Defining the role of novel fungal effector protein **AP5292** during myrtle rust infections to inform management of *Austropuccinia psidii*

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Taonga (treasured) species under threat

New Zealand's largest Pōhutukawa Tree is currently infected with *A. psidii.* [1]

The Pōhutukawa Tree *(Metrosideros excelsa)* is culturally-important in New Zealand.

Māori (indigenous New Zealanders) speak of Pōhutukawa in several pūrakau (cultural narratives). The most common is where the crimson flowers are viewed as the spilt blood of the demigod Tāwhaki who died falling to Earth. One centuries-old tree in Cape Rēinga (the northern tip of New Zealand) guards the entrance to the place where spirits leave New Zealand to journey back to Hawaiki, the Māori spiritual homeland. [2]

Many New Zealanders also use Pōhutukawa as a memorial tree for those that have died.

The Pōhutukawa is also given special importance because its bloom is an iconic symbol of the New Zealand summer. It is therefore sometimes referred to as the New Zealand Christmas tree. [3]

Ohawe Beach Motorcamp with Pōhutukawa in bloom. Photo credit: S. van der Hulst.

A. psidii overview

A. psidii are obligate biotrophic plant pathogens who utilise haustoria (specific infection structures) to interact within the infected host.

A. psidii are dependent on host plants for nutrients diverting these via haustorium. Haustorium within *A. psidii* express and secrete effector proteins (including AP5292) some of which are likely used to establish infection, some are likely avirulence proteins, and others hold currently unknown roles. [4]

Infiltration experiment

1. **AP5292 with a N-terminus YFP tag was transformed into *Agrobacterium tumefaciens.***
2. **Cultures were grown and infiltrated into *Nicotiana benthamiana.***
3. **Viewed via confocal microscopy.**

***Nicotiana benthamiana* is a known model system for transient expression of genes through agrobacterium infiltration.**

In this experiment YFP-AP5292 did not associate to any known plant cellular structure, likely forming protein bodies upon over expression *in planta* (Fig 2). Experiment is currently being repeated to validate results.

Future experiments will proceed with localization assays with a C-terminal YFP tagged AP5292.

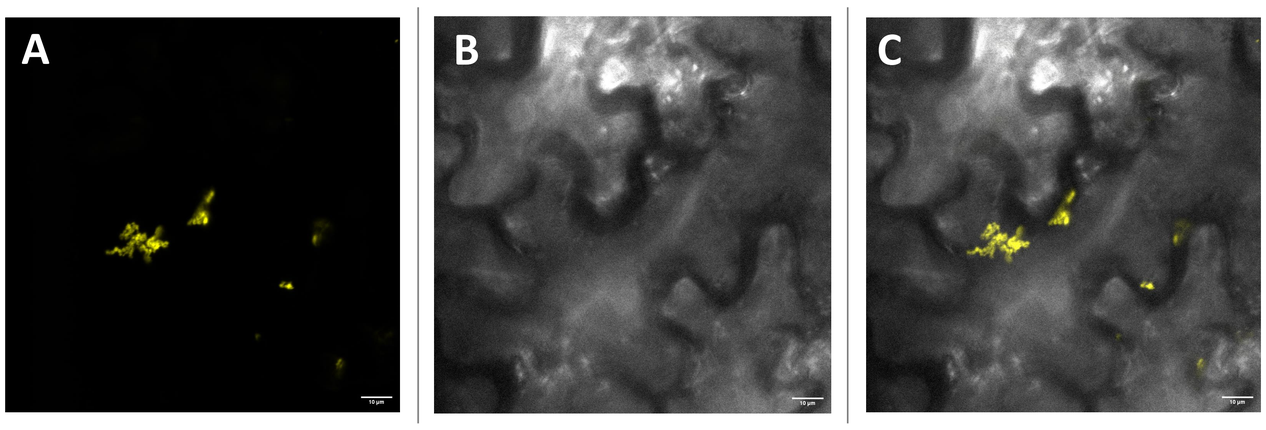

Fig 2. *N. benthamiana* imaged 40 hours after agroinfiltration with AP5292 with a N-terminus YFP tag, via Zeiss 700 confocal microscopy. **A.** AP5292 with a N-terminus YFP tag, **B.** *N. benthamiana,* **C.** Merged image created in ImageJ. Scale bar indicates 10 µm.

What is AP5292?

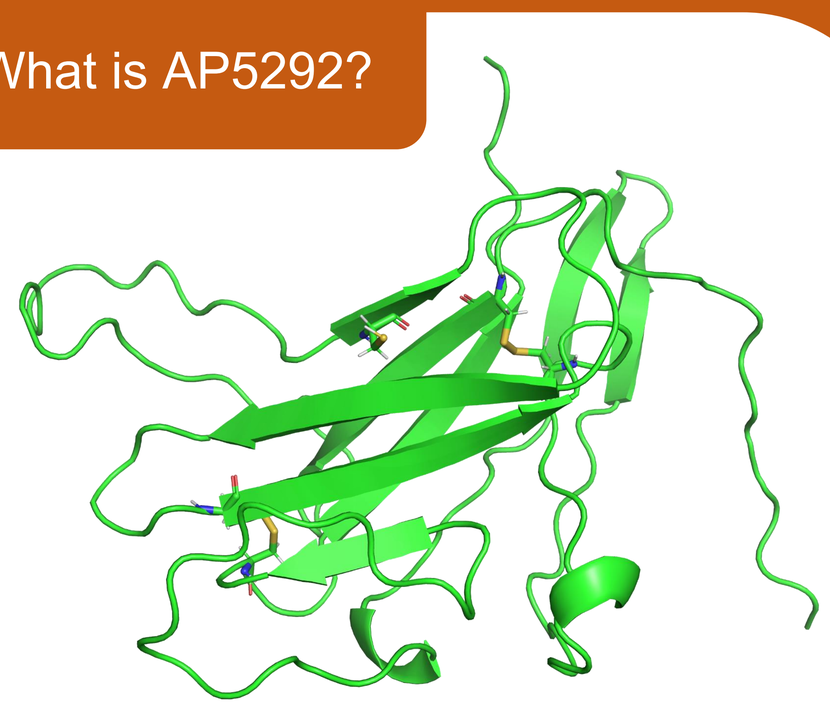

Fig 1. AlphaFold2 folding prediction for effector protein AP5292. Noted features include one free cysteine and two disulfide bonds. The disulfide bonds appear either side of beta sheets.

- AP5292 has one of the almost 370 effectors predicted in the *A. psidii* genome.
- AP5292 has one of four fungal proteins that are significantly differentially expressed between 24 and 48 hours after inoculation.
- AP5292 has homologues within other *Pucciniales*.
- AP5292 contains five cysteines, four are predicted to form disulfide bonds and one free which may suggest a host protein partner is possible (see Fig. 1).

Next steps

Protein purification and extraction.

Further infiltration assays.
X-ray crystallography.
Build a yeast-2-hybrid library.
Identify plant binding partners.

References

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