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What are the traits of *Phelipanche ramosa* (L.) Pomel that contribute to the success of its biological cycle on its host *Brassica napus* L.?

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ABSTRACT

In France, the fact that the holoparasitic plant *Phelipanche ramosa* has adapted to oilseed rape over the past two decades is deeply worrying, as it can cause over 80% yield losses. Its distribution area and the range of its host plants, whether they are cultivated plants or weeds, are expanding dramatically. As no natural regression of the infestation has been recorded so far, we undertook a detailed study of the different steps of the biological cycle of the *P. ramosa*/oilseed rape pathosystem to determine the adaptive traits that favour the parasite's success. We combined experimental approaches involving controlled conditions with *in vitro* and in pot co-cultivation on the one hand and a field trial on the other hand. These experiments allowed us to determine a 4-mm zone around host roots within which oilseed rape root exudates stimulate the germination of *P. ramosa*, the early action of host root exudates and the rapid fixation of the parasite on host roots. We also unveiled that *P. ramosa* was able to tune its biological cycle to that of oilseed rape. The important trophic relationships between the parasite and its host induced phenotypic (dwarfism, leaf chlorosis, silique abortion) as well as agronomical (90% yield losses) consequences on oilseed rape. Our results can constitute a relevant basis for further experimental studies. The research perspectives they open will focus on key-processes of the host–parasite relationship, and more particularly on the trophic relationships that are set up as far as carbon assimilates and minerals such as nitrogen are concerned.

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Introduction

Among crop bioagressors, the *Phelipanche* and *Orobanche* genera are obligate parasites of dicotyledonous roots and are considered as one of the main biotic constraints on economically important crops. They are also known to be the cause of crop losses ranging from 5 to 100% (Joel et al., 2007; Musselman, 1980; Parker and Riches, 1993; Press and Graves, 1995; Press and Phoenix, 2005; Schneeweiss, 2007). *Phelipanche ramosa* (L.) Pomel (Joel, 2009) is most devastating and has by far the widest range of hosts, among which Solanaceae, Brassicaceae and legumes are prominent (Buschmann et al., 2005; Haidar et al., 2003; Joel et al., 2007; Parker and Riches, 1993). In France, *P. ramosa* got remarkably adapted to oilseed rape, causing deeply worrying losses of about 80%, but also to hemp and tobacco. So far the adaptation of *P. ramosa* to oilseed rape seems to have remained limited to the French territory. The distribution area of the parasite and the range of its host plants appear to have dramatically expanded lately, with more than 70 weed species as new hosts (Boulet et al., 2007; Brault et al., 2007; Gibot-Leclerc et al., 2003, 2006, 2009).

The biological cycle of epirhizal parasites is usually composed of two distinct phases (Bouwmeester et al., 2003; Joel et al., 2007; Keyes et al., 2001; Parker and Riches, 1993; Rubiales, 2003; Sauerborn, 1991; Yoder, 2001). Underground, the first phase is initiated by seed germination and goes on with the fixation on host roots and the penetration into host tissues along with the development of an absorption system called a haustorium. Aboveground, the second phase starts with the emergence and then the growth of floral scapes and ends with flowering and fructification. In recent years several authors (Buschmann et al., 2005; Echevarría-Zomeño et al., 2006; Goldwasser et al., 2001; Haidar et al., 2003; Kogan, 1994; Lu et al., 2000; Pérez de Luque et al., 2004; Sallé et al., 2000) have insisted on the need to increase our knowledge about parasitic plant biology in order to make it easier to develop suitable protocols for an efficient control. The knowledge acquired about broomrape so far deals with the biology of the main broomrape/host pathosystems, *i.e. Orobanche cernua*/sunflower, *O. crenata*/faba bean, *O. cumana*/sunflower, *Phelipanche ramosa*/potato, *P. ramosa*/tobacco,

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P. ramosa/tomato. However, a detailed study of the biological cycle of the *P. ramosa*/oilseed rape pathosystem has never been performed. Acquiring knowledge about the biology of the *P. ramosa*/oilseed rape pathosystem is all the more crucial as recent molecular biology works have proved that there exist at least two different *P. ramosa* pathovars with clearcut host specificities, which makes culture management in severely infested plots even more complicated.

In order to determine the adaptive traits that favour the parasite's success, we characterized the biological cycle of the *P. ramosa*/oilseed rape pathosystem. More precisely, we determined (1) the extent of the zone where oilseed rape root exudates stimulate *P. ramosa* seed germination; (2) the success rate of the first fixation steps of *P. ramosa* on host roots following its germination; (3) how the different phases of the *P. ramosa* development cycle tune in with the oilseed rape cycle; (4) the phenotypic and agronomical consequences of the parasitism of *P. ramosa* on oilseed rape. In order to make it easier to study the early development steps of *P. ramosa*, we decided to work with *in vitro* and sandwich co-cultivation methods. In parallel, a field trial was

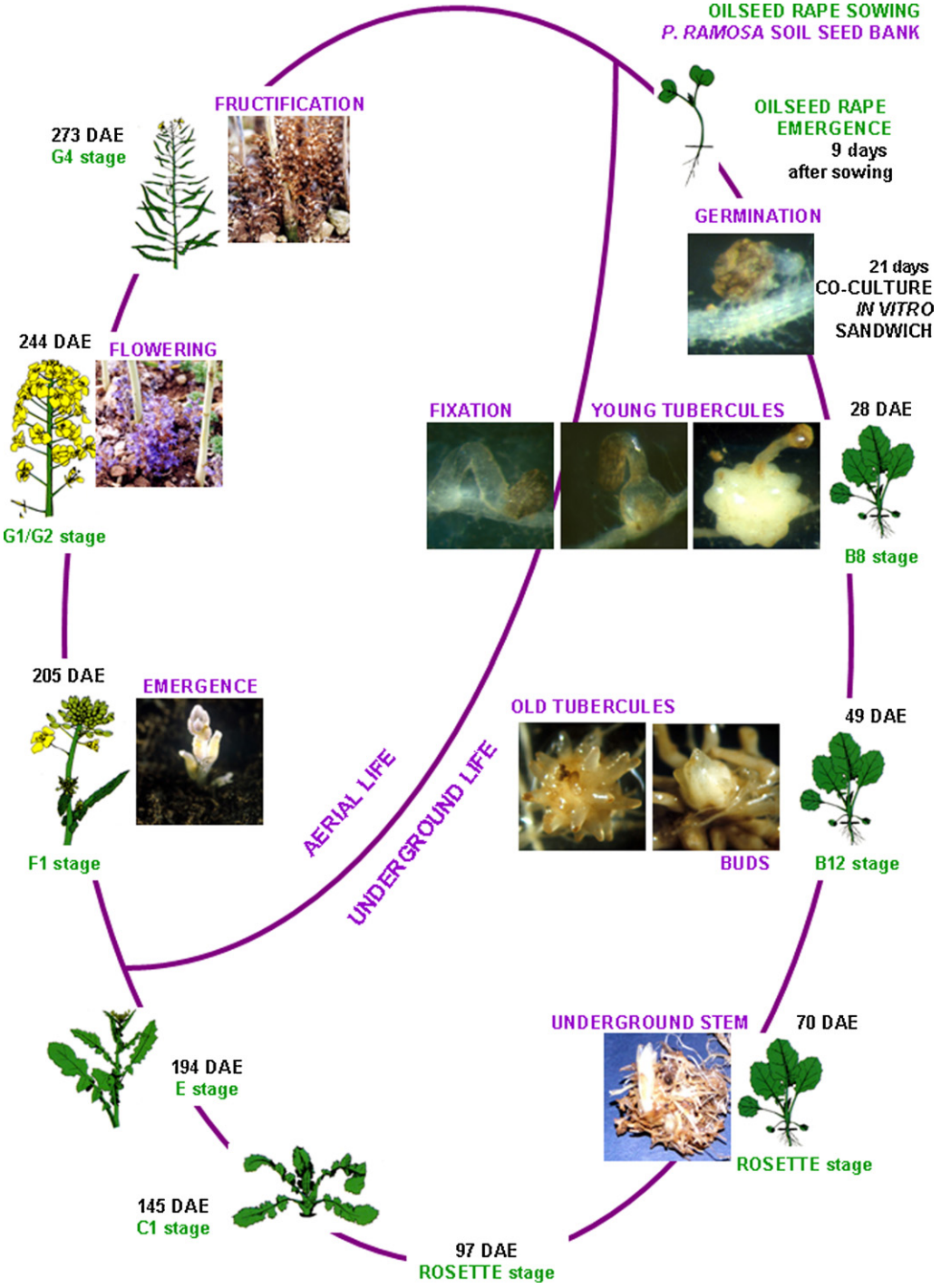


Fig. 1. Development cycle of the *Phelipanche ramosa*/oilseed rape pathosystem in Charente-Maritime (France).

performed to characterize the consequences of *P. ramosa* parasitism on oilseed rape at the whole plant level and at the canopy level.

Materials and methods

Laboratory and field experiments were conducted between 2000 and 2004.

Seed origin

The seeds were collected in 1999 from natural populations of *P. ramosa* that had severely infested fields of oilseed rape (*Brassica napus* L. Brassicaceae) in Saint-Pierre-de-Juillers (Latitude 45,9333; Longitude −0.3667; Charente-Maritime, France). Once harvested, the seeds were sifted to clean them. Oilseed rape seeds (var. Zénith) were provided by the CETIOM (Centre technique des

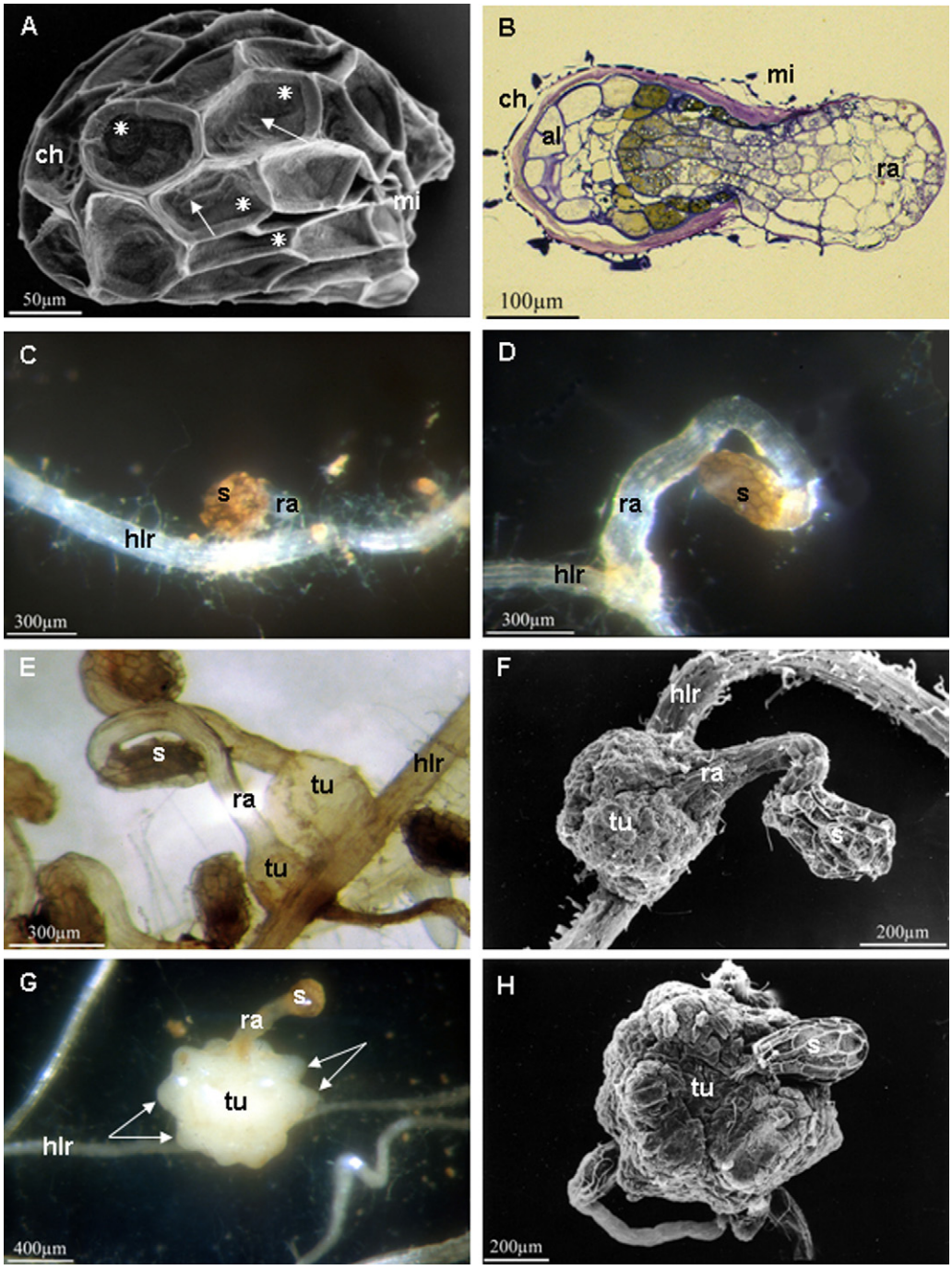


Fig. 2. (A) Morphological aspect of a *Phelipanche ramosa* seed in SEM. We can note the way alveoli are arranged (*) as well as punctuation details (arrows) of the surface cell inner walls. (B) Longitudinal section of a *P. ramosa* germinated 8 days after starting *in vitro* co-cultivation with oilseed rape. (C) *Phelipanche ramosa* germinated 11 days after starting sandwich co-cultivation with oilseed rape. (D) *Phelipanche ramosa* fixation on an oilseed rape root, in the field 28 DAE. (E) Very young *P. ramosa* tubercles 2 months after starting *in vitro* co-cultivation with oilseed rape. (F) Very young tubercle in SEM, in the field 28 DAE. (G) Young tubercle with protuberances (arrows) corresponding to the parasite's future adventitious roots, in the field 28 DAE. (H) Young tubercle in SEM, in the field 28 DAE. al, albumen; ch, chalaza zone; hlr, host lateral root; mi, micropyle; ra, radicle; s, seed; tu, tubercle.

Table 1
Numbers of *Phelipanche ramosa* plants for each of the 5 underground ontogenic stages observed on all 30 infested oilseed rape root systems in a soil that is naturally infested by broomrape in Charente-Maritime (France).

Sampling date (days after oilseed rape emergence (DAE))	*Phelipanche ramosa* ontogenic developmental stages					
	Fixation	Young tubercle	Old tubercle	Bud	Underground stem	Total
7 DAE	0	0	0	0	0	0
12 DAE	0	0	0	0	0	0
19 DAE	0	0	0	0	0	0
28 DAE	**8**	**46**	0	0	0	**54**
49 DAE	**1**	**60**	**36**	**12**	0	**109**
70 DAE	0	**218**	**368**	**21**	**128**	**735**
97 DAE	0	0	**100**	**20**	**90**	**210**
145 DAE	0	0	**417**	**267**	**257**	**941**
194 DAE	0	0	0	**++**	**+++**	**>1500**
244 DAE	0	0	0	0	**+++**	**>1500**

When the host root system was infested by more than 50 broomrapes, the exact number of each of the broomrape developmental stages could not be determined. Their relative frequency is indicated by plus signs: +, low numbers of the parasite's developmental stage; ++, average numbers; +++, high numbers.

oléagineux métropolitains et du chanvre industriel, France). *Phelipanche ramosa* as well as oilseed rape seeds were kept in glass containers at room temperature.

In vitro ***experiment***

After disinfection (70% ethanol, 5 min, and 3% calcium hypochlorite solution, 10 min), 15–25 seeds of *P. ramosa* were laid on Whatman GF/A paper discs (Ø 12 mm). Eight 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. Twenty Petri dishes, containing 120–200 seeds each, were placed in darkness at 20 °C for 14 days to pre-condition the seeds (obligate hydration period). Thereafter, one disinfected oilseed rape seed was placed each in a rectangular plastic box containing MS (1/2) (Murashige and Skoog, 1962) nutrient solution with 1% agar. Twenty boxes prepared that way were placed in a growth chamber, at 23 ± 1 °C (day) and 18 ± 1 °C (night), with a 16-h photoperiod, and 70 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD). Once the oilseed rape root system had developed (*i.e.* 2 weeks after the beginning of the experiment), the 8 discs bearing the preconditioned *P. ramosa* seeds were transferred under the host root system. The *P. ramosa*/oilseed rape co-cultivation was carried out in the same temperature and light conditions. The development of the parasite on oilseed rape roots was monitored for 12 weeks. The observations were carried out under a stereo microscope daily for 4 weeks, and then once a week for the following 8 weeks. For each observation, the percentage of *P. ramosa* germination induced by oilseed rape root exudates was counted, and the early developmental stages of the parasite on its host were recorded. Our observations were also carried out in light and scanning electron microscopy (SEM). The whole experiment was performed twice.

Sandwich experiment

Losner-Goshen and collaborators' method (1998) was applied, with slight modifications. After disinfection (70% ethanol, 5 min, and 3% calcium hypochlorite solution, 10 min), 44 1 mg-*P. ramosa* seed samples (approximately 350 seeds each) were dried under a laminar flow at room temperature for 24 h. Then each sample was sprinkled onto a GF/A sheet (Ø 90 mm) at the bottom of a Petri dish (Ø 90 mm), and then hydrated. The 44 Petri dishes were placed in darkness at 20 °C for 14 days to achieve seed preconditioning. Forty-four oilseed rape seeds were laid individually onto GF/A sheets (Ø 90 mm) at the bottom of Petri dishes (Ø 90 mm), and then hydrated. After oilseed rape seed germination in darkness at 30 °C, each oilseed rape seedling was laid onto a GF/A sheet (Ø 90 mm) in contact with the pre-conditioned *P. ramosa* seeds. A second sheet was added to cover all the seeds. The sandwiched individual oilseed rape seedling and broomrape seeds were placed on pierced plastic Petri dishes (Ø 90 mm) whose sides had been notched. Each of the 44 modified Petri dishes was placed slantwise inside a terra-cotta pot (Ø 15 cm) filled two-thirds full with a 1 vol:1 vol potting substrate and planting substrate soil mix. Twenty ml of MS (1/2) nutrient solution was added to each pot. The co-cultivation of the *P. ramosa*/oilseed rape pathosystem was carried out in the greenhouse [23 ± 1 °C (day) and 18 ± 1 °C (night), and 55% relative humidity]. The natural light was complemented by artificial light (100 μmol m^{-2} s^{-1} PPFD) to have a 16-h photoperiod. Ten ml of nutrient solution was added daily. *Phelipanche ramosa* development on oilseed rape was assayed for 12 weeks. Samples were taken daily during the first week, every 2 days for the next 2 weeks and then once a week for the remaining 9 weeks. For each sampling, the root system of 2 oilseed rape plants was taken out and observed under a stereo microscope. The percentage of *P. ramosa* germination induced by oilseed rape root exudates was counted and the early developmental stages of the parasite on its host were recorded. Our observations were also carried out in light microscopy and SEM. The whole experiment was performed twice.

Field trial

On 28th August 2000, oilseed rape was sowed on a soil, consisting of silt, clay and sand, severely infested by *P. ramosa* and located in Villemorin (Latitude: 46.003887; Longitude: −0.296944, Charente-Maritime, France). The 4-replicate trial was carried out in whole Fischer blocks. The experiment was led through till oilseed rape harvest. Samples were taken at 10 different dates, *i.e.* 7, 12, 19, 28, 49, 70, 97, 145, 194 and 244 DAE (days after emergence). At each sampling date, 30 oilseed rape plants were dug out and observed under a stereo microscope. Oilseed rape developmental stages were determined and infested host roots were counted. Infestation intensity was estimated by counting the number of *P. ramosa* ontogenetic stages fixed on host roots as follows: (a) fixation; (b) young tubercle without adventitious roots; (c) old tubercle with adventitious roots; (d) bud with adventitious roots but without a stem; (e) underground stem; (f) stem emergence; (g) flowering; (h) fructification. Our observations were also carried out in light microscopy and SEM.

Histological and cytological studies under a light microscope and a scanning electron microscope (SEM)

The parasite on the host plant was taken at different development stages, fixed in a 4% glutaraldehyde solution in a pH 7.4 cacodylate buffer for 24 h and then post-fixed in a 1% osmium

tetroxide solution in the same buffer for 1 h (Ledbetter and Porter, 1963). After that, the samples were dehydrated by dipping them into ethanol baths of increasing ethanol concentrations and araldite-included according to Glauert and Glauert's method (1958). Then the included samples were sliced into semi-thin sections for photon microscopy and stained with toluidine blue.

For SEM observations, samples first fixed in a glutaraldehyde solution for several days were dehydrated by dipping them into baths of increasing ethanol concentrations. Then they were CO_2-treated until the critical point and sputter-coated with gold (Guillaumin, 1980).

Results

Fig. 1 gives a comprehensive overview of all results detailed in the following and illustrates the developmental cycle of the parasite and its host.

Underground life stage of Phelipanche ramosa

Phelipanche ramosa seeds are oval-shaped, 350 μm long, 250 μm wide, and weigh 3 μg. They are covered by a hard reticulate coat. The narrowest end corresponds to the micropyle while the roundest opposite end corresponds to the chalaza (Fig. 2A). The testa contains alveolar cavities in it (Fig. 2A, *) which are composed of dehydrated cells aligned along the long axis of the seed. As the cytoplasmic content of cells is gone, the outer wall has collapsed and follows the circular punctuations of the inner wall (Fig. 2A, arrows). The albumen (Fig. 2B) is composed of 3 or 4 layers of cells containing numerous lipid globules and starch granules. It surrounds the primitive embryo devoid of radicle, gemmule or cotyledons. After pre-conditioning, only the *P. ramosa* seeds situated nearby the oilseed rape roots (less than 4 mm in our conditions) were able to germinate. The radicle of *P. ramosa* juts out of the micropyle region (Fig. 2B) and grows towards the host root (Fig. 2C). In the absence of fixation on oilseed rape roots, *P. ramosa* seeds degenerated and died in less than 3 or 4 days. In the *in vitro* method, *P. ramosa* seed germination occurred 3 days (61 growing day-degrees, base 0) after starting the co-cultivation. On the 8th day, *P. ramosa* germination percentage was 50% and it reached 95% 3 days later. With the sandwich method, the lag-time was longer (7 days), the germination percentage was 50% on the 11th day and it reached 90% on the 15th day. Taking into account the different lag-times probably due to the age of the oilseed rape seedlings when parasite and host were put together, we can consider that *P. ramosa* germination occurred when oilseed rape seedlings were 11–14 days old, while the maximal germination percentage was reached when they were 21 days old (Fig. 1).

In the field, oilseed rape emergence occurred 9 days after sowing (Fig. 1). When the first samples were taken 7, 12 and 19 DAE, host roots were not yet infected by *P. ramosa* (Table 1). Yet, the results obtained with the *in vitro* and sandwich methods indicate that the maximal germination percentage for *P. ramosa* seeds is reached when oilseed rape is 21 days old. We can therefore hypothesize that the 21-day-old-oilseed rape root exudates (B6, *i.e.* 6-leaf stage) did induce the germination of numerous *P. ramosa* seeds, but these germinations were very likely lost due to the uprooting and washing steps of the root systems. Parasite fixations and young tubercles were observed from the 4th sampling date onward (Fig. 1, B8, *i.e.* 8-leaf stage, 28 DAE). It is important to note that *P. ramosa* only fixed itself on the lateral roots of oilseed rape. At that time, young tubercles were already 5 times as numerous as fixations (Table 1). Field results were in accordance with those obtained with the *in vitro* and sandwich methods. *Phelipanche ramosa* fixations were detected when oilseed rape was 30 days old and young tubercles only developed 1 month later. In these controlled conditions, *P. ramosa* development did not go further than the young-tubercle stage throughout the 3-month long experiment (Fig. 2 D–H).

When *P. ramosa* first penetrated into oilseed rape roots, meristematic cell proliferation allowed the haustorium, which at that stage was not yet functional, to make its way through the host tissues (Fig. 3A). Later on, only apical cells remained able to proliferate whereas distal cells got organized into a parenchyma and xylem elements differentiated. Once haustorial apical cells had made contact with the host's vascular system cells, they stopped dividing and migrating into the host. They stuck to the host vessel cells without perforating them. The continuity between broomrape haustorium vessels and the host xylem is clearly visible (Fig. 3B).

Old tubercles and buds were observed when oilseed rape was 49 days old or older (Fig. 1, B12, *i.e.* 12-leaf stage). The proliferation of cells located around the caulinary meristem, at the origin of the floral scape, led to the formation of a wreath of adventitious roots (Fig. 4A, arrows) which in some cases were able to make contact with other host roots and produced secondary haustoria. Once adventitious roots were formed, the tubercle rapidly turned into a bud thanks to its apical cells. Inside the bud, the caulinary meristem was protected by scales (Fig. 4B). It is important to note that at 49 DAE only one new fixation was detected, while young and old tubercles were much more numerous than buds (Table 1). From that point onward the buds grew vertically and formed young underground stems which made their way through the soil towards the surface. The first underground stems (Fig. 4C) were observed 70 DAE (Fig. 1, rosette stage). At that time fixations had all disappeared whereas all other stages except the bud stage were in constantly increasing numbers (Table 1). At the following sampling date (97 DAE, rosette stage), young tubercles had disappeared. Till 145 DAE (C1, *i.e.* bolting stage), the numbers of old tubercles, buds and underground stems increased considerably. Conversely, no new fixation or young tubercle was detected. During that period, oilseed rape stopped its vegetative development, and no new *P. ramosa* seed germination leading to the development of new young parasitic developmental stages appeared to have been induced. At 194 DAE (E-stage, *i.e.* separate-flower-bud stage), old tubercles were not visible any more while each root system carried more than 50 buds and underground stems (Table 1).

Aerial phase of P. ramosa

Phelipanche ramosa emerged at the soil surface 205 days after oilseed rape emergence (Fig. 1, F1, *i.e.* early flowering stage). The short, frail, rather hairy, branched stem bulged out at its base (Fig. 4D). It had leaves that contained no chlorophyll pigments and were reduced to 6- to 12 mm long thick, acuminate, alternate scales. This non-chlorophyllic, scaly axis then turned into a floral scape 244 DAE (G1/G2, *i.e.* early fructification stage). The flowers were 1–1.5 cm long. They were inserted individually at the axils of scaly bracts. They had an irregular, curved shape, and a blue-tinted mauve colour. They did not have distinct peduncles and they were grouped in rather long floral scapes when flowering was well advanced (Fig. 5A and B). It is important to note that at that last time-point buds could no longer be found while large numbers of underground stems with surrounding bunches of adventitious roots remained ready to emerge (Table 1). Once fecundation had taken place, each pollinated flower turned into a capsule containing approximately 600–800 seeds each (Fig. 5C). *Phelipanche ramosa* started fructifying 273 DAE (Fig. 1, G4, *i.e.* end-of-fructification stage) and the floral scapes of broomrape were ripe a few days after those of oilseed rape (Fig. 5D).

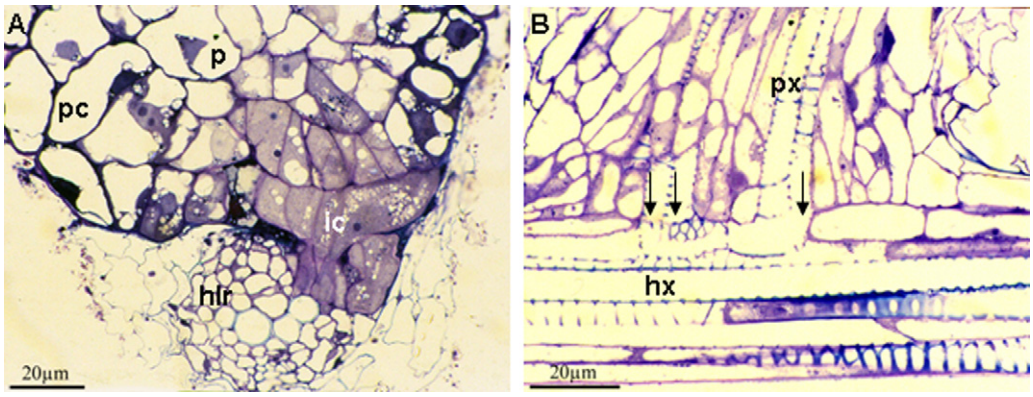


Fig. 3. (A) Longitudinal section of an early penetration stage of *P. ramosa*. The oilseed rape root is cut transversely. (B) Xylem links (arrows) in a longitudinal section of the base of a *P. ramosa* tubercle. hlr, host lateral root; hx, host's xylem; ic, intrusive cells; p, parasite; pc, parenchyma cells; px, parasite's xylem.

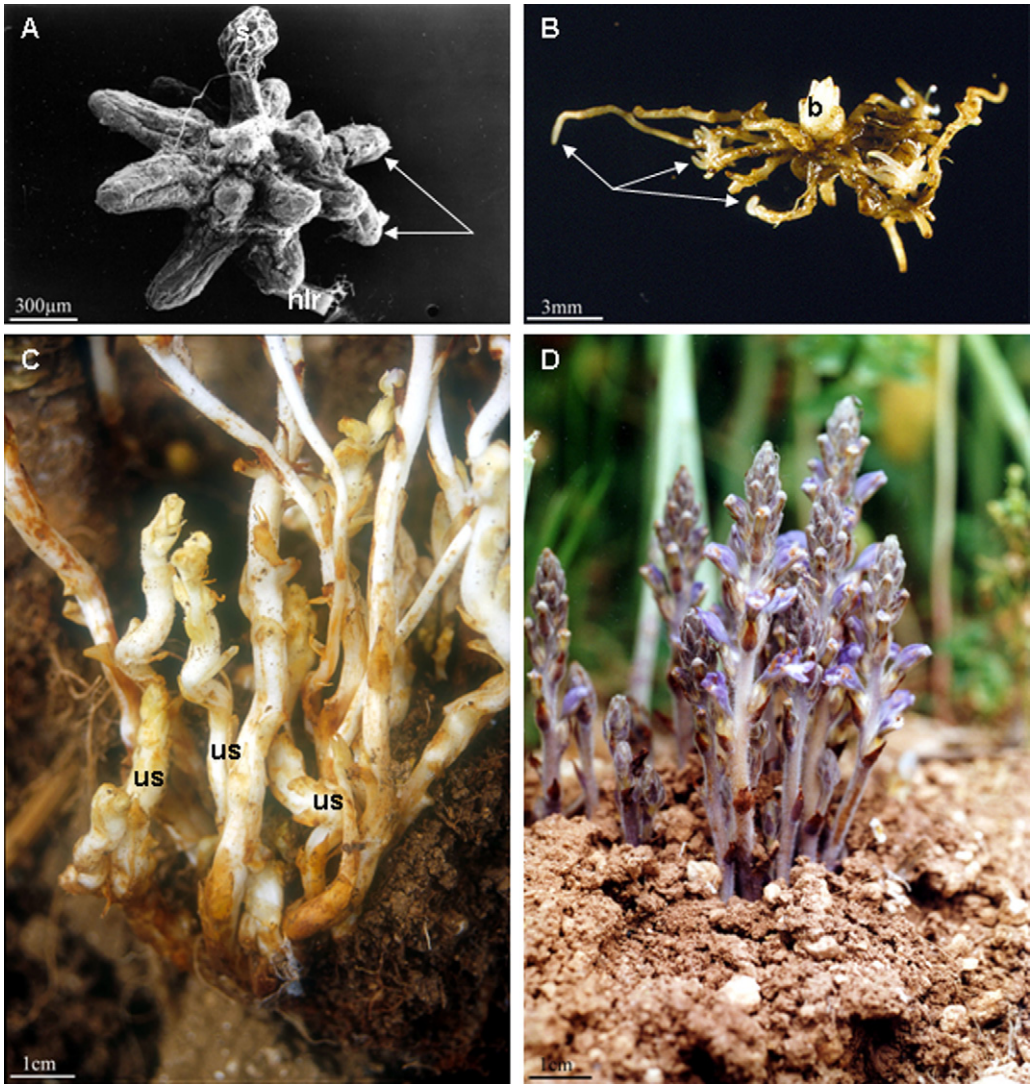


Fig. 4. (A) Old tubercle with young roots (arrows) which hide the caulinary meristem in SEM, in the field 49 DAE. (B) *Phelipanche ramosa* bud with numerous long adventitious roots (arrows) in the field 49 DAE. (C) Bunch of *P. ramosa* underground stems, in the field 70 DAE. (D) *Phelipanche ramosa* emergence, in the field 205 DAE. b, bud; hlr, host lateral root; us, underground stem.

Phenotypic and agronomical consequences on oilseed rape

During the autumn–winter phase of oilseed rape, the shoot part of the host plant showed no visible parasitism-related symptoms. It was only by digging out root systems and examining them closely that we detected the presence of *P. ramosa*. But in the spring phase, the first symptoms became visible when oilseed rape floral peduncles started growing (Fig. 1, E stage). Parasitic attack induced oilseed rape dwarfism, which was visible as a depressive zone at the field level (Fig. 6A, arrows) and leaf chlorosis (Fig. 6B, arrows). Once *P.*



Fig. 5. (A) *Phelipanche ramosa* flowering, in the field 244 DAE. (B) Detail of a *P. ramosa* flower. (C) Young *P. ramosa* capsule. Immature seeds form a yellow compact bulk (arrow). (D) Fructified *P. ramosa*, in the field 273 DAE.

ramosa had emerged, its branched floral scapes were easily identifiable. The most severely infested parts were usually those on the outskirts of the fields (Fig. 6C). In the case of severe infestations, each oilseed rape plant could harbour as many as 15–20 floral scapes; the infested zone appeared then to be fully covered by *P. ramosa* (Fig. 6D). When oilseed rape ripened, the size of the depressive zone increased while the low density of the crop made it possible for weeds to develop. Infested oilseed rapes produced few siliques and many of them aborted (Fig. 6E, arrows). Yield losses were significant: from an average 38 q ha^{-1} when oilseed rape was healthy, they went down to 4 q ha^{-1} in the most severely infested fields. After oilseed rape harvest, only wilted *P. ramosa* floral scapes remained (Fig. 6F), perfectly aligned with the sowing lines.

Discussion

A combination of field and controlled experimental approaches allowed us to characterize precisely the different steps of the *P. ramosa* cycle, at the cell level as well as at the whole plant level. To our knowledge, this is the first such study to be carried out about this pathosystem.

Biological traits of P. ramosa contributing to the success of its biological cycle

Phelipanche ramosa as a parasite of oilseed rape possesses different specific traits that contribute to the success of its cycle. The first trait concerns its dispersion: it produces light, minute seeds in huge numbers. Thus its seeds are easily dispersed by wind, waters, animals, farming machinery, and contaminated seed transport (Berner et al., 1994; Parker and Riches, 1993). The second trait is the toughness of its seed coat. This trait is common to *Orobanche* and *Striga* seeds and could explain why their seeds can retain their germinating capacity in the soil for several years (Parker and Riches, 1993; Worsham, 1987). The third trait lies in the need for seed germination to be stimulated by host root exudates. We observed that *P. ramosa* seed germination could only occur within a short distance from oilseed rape roots. We found that distance to be 4 mm. Oilseed rape root exudates thus appear to have a stimulating effect within a limited zone around the roots compared to the 10 mm with the faba bean/*O. crenata* pathosystem (Sallé et al., 1984). Such a system could prevent the seeds from germinating in the absence of a host and avoid *P. ramosa* seed germination at too great a distance from oilseed rape roots.

The three biological traits of *P. ramosa* as a parasite of oilseed rape mentioned above complete other traits already mentioned in the literature, namely: the absence of a primary dormancy, or a very early break of primary dormancy, as *P. ramosa* seeds can germinate as early as harvest time. Apart from the indispensable presence of a susceptible host plant, *P. ramosa* seed pre-conditioning and germination do not require any specific environmental conditions (Gibot-Leclerc et al., 2004, 2006).

Early efficacy of root exudates on P. ramosa germination

Our results show that whichever co-cultivation technique was used, *P. ramosa* seed germination was massively induced 21 DAE, while oilseed rape had only reached the 6-leaf stage. Oilseed rape



Fig. 6. (A) Depressive zone (arrows) due to slowed growth of infested oilseed rape. (B) Chlorosis in the leaves of infested oilseed rape (arrows). (C) *Phelipanche ramosa* floral scapes located on the outskirt of an oilseed rape plot. (D) Very high *P. ramosa* density in a severely infested oilseed rape plot. (E) Top part of an infested oilseed rape flower bunch with aborted siliques (arrows). (F) Harvested infested oilseed rape plot.

root exudates therefore appear to be effective in the early stages of its growth. For *P. ramosa*, such earliness has been shown with tobacco (Labrada, 1994). It is noteworthy that no new fixation or young tubercle was observed while oilseed rape was between the rosette stage (97 DAE) and the shooting stage (145 DAE). Taken together, these results suggest a tight correlation between the developmental stage reached by the host and the efficacy of its root exudates.

Fixation, a fundamental process for resource acquisition by P. ramosa

Once it has germinated, *P. ramosa* very quickly fixes itself (28 days) on oilseed rape host roots. A similar precocity was previously reported on hemp and tobacco roots (Brault et al., 2007; Labrada, 1994). Broomrape fixation has to be completed for the parasite to be able to penetrate host tissues later and draw the carbon, mineral and water resources needed for its growth. A continuum between oilseed rape and *P. ramosa* xylems was highlighted. Such xylem-to-xylem contacts have also been evidenced in the case of the *P. ramosa*/carrot pathosystem (Zehhar et al., 2003). Our light-microscopy observations did not allow us to determine whether host and parasite xylem vessels were simply adjacent or had made direct lumen contact (Hibberd and Jeschke, 2001).

Xylem-mediated solute supply to the parasite is likely to be low due to the low transpiration rate of the parasite that generates only a weak driving force for the solute flux within the xylem (Hibberd et al., 1999). Yet we did not observe any phloem-to-phloem connexions. But phloem tissues are found in broomrape haustoria (Dörr and Kollmann, 1975; Hibberd and Jeschke, 2001). The host phloem is known to provide almost all sugars (>99%) and most minerals such as nitrogen, magnesium and potassium (Abbes et al., 2009; Hibberd and Jeschke, 2001; Hibberd et al., 1999).

Tuning of the host and parasite development cycles

Phelipanche ramosa tightly tunes the duration of its biological cycle to its host's (273 days). Such tuning was found for *P. ramosa* parasiting different species (Brault et al., 2007; Kogan, 1994; Neumann and Sallé, 2000), with a biological cycle that can last 45–150 days according to the species considered. In our study, *P. ramosa* emerged, *i.e.* started the aerial phase of its cycle, when oilseed rape started flowering, in accordance with common observations on different host plants, such as hemp, eggplant, tobacco, tomato or water melon (Brault et al., 2007; Kogan, 1994; Labrada, 1994). Another aspect of cycle tuning was when *P. ramosa* floral scapes started growing at the same time as oilseed rape started forming its siliques. Such tuning is believed to enable *P. ramosa* to deprive the host of nutrients as optimally as possible by diverting water and nutrients from the host for its own use.

Consequences of nutrient diversion on the growth and agronomical performances of oilseed rape

The broomrape biological cycle phase that occurs between seed germination and haustorium penetration takes place exclusively underground, so it cannot be detected by outsiders. Besides, the symptoms of *P. ramosa* parasitism on oilseed rape become detectable late in the oilseed rape life cycle, *i.e.* only once its floral peduncles start elongating. An overall slower growth of oilseed rape could be observed, along with leaf chlorosis, low silique production, and numerous abortions. Such symptoms are often seen in host/parasite relationships (Sallé et al., 1995).

By developing on host roots, the parasite competes with the host for water, minerals and sugars. Sometimes, the decrease in host biomass is not fully compensated for by parasite biomass (Grenz et al., 2008). In this case, photosynthetic protein diversion is likely to prevent the host from maintaining its photosynthetic level. This phenomenon could be at the origin of the oilseed rape leaf chlorosis and biomass drop observed in our study. Besides, following the source-sink concept, Manschadi et al. (2001) proposed that the parasite could be a priority sink compared to host vegetative organs and newly formed pods, whereas pods in the filling stage could be priority sinks compared to the parasite. This could account for the low numbers of siliques and the numerous abortions observed in our study. The high yield losses of oilseed rape that we observed are in accordance with other studies (Bernhard et al., 1998; Manschadi et al., 2001; Mesa-García and García-Torres, 1986).

Last of all, hormonal imbalance, characterized by a decrease in gibberellin and cytokinine content and an increase in abscisic acid content, could cause the growth delay at the origin of the infested plants' dwarfism. The imbalance could also result from wounds and water stress caused by the parasite's setting in (Watling and Press, 1997).

Conclusion

The highly specialized parasitic way of life set up by *Phelipanche ramosa* on oilseed rape highlights different adaptive traits that favour its success. These traits concern *P. ramosa* germination and fixation, with the determination of a 4-mm influence zone of oilseed rape root exudates on parasitic seed germination and an early effect of exudates followed by a very rapid fixation of the parasite on host roots. Consequently, an ever-increasing parasitic seed bank is maintained. These traits are more globally related to the whole biological cycle of *P. ramosa* since the different steps of its cycle depend little on weather conditions but completely on the presence of a host. Our study evidenced a fine tuning between oilseed rape and *P. ramosa* cycle phases, which led to a synchronization of their fructification stages. Although the way *P. ramosa* germinates and fixes itself on host roots is well characterized, the physiological responses that induce the fine tuning of the parasite's cycle phases to its host's remain to be elucidated. Besides, the fulfilment of *P. ramosa*'s biological cycle induces consequences on oilseed rape, at the phenotypic and the agronomical levels.

Our study can constitute a relevant basis for future experimental studies. Knowing the number of days needed for *P. ramosa* to reach its early developmental stages, which take place underground and are thus invisible to outsiders, will make it possible to better plan oilseed rape root sampling in order to get *P. ramosa* plants at the wanted stage. Concerning *P. ramosa* emergence, it will be possible to plan observations from oilseed rape phenology once it has reached the early flowering stage. As for the parasite's later developmental stages, they are visible to the naked eye, which solves the problem. In addition to these practical applications, our work opens onto new research perspectives that can focus on key-processes of the host–parasite relationship. In particular, the trophic relationships concerning carbon assimilates and minerals such as nitrogen need to be further investigated in order to better understand the competition that takes place between host and parasite in this pathosystem.

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