR24 was different. To optimize the estimation of parameters of the cumulative germination curves based on equations defined below, we have, therefore, adjusted the concentration ranges of GR24. Eleven concentrations of GR24 to the population R (10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}, 10^{-1} and 1 mg l^{-1}) and 12 concentrations of GR24 to the population T (10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 2×10^{-3}, 4×10^{-3}, 6×10^{-3}, 8×10^{-3}, 10^{-2}, 10^{-1}, 1, 10 mg l^{-1}) were tested, as well as a control without GR24 for both populations. For preparing these solutions, 1 mg of GR24 was dissolved in 1 ml of acetone and then diluted in distilled water to obtain the appropriate concentrations. The Petri dishes were placed at 20°C in darkness. One Petri dish was prepared for each GR24 concentration and each *P. ramosa* population. Each Petri dish contains on average 290 ± 85 (mean ± SD) seeds for population R and on average 192 ± 42 seeds for population T. The cumulative number of germinated seeds of *P. ramosa* was counted 3, 4, 5, 6, 7 and 10 d after application of the treatment condition using a stereoscopic microscope (1.95–250×). A seed was considered germinated when the radicle had pierced the seed coat.

Statistical analyses

Cumulative germination curves were analysed using a three-parameter log-logistic model and a time-to-event approach (Onofri et al., 2010; Ritz et al., 2013). In this approach, characterizing the temporal pattern of germination of *P. ramosa* seeds, we have modelled the time taken by a seed to germinate.

The three-parameter log-logistic equation used is as follows, according to Ritz et al. (2013):

$$F(t) = \frac{G_{\max}}{1 + \exp[b\log(t) - \log(t_{50})]}$$

with b, the slope around the inflexion point, $G_{\max}$ the proportion of germinated seeds at the end of the experiment (higher asymptote, also named upper limit parameter d) and t_{50}, the median

https://doi.org/10.1017/S0960258521000143 Published online by Cambridge University Press

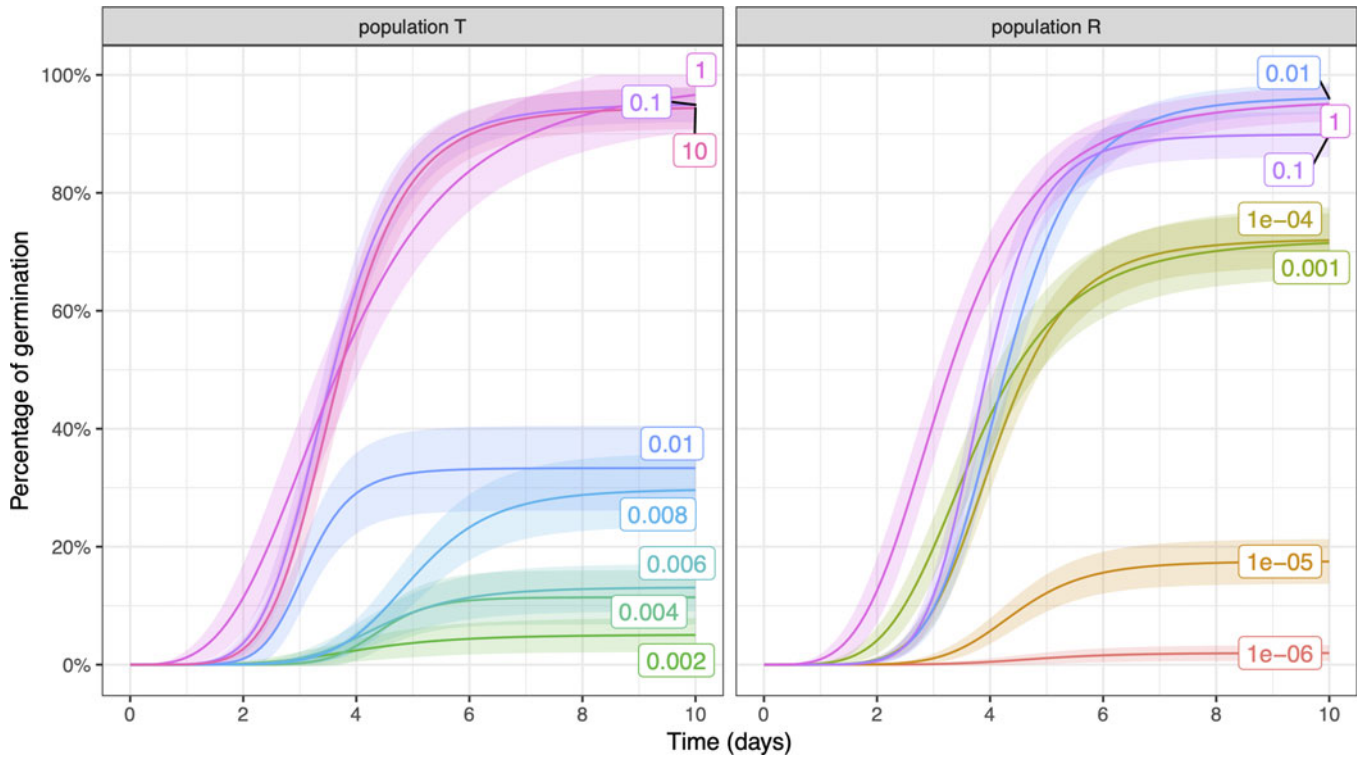

Fig. 1. Time-to-event approach of seed germination for *P. ramosa* population T (left) and population R (right). Cumulative fitted germination curves, and estimated 95% confidence interval based on the time-to-event model.

time (also named inflexion point), that is the time to have 50% of the maximum germination ($G_{max}/2$). In this model, it was assumed that the lower asymptote was equal to 0. The time-to-event approach accounts for particular features characterizing the type of data, that is right-censored observations and monitoring intervals (Ritz et al., 2013). In our study, the concentrations of GR24 tested differing partially between the populations R and T, and the time-to-event approach was carried out separately for each population. The fitted models were furthermore used to derive the germination time for the 10th, 20th, 30th and 50th percentiles (thereafter named T_{10}, T_{20}, T_{30} and T_{50}) of the total seed population. The choice of using different percentiles was based on the fact that in our study, the G_{max} differed widely among GR24 concentrations and in several cases, G_{max} was thus lower than 50%. All analyses were made with R version 4.0.2 (R Core Team, 2020) and the drc package version 3.0-1 (Ritz et al., 2015).

Results

No seed germination of *P. ramosa* was observed for the control without GR24, as well as for concentrations of GR24 lower than or equal to 10^{-3} mg l^{-1} for population T (tobacco) and 10^{-7} mg l^{-1} for population R (oilseed rape). In the following, only the concentrations of GR24 that induced seed germinations are shown as cumulative germination curves (Fig. 1). Moreover, for both populations and the various concentrations retained, all parameters of the three-parameter log-logistic model were significantly different from 0 ($p < 0.001$).

Concerning population T, the concentrations of GR24 ≥ 0.1 mg l^{-1} induced a maximum percentage of germinated seeds 10 d after application (G_{max}) higher than 90%, while for lower concentrations, G_{max} was consistently lower than 50% (Table 1, Fig. 1). Moreover, the time required to reach 50% of the maximum germination (t_{50}) ranged between 3.5 and 3.7 d for the concentrations of GR24 ≥ 0.1 mg l^{-1}, while more than 4 d for the concentrations of GR24 < 0.01 mg l^{-1} (Table 1). The 0.01 mg l^{-1} GR24 concentration exhibited an intermediate result with a G_{max} value close to that of lower concentrations and a t_{50} close to that of higher concentrations (Table 1). For concentrations of GR24 < 0.1 mg l^{-1}, the time required to reach the 10th, 20th and 30th percentiles (T_{10}, T_{20} and T_{30}) of the total seed population was longer than for higher concentrations tested (Table 1). Moreover, T_{50} could not be calculated for concentrations of GR24 < 0.1 mg l^{-1}, but appeared quite similar to t_{50} for concentrations of GR24 ≥ 0.1 mg l^{-1}.

Concerning population R, the concentrations of GR24 ≥ 0.01 mg l^{-1} induced a G_{max} higher than 90%, and the concentrations ≥10^{-4} mg l^{-1} a G_{max} higher than 70%, while only the lowest concentrations of GR24 tested induced less than 20% of germinated seeds 10 d after application (Table 1, Fig. 1). Moreover, the median time (t_{50}) showed a decreasing trend between 10^{-6} and 10^{-3} mg l^{-1}, followed by an increase for 10^{-2} mg l^{-1}, and then a further decrease (Table 1). The lowest median time was reached for the highest concentration of GR24 tested (Table 1). For concentrations of GR24 < 0.01 mg l^{-1}, the time required to reach the 10th, 20th, 30th and 50th percentiles (T_{10}, T_{20}, T_{30} and T_{50}) of the total seed population were often longer than for higher concentrations tested, with however several exceptions (Table 1). As for population T, T_{50} appeared quite similar to t_{50} for concentrations of GR24 ≥ 0.01 mg l^{-1}.

Discussion

No germination of *P. ramosa* seeds was observed either in the absence, or at too low concentrations of the germination

https://doi.org/10.1017/S0960258521000143 Published online by Cambridge University Press

Table 1. Germination time (d, with 95% IC in brackets) for different percentiles (T_{10}, T_{20}, T_{30} and T_{50} calculated on the total seed population) and t_{50} (the median time, that is the time to have 50% of the maximum germination G_{max}), and G_{max} (the percentage of germinated seeds of *P. ramosa* at the end of the experiment, that is 10 d) for population T (top) and population R (bottom). More details about the parameters in the main text

GR24 concentrations (mg l^{-1})	Germination time (days)					G_{max} (%)
	T_{10}	T_{20}	T_{30}	T_{50}	t_{50}	
Population T						
2×10^{-3}	–	–	–	–	4.09 [3.16; 5.02]	5 [2; 8]
4×10^{-3}	5.39 [4.79; 5.99]	–	–	–	4.41 [4.06; 4.76]	11 [7; 16]
6×10^{-3}	5.35 [4.76; 5.95]	–	–	–	4.44 [4.01; 4.87]	13 [9; 17]
8×10^{-3}	4.55 [4.24; 4.87]	5.55 [5.16; 5.93]	–	–	5.02 [4.69; 5.34]	30 [24; 36]
0.01	2.81 [2.59; 3.02]	3.29 [3.08; 3.51]	4.14 [3.72; 4.57]	–	3.13 [3.37; 3.68]	33 [26; 41]
0.10	2.42 [2.25; 2.59]	2.79 [2.64; 2.95]	3.08 [2.93; 3.22]	3.59 [3.43; 3.74]	3.53 [3.37; 3.68]	95 [92; 98]
1.0	1.92 [1.59; 2.25]	2.44 [2.11; 2.77]	2.87 [2.53; 3.20]	3.69 [3.33; 4.05]	3.69 [3.33; 4.05]	100 [93; 100]
10.0	2.54 [2.33; 2.75]	2.92 [2.73; 3.11]	3.20 [3.03; 3.38]	3.72 [3.54; 3.90]	3.65 [3.47; 3.83]	95 [91; 98]
Population R						
10^{-6}	–	–	–	–	4.82 [3.83; 5.81]	2 [1; 3]
10^{-5}	4.63 [4.33; 4.93]	–	–	–	4.45 [4.16; 4.73]	18 [14; 21]
10^{-4}	3.03 [2.88; 3.19]	3.49 [3.35; 3.63]	3.86 [3.72; 4.00]	4.66 [4.48; 4.84]	4.08 [3.94; 4.23]	72 [68; 77]
10^{-3}	2.48 [2.24; 2.72]	3.01 [2.79; 3.23]	3.44 [3.22; 3.67]	4.44 [4.12; 4.76]	3.72 [3.48; 3.95]	72 [66; 78]
0.01	3.02 [2.85; 3.20]	3.43 [3.27; 3.59]	3.74 [3.58; 3.89]	4.28 [4.12; 4.44]	4.23 [4.07; 4.39]	96 [94; 99]
0.10	2.93 [2.79; 3.07]	3.26 [3.13; 3.39]	3.51 [3.39; 3.63]	3.96 [3.83; 4.09]	3.84 [3.72; 3.97]	90 [86; 94]
1.0	1.87 [1.62; 2.13]	2.30 [2.07; 2.54]	2.64 [2.42; 2.86]	3.29 [3.07; 3.51]	3.22 [3.01; 3.44]	96 [93; 99]

stimulant, confirming that the induction of seed germination of this parasitic plant requires a minimum concentration of stimulant, in accordance with previous studies (Gibot-Leclerc et al., 2004; Fernández-Aparicio et al., 2011; Matusova et al., 2014; Huet et al., 2020). In our study, following a conditioning period, the germination of *P. ramosa* seeds was induced by concentrations of GR24 as low as 10^{-6} mg l^{-1}, comparable to GR24 concentrations observed in other studies (Fernández-Aparicio et al., 2011; Matusova et al., 2014), although lower concentrations can also induce germination (Huet et al., 2020). Moreover, we highlighted a marked difference in sensitivity to the germination stimulant between the two *P. ramosa* populations studied, the *P. ramosa* seeds of the R (oilseed rape) population appearing more sensitive to GR24 than the *P. ramosa* seeds of the T (tobacco) population by a factor 10^4. *P. ramosa* populations are well known to be characterized by highly variable germination rates between host species. Thus, Gibot-Leclerc et al. (2016) showed that in the Brassicaceae family, the germination rate of *P. ramosa* depends on the origin of the seeds (oilseed rape or tobacco) and host species with germination rates higher than its crop hosts depending of the species, a similar pattern being observed in the Fabaceae family (Perronne et al., 2017). The marked difference in sensitivity to a germination stimulant could, therefore, be due to the ability of *P. ramosa* to produce seeds able to infest and develop on different hosts during the crop sequence. In poorly diversified crop consequences (i.e. tobacco monoculture or rapeseed-wheat rotation), this ability can result in the selection of populations that are highly adapted to the crop, as observed on other parasitic plants (Dor et al., 2020).

The median time (t_{50}) also differed both within and between *P. ramosa* populations under the different GR24 concentrations tested, a result confirmed by calculating the germination time for different percentiles of the total seed population (T_{10}, T_{20}, T_{30} and T_{50}) when possible. For these two calculation methods, the germination speed appears high at the higher concentrations of GR24 tested and overall low at lower concentrations for both *P. ramosa* populations, although values do not change monotonically with the level of GR24 concentration. Moreover, the germination speed partly differed between the two *P. ramosa* populations for a similar GR24 concentration, for example from around 3.7 d for population T to around 3.3 d for population R at 1 mg l^{-1}, even though at these high concentrations, values of t_{50} and T_{50} were broadly similar. At concentrations higher than 0.1 mg l^{-1}, for which the maximum germination rate was higher than 90% for both *P. ramosa* populations, the germination speed always seemed to be less than 4 d. A fast germination appears particularly crucial for *P. ramosa* because, in the absence of fixation on host roots, *P. ramosa* seeds degenerated and died within a few days (Gibot-Leclerc et al., 2012), thus potentially explaining faster germination at high concentrations.

For both *P. ramosa* populations and all GR24 concentrations, the germination rate reached a maximum before the end of the experiment (10 d). For high GR24 concentrations, the germination percentage was around 90%. These results are in accordance with previous dose-response studies showing that *P. ramosa* seeds reach a higher asymptote, frequently higher than 90%, in response to GR24 (Gibot-Leclerc et al., 2004; Matusova et al., 2014; Huet et al., 2020). For low GR24 concentrations, the maximum germination rate was lower, to be compared to a viability of seed lots ranging from 87 to 97% in our study, suggesting that the remaining viable seeds are no longer stimulated by GR24. Moreover, the maximum germination can greatly differ between the two *P.*

https://doi.org/10.1017/S0960258521000143 Published online by Cambridge University Press

ramosa populations for a similar GR24 concentration, for example from 33% for population T to 96% for population R at 0.01 mg l^{-1}. These differences were also observed by Matusova et al. (2014) between two geographically distinct *P. ramosa* populations harvested from different host crops. Our observations of a higher asymptote (G_{max}) differing between concentrations of the germination stimulant for the same *P. ramosa* population, or between *P. ramosa* populations for a same GR24 concentration, suggest a response to a limiting factor for which the minimum level to reach a high maximum germination rate is variable between populations. This suggestion might be consistent with the hypothesis that the strigolactone receptor of parasitic plants could use a mode of signal perception involving the hydrolysis of the bound hormone (Brun et al., 2018; Bürger and Chory, 2020). Following this hypothesis, we could suppose both that the GR24 concentration in the environment allows the induction of germination of most seeds only from a given non-limiting threshold of this strigol analogue, and that the receptors of the *P. ramosa* population R require a lower concentration compared with the population T, thus achieving a high germination rate at a lower threshold. Moreover, this hypothesis would be consistent with the observations presented above related to the differences in sensitivity to the germination stimulant between *P. ramosa* populations.

To summarize, our study showed that the germination rate of *P. ramosa* seeds depended on the GR24 concentration and the duration of stimulation. In addition, this study highlighted that these two parameters could greatly vary according to the origin of the *P. ramosa* seeds. The synthetic germination stimulant GR24 being widely used as a standard in germination studies of parasitic plants to assess the germination rate, and it follows that its optimal use requires preliminary analyses to define the duration and the optimal concentration. Moreover, studies have also shown the influence of the GR24 used, that is stereoisomers or mix of racGR24, on the germination rate at different concentrations of the germination stimulant (e.g. Huet et al., 2020). Finally, it is important to precise that tobacco and oilseed rape produce germination stimulants that differ between these crops, as well as compared with GR24 (Xie et al., 2007; Auger et al., 2012), and that a cross-evaluation of the effect of these stimulants would also allow characterizing host specificities.

The presence of ecotypes in *P. ramosa* with different life cycles duration has already been observed. For example, the *P. ramosa* tobacco population was reported to be able to reproduce on both oilseed rape and tomato, whereas the tomato lifespan was too short for the *P. ramosa* oilseed rape population to produce seeds over its life cycle (Gibot-Leclerc et al., 2013). The difference in germination speed between *P. ramosa* populations observed in our study shows further distinct responses at the intraspecific scale, in addition to distinct seasonal variation of seed dormancy (Pointurier et al., 2019), aggressiveness (Gibot-Leclerc et al., 2013) already reported among these populations. Our study thus suggests that the specialization of *P. ramosa* probably occurs at least from the first stage of the cycle, that is the underground contact between the seeds of the broomrape and host plant root exudates (Fernández-Aparicio et al., 2011; Gibot-Leclerc et al., 2016; Perronne et al., 2017).

More recently, the populations studied have been identified as genetically differentiated (Stojanova et al., 2019). This type of differentiation has also been described in native populations of *Orobanche minor* where ISSR markers provided preliminary evidence of host-driven divergence of the coastal clade *O. minor* ssp. *maritima* growing on sea carrot (*Daucus carota* ssp. *gummifer*) from the host generalist lineage *O. minor* var. *minor* growing on clover (*Trifolium pratense*) (Thorogood et al., 2008, 2009).

From an evolutionary point of view, the acquisition of this response could reflect an optimization of the expansion strategy of *P. ramosa* by broadening its spectrum of potential hosts due to an intraspecific specialization.

Acknowledgements. We thank Philippe Simier and Jean-Bernard Pouvreau (LBPV, University of Nantes, France) for their safe contribution to the sampling of oilseed rape-infested areas and Sarah Huet (Agroécologie, Dijon, France) for our discussion on this work.

Financial support. This work was supported by CASDAR C201307 PHERAFAB and CASDAR FAM 201939 ELIOT.

Conflicts of interest. The authors declare that there are no conflicts of interest regarding the publication of this paper.

References

Auger B, Pouvreau JB, Pouponneau K, Yoneyama K, Montiel G, Le Bizec B, Yoneyama K, Delavault P, Delourme R and Simier P (2012) Germination stimulants of *Phelipanche ramosa* in the rhizosphere of *Brassica napus* are derived from the glucosinolate pathway. *Molecular Plant-Microbe Interactions* **25**, 993–1004. doi:10.1094/MPMI-01-12-0006-R.

Brault M, Betsou F, Jeune B, Tuquet C and Sallé G (2007) Variability of *Orobanche ramosa* populations in France as revealed by cross infestations and molecular markers. *Environmental and Experimental Botany* **61**, 272–280. doi:10.1016/j.envexpbot.2007.06.009.

Brun G, Braem L, Thoiron S, Gevaert K, Goormachtig S and Delavault P (2018) Seed germination in parasitic plants: what insights can we expect from strigolactone research? *Journal of Experimental Botany* **69**, 2265–2280. doi:10.1093/jxb/erx472.

Brun G, Thoiron S, Braem L, Pouvreau JB, Montiel G, Lechat MM, Simier P, Gevaert K, Goormachtig S and Delavault P (2019) CYP707As are effectors of karrikin and strigolactone signalling pathways in *Arabidopsis thaliana* and parasitic plants. *Plant Cell & Environment* **42**, 2612–2626. doi:10.1111/ pce.13594.

Bürger M and Chory J (2020) The many models of strigolactones signaling. *Trends in Plant Science* **25**, 395–405. doi:10.1016/j.tplants.2019.12.009.

Clarke CR, Timko MP, Yoder JI, Axtell MJ and Westwood JH (2019) Molecular dialog between parasitic plants and their hosts. *Annual Review of Phytopathology* **57**, 279–299. doi:10.1146/annurev-phyto-082718-100043.

Dor E, Plakhine D, Joel DM, Larose H, Westwood JH, Smirnov E, Ziadna H and Hershenhorn J (2020) A new race of sunflower broomrape (*Orobanche cumana*) with a wider host range due to changes in seed response to strigolactones. *Weed Science* **68**, 134–142. doi:10.1017/wsc.2019.73.

Fernández-Aparicio M, Flores F and Rubiales D (2009) Recognition of root exudates by seeds of broomrape (*Orobanche* and *Phelipanche*) species. *Annals of Botany* **103**, 423–431. doi:10.1093/aob/mcn236.

Fernández-Aparicio M, Yoneyama K and Rubiales D (2011) The role of strigolactones in host specificity of *Orobanche* and *Phelipanche* seed germination. *Seed Science Research* **21**, 55–61. doi:10.1017/S0960258510000371.

Gauthier M, Véronési C, El-Halmouch Y, Leflon M, Jestin C, Labalette F, Simier P, Delourme R and Delavault P. (2012) Characterisation of resistance to branched broomrape, *Phelipanche ramosa*, in winter oilseed rape. *Crop Protection* **42**, 56–63. doi:10.1016/J.CROPRO.2012.07.002.

Gibot-Leclerc S, Corbineau F, Sallé G and Côme D (2004) Responsiveness of *Orobanche ramosa* L. Seeds to GR 24 as related to temperature, oxygen availability and water potential during preconditioning and subsequent germination. *Plant Growth Regulation* **43**, 63–71. doi:10.1023/B:GROW.0000038242.77309.73.

Gibot-Leclerc S, Sallé G, Reboud X and Moreau D (2012) What are the traits of *Phelipanche ramosa* (L.) Pomel that contribute to the success of its biological cycle on its host *Brassica napus* L.? *Flora – Morphology, Distribution, Functional Ecology of Plants* **207**, 512–521. doi:10.1016/j.flora.2012.06.011.

Gibot-Leclerc S, Dessaint F, Reibel C and Le Corre V (2013) *Phelipanche ramosa* (L.) Pomel populations differ in life-history and infection response to hosts. *Flora – Morphology, Distribution, Functional Ecology of Plants* **208**, 247–252. doi:10.1016/j.flora.2013.03.007.

Gibot-Leclerc S, Perronne R, Dessaint F, Reibel C and Le Corre V (2016) Assessment of phylogenetic signal in the germination ability of *Phelipanche ramosa* on Brassicaceae hosts. *Weed Research* **56**, 452–461. doi:10.1111/wre.12222.

Huet S, Pouvreau JB, Delage E, Delgrange S, Marais C, Bahut M, Delavault P, Simier P and Poulin L (2020) Populations of the parasitic plant *Phelipanche ramosa* influence their seed microbiota. *Frontiers in Plant Sciences* **11**, 1075. doi:10.3389/fpls.2020.01075.

Joel DM (2009) The new nomenclature of *Orobanche* and *Phelipanche*. *Weed Research* **49**, 6–7. doi:10.1111/j.1365-3180.2009.00748.x.

Kokla A and Melnyk CW (2018) Developing a thief: haustoria formation in parasitic plants. *Developmental Biology* **442**, 53–59. doi:10.1016/j.ydbio.2018.06.013.

Le Corre V, Reibel C and Gibot-Leclerc S (2014) Development of microsatellite markers in the branched broomrape *Phelipanche ramosa* L. (Pomel) and evidence for host-associated genetic divergence. *International Journal of Molecular Science* **15**, 994–1002. doi:10.3390/ijms15010994.

Mangnus EM, Dommerholt FJ, De Jong RLP and Zwanenburg B (1992a) Improved synthesis of strigol analog GR24 and evaluation of the biological activity of its diastereomers. *Journal of Agricultural and Food Chemistry* **40**, 1230–1235. doi:10.1021/jf00019a031.

Mangnus EM, Stommen PLA and Zwanenburg B (1992b) A standardized bioassay for evaluation of potential germination stimulants for seeds of parasitic weeds. *Journal of Plant Growth Regulation* **11**, 91. doi:10.1007/BF00198020.

Matusova R, Kullacova D and Tóth P (2014) Response of weedy and non-weedy broomrapes to synthetic strigolactone analogue GR24. *Journal of Central European Agriculture* **15**, 72–82. doi:10.5513/JCEA01/15.4.1511.

Onofri A, Gresta F and Tei F (2010) A new method for the analysis of germination and emergence data of weed species. *Weed Research* **50**, 187–198. doi:10.1111/j.1365-3180.2010.00776.x.

Parker C (2009) Observations on the current status of *Orobanche* and *Striga* problems worldwide. *Pest Management Science* **65**, 453–459. doi:10.1002/ps.1713.

Perronne R, Gibot-Leclerc S, Dessaint F, Reibel C and Le Corre V (2017) Is induction ability of seed germination of *Phelipanche ramosa* phylogenetically structured among hosts? A case study on Fabaceae species. *Genetica* **145**, 481–489. doi:10.1007/s10709-017-9990-x.

Pointurier O, Gibot-Leclerc S, Le Corre V, Reibel C, Strbik F and Colbac N (2019) Intraspecific seasonal variation of dormancy and mortality of *Phelipanche ramosa* seeds. *Weed Research* **50**, 407–418. doi:10.1111/wre.12378.

R Core Team (2020) *R: a language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from: http://www.R-project.org/.

Ritz C, Pipper CB and Streibig JC (2013) Analysis of germination data from agricultural experiments. *European Journal of Agronomy* **45**, 1–6. doi:10.1016/j.eja.2012.10.003.

Ritz C, Baty F, Streibig JC and Gerhard D (2015) Dose-response analysis using R. *PLoS One* **10**, e0146021. doi:10.1371/journal.pone.0146021.

Stojanova B, Delourme R, Duffé P, Delavault P and Simier P (2019) Genetic differentiation and host preference reveal non-exclusive host races in the generalist parasitic weed *Phelipanche ramosa*. *Weed Research* **59**, 107–118. doi:10.1111/wre.12353.

Thorogood CJ, Rumsey FJ, Harris SA and Hiscock SJ (2008) Host-driven divergence in the parasitic plant *Orobanche minor* Sm. (Orobanchaceae). *Molecular Ecology* **17**, 4289–4303. doi:10.1111/j.1365-294X.2008.03915.x.

Thorogood CJ, Rumsey FJ, Harris SA and Hiscock SJ (2009) Gene flow between alien and native races of the holoparasitic angiosperm *Orobanche minor* (Orobanchaceae). *Plant Systematics and Evolution* **282**, 31–42. doi:10.1007/s00606-009-0204-6.

Wang Y and Bouwmeester HJ (2018) Structural diversity in the strigolactones. *Journal of Experimental Botany* **69**, 2219–2230. doi:10.1093/jxb/ery091.

Xie X, Kusumoto D, Takeuchi Y, Yoneyama K, Yamada Y and Yoneyama K (2007) 2'-epi-orobanchol and solanacol, two unique strigolactones, germination stimulants for root parasitic weeds, produced by tobacco. *Journal of Agricultural and Food Chemistry* **55**, 8067–8072. doi:10.1021/jf0715121.

Yoshida S, Cui S, Ichihashi Y and Shirasu K (2006) The haustorium, a specialized invasive organ in parasitic plants. *Annual Review of Plant Biology* **67**, 643–667. doi:10.1146/annurev-arplant-043015-111702.

Zehhar N, Labrousse P, Arnaud MC, Boulet C, Bouya D and Fer A (2003) Study of resistance to *Orobanche ramosa* in host (oilseed rape and carrot) and non-host (maize) plants. *European Journal of Plant Pathology* **109**, 75–82. doi:10.1023/A:1022060221283.