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科研費 KAKENHI

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研究課題名（和文）Carbon / nitrogen interactions between legumes and parasitic plants

研究課題名（英文）Carbon / nitrogen interactions between legumes and parasitic plants

研究代表者
Ｉｒｖｉｎｇ　Ｌｏｕｉｓ（IRVING, Louis）

筑波大学・生命環境系・助教

研究者番号：６０５９７１７８

交付決定額（研究期間全体）：（直接経費）　　2,800,000円

研究成果の概要（和文）：We discovered that parasitism in the Phtheirospermum / alfalfa system can be suppressed by supplying the host plant with nitrogen. We also discovered that Phtheirospermum is first discovered carbon-feeding hemiparasite. Parasitism by Phtheirospermum can cause a 50% upregulation in photosynthesis.

研究成果の概要（英文）：Our understanding of hemiparasitic plants is that they steal nutrients, hormones and water, but little carbon. In the last year, we have made three major discoveries about the parasite Phtheirospermum japonicum and its host, Medicago sativa. P. japonicum is a carbon-feeding hemiparasite. It does not steal nutrients from the host, but supplements its growth with host-derived photoassimilate. Parasitic intensity in the Phtheirospermum / Medicago system is mediated by host nutrient status. Where the host is supplied nitrogen, it is able to resist the parasite, and suffers no damage. The parasite receives no benefit of attachment to the host. Under nutrient-deprived conditions, host growth is decreased by 75%, and the parasite increased by 33%. Parasite will not grow without N, thus, we must separate out host and parasite nutrient supply. Previous experiments have shown increased host photosynthesis caused by parasite attachment to clover, but only under nutrient deprived conditions.

研究分野：Plant Science

キーワード：hemiparasite　Phtheirospermum　carbon　N status　conditional parasitism

１．研究開始当初の背景

Parasitic plants have been estimated to cause over $10 billion of damage to agriculture annually. Some species can cause the complete loss of a crop, causing hunger and poverty in developing countries. However, the mechanisms by which parasites cause their damage are poorly known. Previous studies have shown that this can be due to resource abstraction by the parasite or decreased host-plant photosynthesis.

Parasitic plants can be split into two main groups: holoparasites, which steal all resources including carbon from the host plant (e.g. *Orobanche*), and hemiparasites, which steal nutrients, hormones and water, but only take low levels of carbon (e.g. *Rhinanthus*). Legumes are generally thought to be high-quality hosts for the parasite, but the reasons are not understood.

２．研究の目的

Our primary aim to understand the importance of nutrient availability on the legume – parasite system. We wanted to do a direct comparison of the effects of the holoparasite *Orobanche* with those of a native hemiparasite, *Phtheirospermum*.

As a secondary goal, we aimed to understand the importance of exogenous N supply vs. nitrogen fixed by the *Rhizobium* symbionts of the legume host.

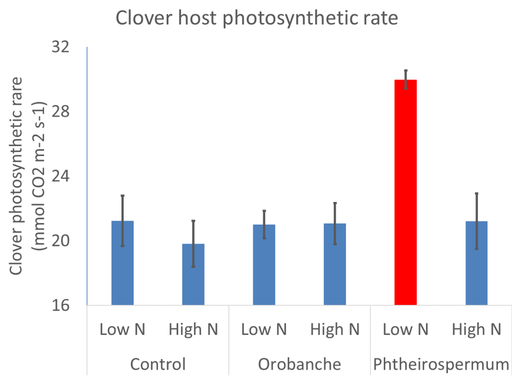
３．研究の方法

Our primary method was to grow the host and parasitic plant together or separately at a variety of different exogenous N levels, then monitor the photosynthetic rates, tissue N concentrations and growth rates of the host and parasitic plant either separately or together.

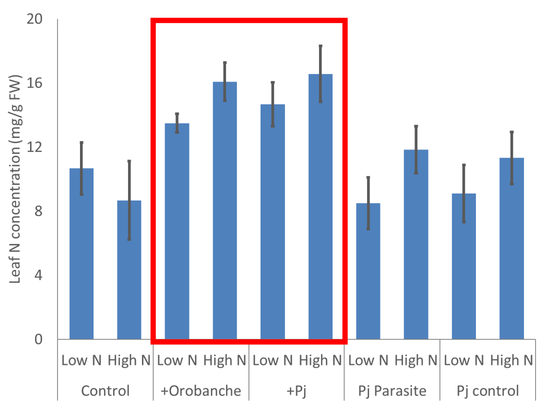
In addition, carbon and nitrogen isotopic labelling would be used to quantify the C and N fluxes between the host plant and the parasite.

４．研究成果

During the first 18 months of the project we tried a pot experiment where the clover host plant was grown by itself, or with either of the two parasites (*Orobanche* or *Phtheirospermum*), at high and low nitrogen levels. While we could find a strong negative influence of *Orobanche* parasitism on clover growth, we could not find any influence of *Phtheirospemum* parasitism on biomass. Indeed, *Phtheirospermum* plants grown by themselves were generally larger than when they were grown with clover plants. However, although we could find no influence of the clover host on parasite growth, we found that *Phtheirospermum* parasitism caused a 50% increase in host photosynthesis under low N conditions.



Although this effect was very strong, we had no explanation for this data. We also found that the parasitized plants contained 50% higher leaf N content.

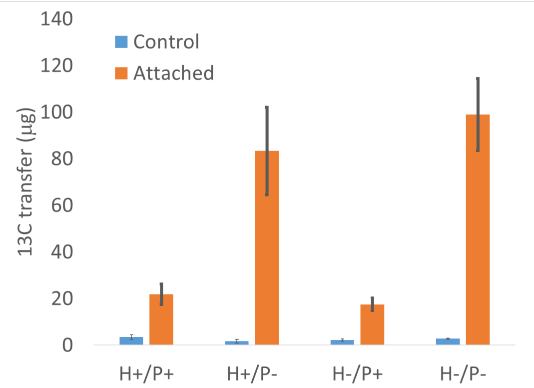


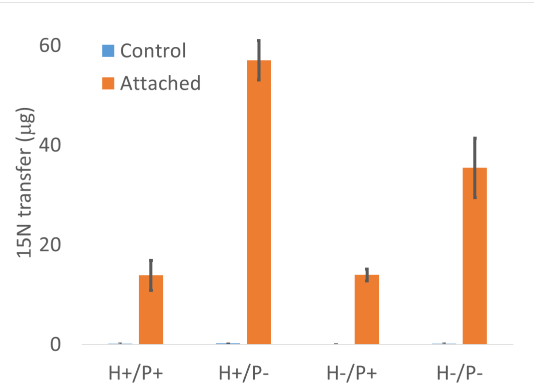
However, we could not connect these to any growth differences in either the host or the parasite. We repeated the experiment several times, but got variable results: sometimes we could repeat the phenomenon, and other times we could not.

Following this, it was decided to try to separate the effects of host and parasite nutrition, as there is obviously a nutrient effect in our data. For these experiments we used alfalfa as a host, as it would eb easier for us to apply isotope labelling. We spent several months prototyping various different root boxes, which would allow us to separate host and parasite nutrition. We also developed a mechanism for supplying 13CO2 and 15NO3 to our hosts, so that we could track the fate of N and C in our plants.

We developed a root box split into three sections; in one section the parasite would grow. In a second section, only the parasite roots could grow. In the middle, there was an "interaction" section, where the parasite and host roots could grow together. This design allowed us to control the N levels to each of the three sections independently.

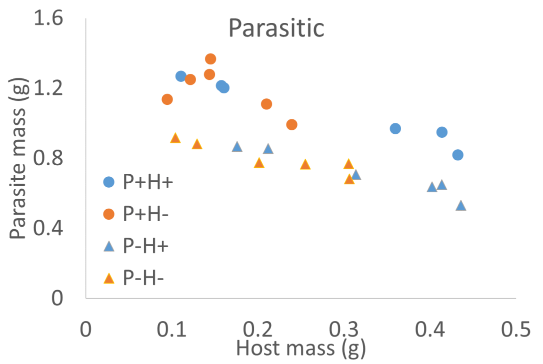
We grew *Phtheirospermum* plants in these boxes either together or separate from their alfalfa hosts, until they were 5 weeks old. At that time, we imposed control and N-free treatments on both the host and the parasite in a fully factorial design for one week before 13C / 15N labelling.

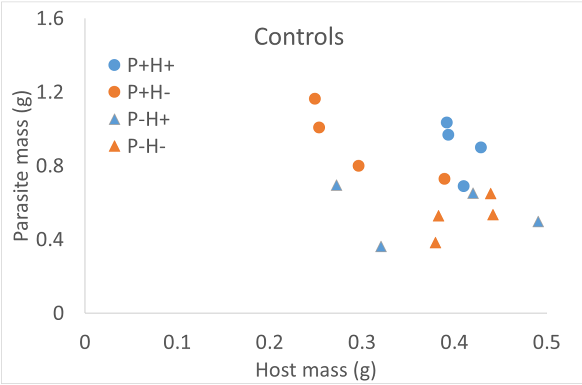




We were able to show a strong transfer of carbon and nitrogen to the parasite, but only 1) when the parasite was able to connect to the host, and 2) when the parasite was nutrient deprived. If the parasite was given nitrogen, the level of resource abstraction was significantly reduced.

It was important for us to demonstrate a functional basis for this behavior at this point, so we ran a growth experiment. We used the same root boxes that we used for the isotope labelling, but we extended the duration of the nutrient treatment to 5 weeks.

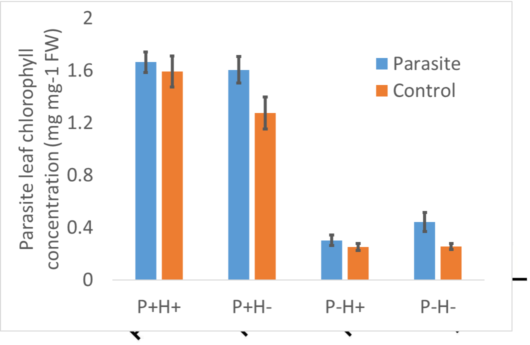




We found a strong negative relationship between host (alfalfa) and parasite mass in the parasitic plants, but not in the controls. In the parasitic treatments, the host plants suffer a growth reduction, where the host is receiving low nutrients, but the nutrient status of the parasite is not important. Our growth data strongly contradicts our isotope labelling data, which suggests there may be different short-term and long-term effects.

We also noticed in our long-term experiment, some negative effect of the clay we used to separate the roots. We decided to redo this experiment, with an improved protocol, and to do a second set of isotope labelling after 4 weeks of the treatment. We have generated superior growth data to our previous experiment, and we are currently measuring the 13C / 15N abundance. We expect to find high levels of C transfer under low N conditions for the host plant.

Our biomass data shows that supplying N to the host plant significantly reduces parasitism. We hypothesize that *Phtheirospermum* is parasitic for carbon, but not nitrogen. *Phtheirospermum* exhibited the ability to maintain growth rates, even when its chlorophyll levels have declined by 80%.



This is the first report of a hemiparasitic plant which is parasitic primarily for carbon, but not nitrogen.

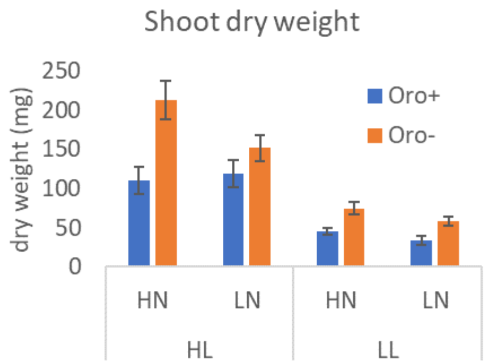
Furthermore, this is the first report of the suppression of parasitism by host nutrient status.

Our parasite requires nitrogen for growth, but if the host is supplied N, it will resist the parasite. This explains the phenomena discovered in our early work. If we gave too little N, the parasite could not grow. If we gave too much, the host would resist parasitism.

We aim to publish our current work within the next 3 months in a suitably high-ranked journal, given the extreme novelty and importance of this work.

We are currently revisiting our early work, in order to demonstrate the elevated CO2 fixation, and complete our project to compare the effects of two different parasites on clover photosynthesis and growth.

In a second stream, we have also been looking at the effects of environmental conditions on the clover / *Orobanche* system. We have explored the effects of light levels and N supply on growth and photosynthesis. Although parasitism caused a decrease in host shoot growth under all conditions, this decrease was minimized under low N conditions. *Orobanche* parasite mass was strongly influenced by light intensity, but N supply has no effect on the parasite mass or N content.



Clover plant shoot biomass
HL / LL = high light / low light
HN / LN = high N / low N

We are currently preparing this manuscript for publication, and expect it to be published within this year.

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔学会発表〕（計 1 件）

1. “Effects of holo- and hemiparasitism on host C/N economy
   IRVING, LJ”
   Parasitic Plant Workshop at Nara Institute of Science and Technology, 2016

６．研究組織
(1)研究代表者

IRVING, Louis
University of Tsukuba・Faculty of Life and Environmental Sciences・
Assistant Professor
研究者番号：60597178