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RESEARCH ARTICLE

Unexpected fast development of branched broomrape on slow-growing Brassicaceae

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Abstract In France, oilseed rape is getting highly infected since 1990 by the branched broomrape *Phelipanche ramosa* (L.) Pomel. Branched broomrape infection causes serious yield losses ranging from 5 to 100 %, notably in the Mediterranean area. *P. ramosa* is parasiting the plant roots. The growth of *P. ramosa* on Brassicaceae weeds has not been studied quantitatively so far, except for the model species *Arabidopsis thaliana*. Since *P. ramosa* has a fast development rate on the fast-growing *A. thaliana*, *P. ramosa* development should be slower on other slower-growing Brassicaceae species. Here, we cultivated in the laboratory seven Brassicaceae weed species including *Capsella bursa-pastoris*, *Capsella rubella*, *Cardamine hirsuta*, *Lepidium campestre*, *Lepidium draba*, *Sinapis arvensis* and *A. thaliana* as control, during 3 weeks. We counted the number of *P. ramosa* individuals that have reached the following growth stages: germination, attachment, tubercle, bud and underground stem. We then assessed the development rate of *P. ramosa* by calculating the odds ratio of attachment or higher development stages of *P. ramosa* on Brassicaceae, with *A. thaliana* as the reference. We found that five Brassicaceae species had an odds ratio ranging from 0.9 to 2.4. These ratios are thus similar or higher than that of the *A. thaliana* reference. This finding shows for the first time that *P. ramosa* develops faster on the five Brassicaceae species. This finding is also unexpected because *A. thaliana* is a fast-growing plant, whereas the five Brassicaceae species have a longer life cycle. Therefore, this observation demonstrates for the first time that *P. ramosa* development depends on others factors than the speed of plant host development.

Keywords *Phelipanche ramosa* · Parasitic plant · *Arabidopsis thaliana* · *Capsella* · *Cardamine* · *Lepidium* · *Sinapis*

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1 Introduction

Among flowering plants, approximately 3,000 species (1 %) are parasitic. These parasitic plants form a close connection with the vascular system of their host plant through a specialised organ, the haustorium, through which they remove water, mineral salts and carbon elements (Parker and Riches 1993; Press and Graves 1995). Branched broomrape—*Phelipanche ramosa* (L.) Pomel (syn. *Orobanche ramosa*)—is a root-holoparasitic angiosperm known to be the cause of crop losses ranging from 5 to 100 %, particularly in countries surrounding the Mediterranean basin (Parker 2009; Press and Phoenix 2005). In Central Europe, populations have mainly infested tobacco (*Nicotiana tabacum* L., Solanaceae) and hemp (*Cannabis sativa* L., Cannabinaceae) fields (Buschmann et al. 2005). In France, oilseed rape (*Brassica napus* L., Brassicaceae) is a new preferred *P. ramosa* host, with a massive extension of the parasite since the beginning of the 1990s and yield losses over 80 % (Gibot-Leclerc et al. 2003, 2012, 2013a). This crop has even become the primary host for the parasite, along with *C. sativa* and *N. tabacum* (Benharrat et al. 2005; Brault et al. 2007). Several authors reported a fine-tuning between the host crop and parasite development cycles, depending on the host species considered, with a corresponding adjustment of growth and an ability to adjust flowering according to the amount of

resources gained from the host (Brault et al. 2007; Gibot-Leclerc et al. 2012; Kogan 1994; Labrada 1994).

Phelipanche ramosa can also infect more than 70 weed species in *B. napus* fields strongly infested by the parasite and thus persist and even proliferate in the absence of host crops (Boulet et al. 2001; Gibot-Leclerc et al. 2003, 2013b). Numerous Brassicaceae weed species are known to be both infected by *P. ramosa* and abundant within *B. napus* fields since herbicides selective for oilseed rape have a low efficacy on these closely related species (Fried and Reboud 2007; Gibot-Leclerc et al. 2003). Among Brassicaceae weeds, thale cress (*Arabidopsis thaliana* (L.) Heynh.) is widely used as a useful model for the study of plant biology, including plant–plant interactions (Mienke et al. 1998; Goldwasser et al. 2002; Bouwmeester et al. 2003). *P. ramosa* infection on *A. thaliana* is similar to that on agricultural hosts. However, the parasite developing on *A. thaliana* has a proportionally faster development rate (45 days) and smaller size at maturity (1–4 flowers) than *P. ramosa* developing on host crops (Goldwasser and Yoder 2001; Goldwasser et al. 2002, 2008; Neumann and Sallé 2000). The dynamics of growth and development of *P. ramosa* on other Brassicaceae weeds has not been described so far. In this study, we analyse how fast *P. ramosa* can grow and develop on representative Brassicaceae weeds, in comparison to *A. thaliana*. Our results provide new insights about the likely influence of Brassicaceae weeds on the parasite extension in *B. napus* fields.

2 Materials and methods

2.1 Seed material

Parasite seeds were collected from natural populations of *P. ramosa* that had severely infested tobacco fields in Priaires (46° 08′ 31″ N, 00° 36′ 24″ W; Deux-Sèvres, France) in 2002. Once harvested, the seeds were sifted for cleaning. We chose to study the development of *P. ramosa* on six Brassicaceae weed species that are commonly found in oilseed rape fields (Fried and Reboud 2007; Gibot-Leclerc et al. 2003): shepherd's purse (*Capsella bursa-pastoris* (L.) Medik.), red shepherd's purse (*Capsella rubella* Reut.), hairy bittercress (*Cardamine hirsuta* L.), field pepperweed (*Lepidium campestre* (L.) R. Br.), whitetop (*Lepidium draba* L.) and wild mustard (*Sinapis arvensis* L.). *A. thaliana* was included as a control since it was previously used to study the germination, haustorium development and growth of *P. ramosa* under controlled conditions (Goldwasser and Yoder 2001; Goldwasser et al. 2002, 2008; Neumann and Sallé 2000). Seeds of the seven Brassicaceae were bought from the seed company Herbiseed in 2010. After collection, all seeds were kept in watertight glass containers at room temperature (approximately 20 °C) until the beginning of the experiments.

2.2 In vitro experiments

After disinfection, 75–100 seeds of *P. ramosa* were laid on Whatman GF/A paper discs (Ø 12 mm). Five discs prepared that way were placed on a Whatman GF/A paper sheet (Ø 90 mm) at the bottom of a Petri dish (Ø 90 mm), and then hydrated. Three Petri dishes, containing 375–500 seeds each, were placed in darkness at 20 °C for 14 days to pre-condition the seeds. Thereafter, three disinfected Brassicaceae weed seeds were placed in a rectangular plastic box containing MS (1/2) (Murashige and Skoog 1962) nutrient solution with 1 % agar. The box prepared that way was placed in a growth chamber, at 23±1 °C (day) and 18±1 °C (night), with a 16-h photoperiod and 70 mol m^{-2} s^{-1} photosynthetic photon flux density (PPFD). Once the Brassicaceae weed root system had developed (i.e. 2 weeks after the beginning of the experiment), the five discs bearing the pre-conditioned *P. ramosa* seeds were transferred under the host root system. The *P. ramosa*/Brassicaceae weed co-cultivation was carried out in the same temperature and light conditions (Fig. 1). The development of the infected Brassicaceae weed roots was assessed 3 weeks later under a stereo microscope. The pre-emergent ontogenic stages of the parasite on Brassicaceae weed hosts were recorded. The development of the parasite was thus described by counting the number of individuals having reached the following pre-emergent ontogenic stages according to Gibot-Leclerc et al. (2012): germination, attachment on host root, young tubercle, old tubercle, bud and underground stem. The experiment was performed for all the seven Brassicaceae weed species. For each species, the experiment was repeated three times in exactly the same experimental conditions, starting on January 17, 2011. In total, three boxes were thus prepared per plant species, each containing three plants and five paper discs with 75–100 parasite seeds per disc, resulting in a total of 45 parasite discs and approximately 3,900 parasite seeds per plant species.

2.3 Data analysis

For each species, we calculated the odds. The 'odds' of an event are defined as the probability of the outcome event occurring (p) divided by the probability of the event not occurring ($1-p$). In this case, p was the probability of the *P. ramosa* stage being "attachment" or higher and $1-p$, the probability of *P. ramosa* stage being "germination". Then we calculated the odds ratios (ORs) with *A. thaliana* as the reference and their 95 % confidence intervals. An odds ratio of less than 1 indicates that the probability to reach "attachment" or higher stages is more likely in the *A. thaliana* species, whilst an odds ratio greater than 1 indicates that the probability is more likely in the other weed species. All analyses were done with R version 3.0.1 (R Development Core Team 2013).

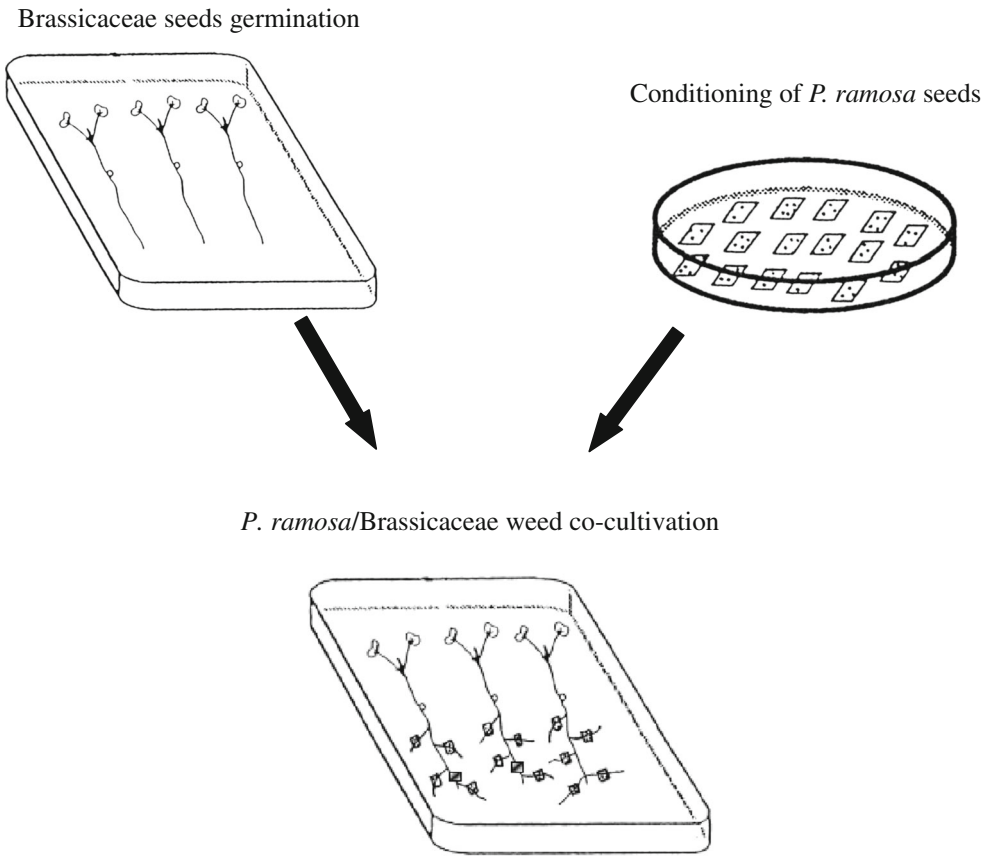

Fig. 1 Co-culture in vitro experiment to study *P. ramosa* infection on Brassicaceae weeds. After disinfection, 75–100 seeds of *P. ramosa* were laid on paper discs. Five discs prepared that way were placed on a paper sheet at the bottom of a Petri dish, and then hydrated. Three Petri dishes, containing 375–500 seeds each, were placed in darkness at 20 °C for 14 days to pre-condition the seeds. Thereafter, three disinfected Brassicaceae weed seeds were placed in a rectangular plastic box containing MS (1/2) nutrient solution with 1 % agar. The box prepared that way was placed in a growth chamber, at 23±1 °C (day) and 18±1 °C (night), with a 16-h photoperiod and 70 mol m^{-2} s^{-1} PPFD. Once the Brassicaceae weed root system had developed (i.e. 2 weeks after the beginning of the experiment), the five discs bearing the pre-conditioned *P. ramosa* seeds were transferred under the host root system. The *P. ramosa*/Brassicaceae weed co-cultivation was carried out in the same temperature and light conditions. The development of the infected Brassicaceae weed roots was assessed 3 weeks later under a stereo microscope. The pre-emergent ontogenic stages of the parasite on Brassicaceae weed hosts were recorded. The development of the parasite was thus described by counting the number of individuals having reached the following pre-emergent ontogenic stages according to Gibot-Leclerc et al. (2012)

3 Results and discussion

We described the advancement of pre-emergent ontogenic stages of *P. ramosa* based on our controlled experiments associating parasite seeds with the root system of seven *Brassicaceae* species. The development observed on six weeds was compared with that observed on the model plant *A. thaliana*.

All Brassicaceae weed species induced germination and subsequent attachment of *P. ramosa* on their roots (Table 1). After 3 weeks, the odds of attachment or higher stages were

Table 1 Numbers of *P. ramosa* plants for each of the five underground ontogenic stages observed on Brassicaceae weed root systems after the 3-week in vitro experiment. Plant species are sorted top to bottom according to *P. ramosa* speed of development

Brassicaceae weeds species	*Phelipanche ramosa* ontogenic developmental stages					
	Attachment	Young tubercule	Old tubercule	Bud	Underground stem	Total
Capsella bursa-pastoris	58		1	1	5	65
Capsella rubella	35		2	2		39
Lepidium campestre	26			1		27
Cardamine hirsuta	65		2			67
Sinapis arvensis	0		2			2
Arabidopsis thaliana	40					40
Lepidium draba.	15					15

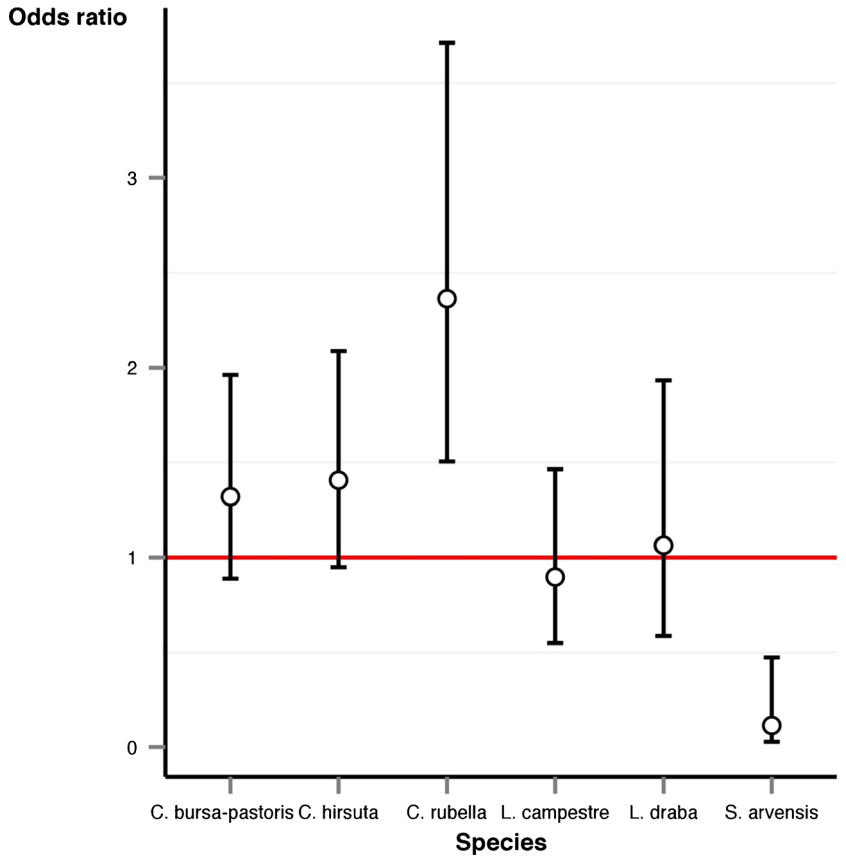

Fig. 2 Odds ratios and 95 % confidence interval (*A. thaliana* as reference). An odds ratio equal to 1 indicates that the probability to reach "attachment" or higher stages is equally likely in the two species. An odds ratio greater than 1 indicates that the probability is more likely in the other weed species and an odds ratio lesser than 1 indicates that the probability is more likely in *A. thaliana*. The *red line* indicates OR=1. We found that five Brassicaceae species had an odds ratio ranging from 0.9 to 2.4. These ratios are thus similar or higher than that of the *A. thaliana* reference. This finding shows for the first time that *P. ramosa* develops faster on the five Brassicaceae species

1.1 % for *A. thaliana*, 1.2 % for *L. draba*, 1.5 % for *Capsella bursa-pastoris*, 2.6 % for *Capsella rubella*, 1.6 % for *Cardamine hirsuta*, 1.0 % for *L. campestre* and 0.1 % for *S. arvensis*. Our experiments thus confirmed that *P. ramosa* is able to infect the seven Brassicaceae species tested. The ratio of the number of fixations or higher stages to the number of germinated seeds was similar between the Brassicaceae weeds and *A. thaliana*, except for *Capsella rubella*, which showed a significantly higher ratio and *S. arvensis*, which showed a significantly smaller ratio (Fig. 2). Records of the ontogenic stages observed during 3 weeks revealed that the rate of development of the parasitic plant was as fast or faster on each of the studied weed species than it was on *A. thaliana*. For all species except *L. draba* (Table 1), we observed more advanced stages such as 'old tubercle' (on *Cardamine hirsuta* and *S. arvensis*), 'bud' (on *Capsella rubella* and *L. campestre*) and even 'underground stem' (on *Capsella bursa-pastoris*). These stages were not present in *A. thaliana*.

The observation that all the Brassicaceae species studied could be infected by *P. ramosa* is consistent with the known host range of the parasite on agricultural crops: oilseed rape (*B. napus*), brown mustard (*Brassica juncea*), cabbage (*Brassica oleracea*) and white mustard (*Sinapis alba*) are all susceptible to it (Foy et al. 1989; Parker and Riches 1993). Concerning Brassicaceae weeds, infection by *P. ramosa* has been reported for *Capsella bursa-pastoris*, *Cardamine hirsuta*, *L. draba* and *S. arvensis* (Boulet et al. 2001; Gibot-Leclerc et al. 2009). Our study is the first to report infection of *Capsella rubella* and *L. campestre* by *P. ramosa*. The faster rates of development observed on weed species, and especially on *Capsella* sp., as compared to *A. thaliana* were surprising given that all these species have longer life cycle duration than the fast-growing *A. thaliana*. For example, under favourable long-day conditions the flowering time for *Capsella bursa-pastoris* varies between 36 and 200 days (Ceplitis et al. 2005), while under the same

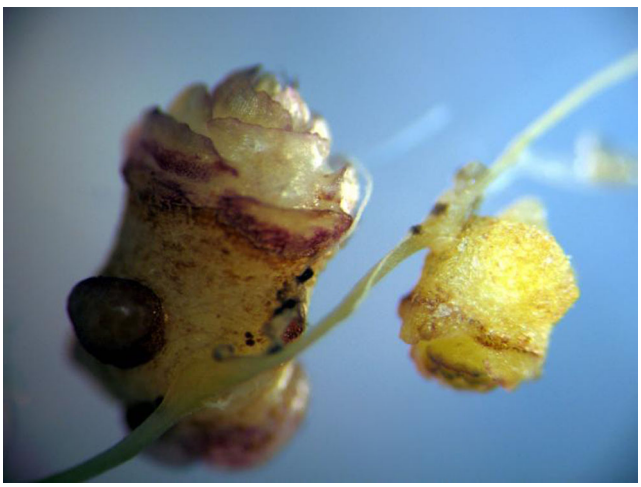

Fig. 3 Reduced underground stem of *P. ramosa* as developed on *C. bursa-pastoris* roots. Magnification ×33

conditions, the 'rapid-cycling'*A. thaliana* ecotype Col-0 flowers in only 32 days (Boyes et al. 2001). Our results thus indicate that the speed of development of the parasitic plant does not strictly depend on the speed of development of its host but also on other factors, such as the amount of resources that can be obtained from the host. In any case, our results suggest that *P. ramosa* is able to rapidly complete its life cycle on various Brassicaceae weeds. This rapid development is, however, associated with a much reduced size (Fig. 3) and, as a consequence, a much reduced seed production. One can therefore question what influence the presence of Brassicaceae weeds might have on the population dynamics of *P. ramosa* within cultivated environments. Noticeably, many Brassicaceae weeds are particularly common and ubiquitous within agricultural landscapes. For example, *Capsella* sp. is able to develop within cultivated fields as well as within various uncultivated habitats, including the crop edges and field boundaries. Moreover, *Capsella* sp. seeds can germinate throughout the year and up to three generations can be observed within a single year (Aksoy et al. 1998). *Capsella* sp. and similar Brassicaceae weeds are therefore commonly observed during inter-cropping. Thus, the fast development and low seed output associated with fixation on those weeds can be compensated for by a higher probability of survival of the parasitic plants, as they escape the management practices implemented within crops.

4 Conclusion

Our present work confirms that the parasitic plant is able to infect various weedy species that belong to the Brassicaceae family and commonly occur within *B. napus* fields. Importantly, our results reveal that the rate of development of *P. ramosa* is faster on these species than it is on the fast-growing plant *A. thaliana*. We therefore suggest that more attention should be paid to the role of Brassicaceae weeds that grow besides or after the crops as alternative hosts and to their importance for the demography of *P. ramosa*.

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