

## Supplementary Information

### **From community analysis to field application: control of apple canker (*Neonectria ditissima*) with endophytic *Epicoccum nigrum* strain B14-1**

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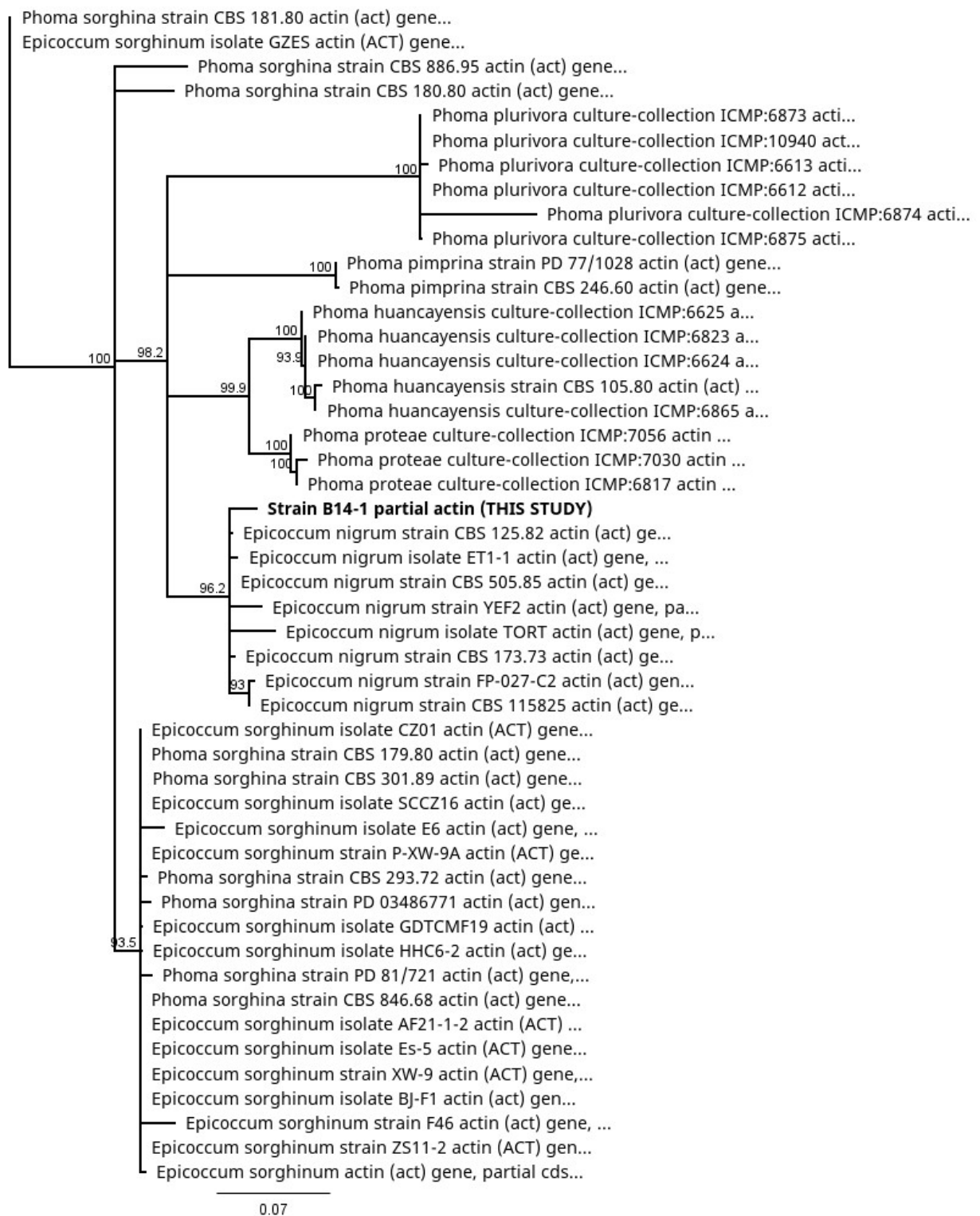
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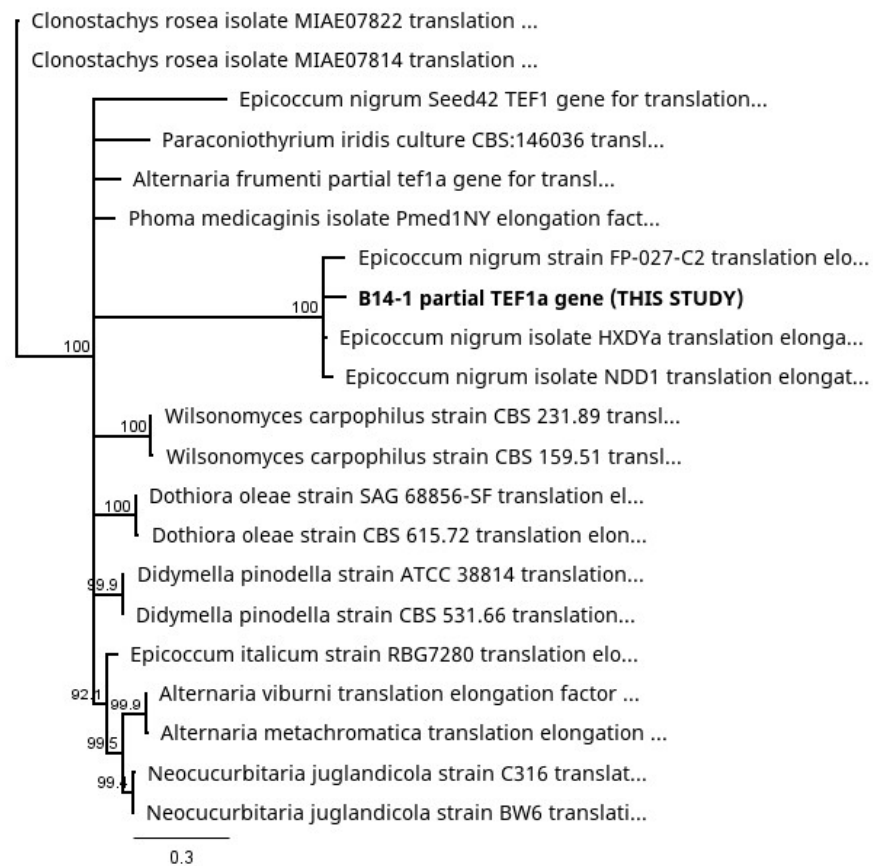
#### *Preliminary E. nigrum sporulation assessment*

Dry peat, vermiculite, and ground lentils were mixed with water in a 1 : 1 : 0.5 : 1 ratio and approximately 500 g of mixture autoclaved (30 min at 121° C) twice in polypropylene bags with 0.5 micron air filter patches (Unicorn, Type 14a). Sterile water was added in a 1:1 (mass : volume) ratio after autoclaving. Bags were inoculated with mycelial plugs from 14 day old *Epicoccum* PDA plates at a rate of one whole plate per bag and incubated at 22 ± 4°C and 12 h day conditions. Media was uniformly colonised by all three *E. nigrum* strains 2-3 weeks after inoculation. Sporulation and spore germination rate (after 48 h of incubation in water) were assessed weekly for 10 weeks. The media was mixed with sterile distilled water in a 1:1 ratio and shaken vigorously for 5 min, homogenate filtered through 2 layers of sterile muslin cloth and centrifuged at 4500× g for 15 min. The spore pellet was resuspended in sterile water. Spore germination was estimated by incubating spores in water at 20 ± 2 °C and 12h daylight for 48 h after which the spores with visible germination tubes longer than the width of the spore were counted as germinated. Spores with viability above 75% were produced between weeks three and five post inoculation, after which the germination decreased to less than 20% by week 10 post inoculation. B14-1 consistently produced > 10<sup>6</sup> spores per 50 g of media and was thus chosen for field trials. Strains C14 and C29 sporulated poorly (500-5000 spores per 50 g), so were not suitable for field trials. B14-1 spores were harvested at 5-7 weeks after inoculation and used within 6 h.

## Supplementary figures



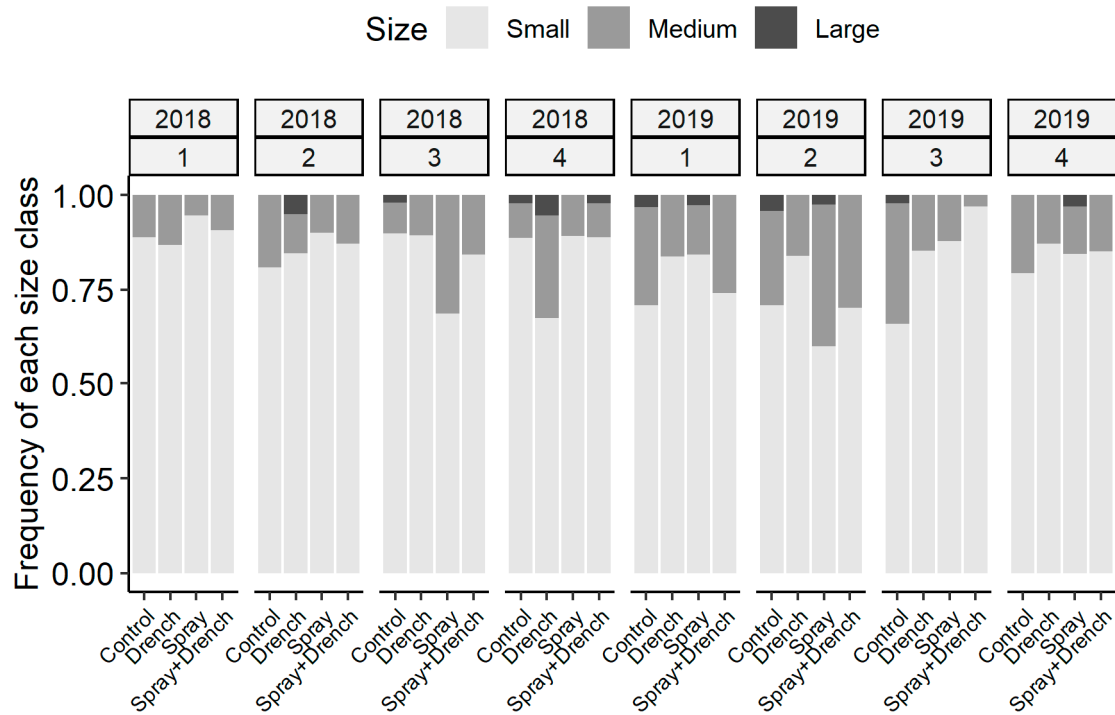
Supplemental Figure S1. Neighbour-joining tree based on partial actin sequences (Jukes-Kantor distance model, 1000 boot-strap iterations).



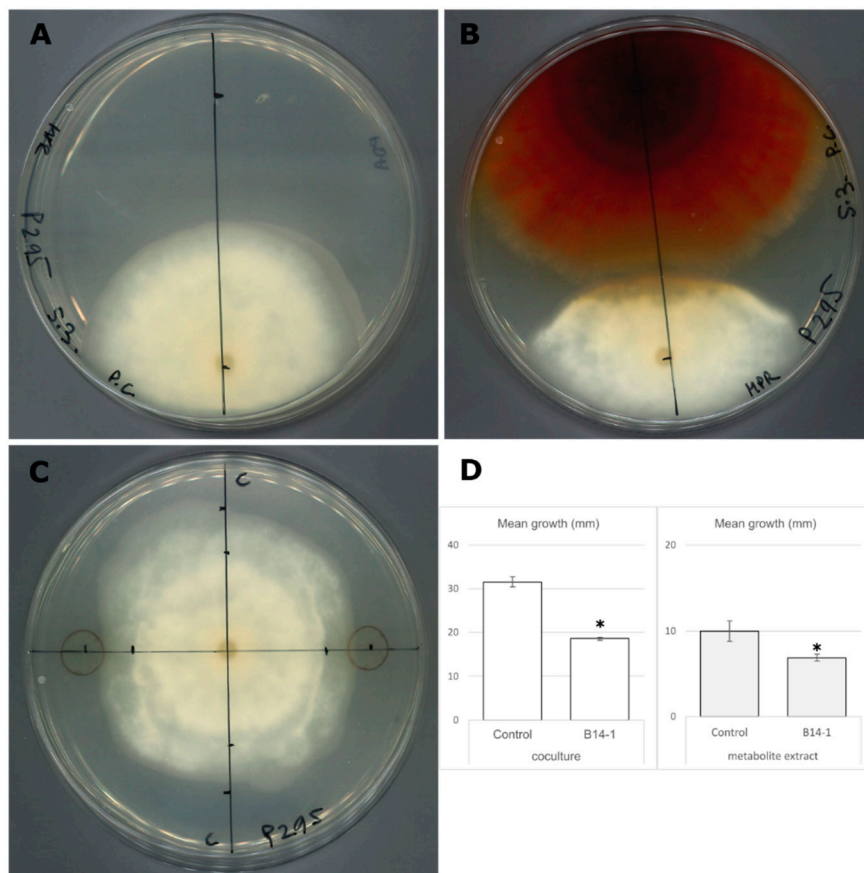
Supplemental Figure S2. Neighbour-joining tree based on partial transcription elongation factor sequences (Jukes-Kantor distance model, 1000 boot-strap iterations).



Supplemental Figure S3. Representative plant phenotypes observed in B14-1 leaf scar protection trial (A-D) and M9 rootstocks augmentation trial (E). Leaf scars inoculated in October 2019 with: A) water control, B) *N. ditissima*, C) B14-1 and *N. ditissima* and D) B 14-1. Images were thaken in June 2020. Canker leasion can be seen on *N. ditissima* inoculated leafscar (B). M9 rootstock shoots ca. 2 months after ammended with B14-1 (2019 trial) are shown in panel E.



Supplemental Figure S4. Rootstock diameter class data across two experiments (2018, 2019) each consisting of four randomised blocks (1-4) with four treatments: control, B14-1 root drench, B14-1 foliar spray or combined root drench and foliar spray. Size classes are: small < 9mm, medium 9mm - 11 mm, and large > 11mm.



Supplemental Figure S5. Representative images the antagonistic effect of B14-1 on *Phytophthora cactorum* isolate 295. *P. cactorum* 295 control (A), *P. cactorum* 295 in dual co-culture assay with B14-1 (B), and B14-1 soluble metabolite assay (C). Both assays were set up in the same way as for *N. ditissima*. The reduction of growth of *P. cactorum* 295 in co-culture (at nine days post assay set up) and in metabolite assays (ethyl acetate extraction, at four days post assay set up) is shown in panel D. \* indicates a significant reduction in comparison to the control (p(t test) < 0.01).