



Exploring resistance to *Ca. Phytoplasma solani* and *Ca. Liberibacter solanacearum* in potato

Jasmin Wiedmer^{1,2}, Doret Wouters¹, Daniel Moñino López¹, Richard G. F. Visser¹, Vivianne G.A.A. Vleeshouwers¹

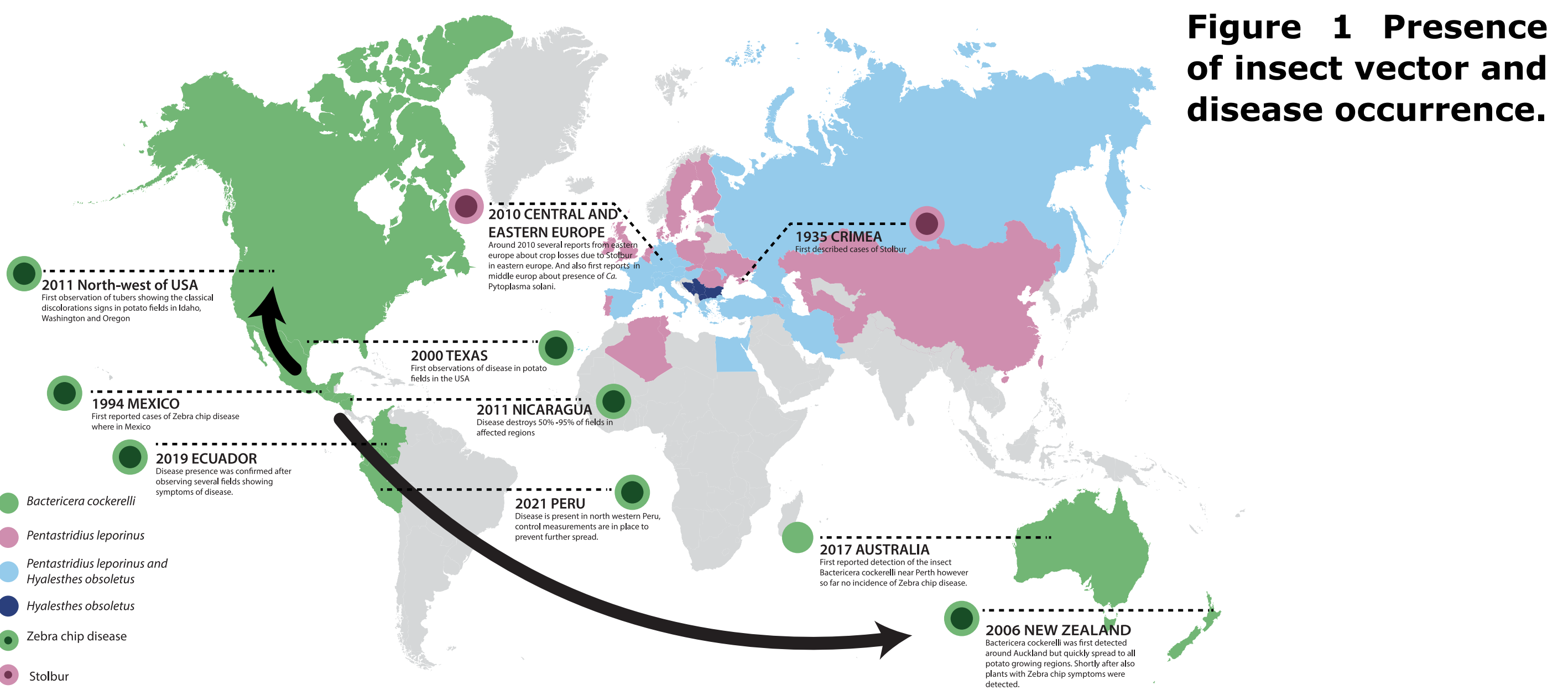
¹ Plant Breeding, Wageningen University and Research, The Netherlands

² Graduate School Experimental Plant Science

Background

Emerging phloem-limited diseases are a major problem all over the world (Figure 1). Potato is threatened by Stolbur caused by *Ca. Phytoplasma solani* (Figure 2), and by Zebra chip caused by *Ca. Liberibacter solanacearum*. So far there is no resistance known against either of these diseases. Our **objectives** for this project are:

- Explore resistance within wild tuber-bearing *Solanum* genotypes
- Identify and characterise effectors
- Identify resistance genes and molecular markers



Approach

Both bacteria cannot be cultured in vitro. As a consequence, disease tests are often only done by assays including the transmitting insect, which are restricted by quarantine regulations, require much space and time, and it remains unclear whether detected resistance targets the insect or the bacteria. Therefore, we suggest an approach combining effectoromics, grafting and diseases test with the insect (Figure 2).

Symptoms on Phytoplasma grafted plants

Grafting experiments with *Ca. Phytoplasma solani*-infected shoots show that potato plants develop diverse symptoms of Stolbur (Figure 3).

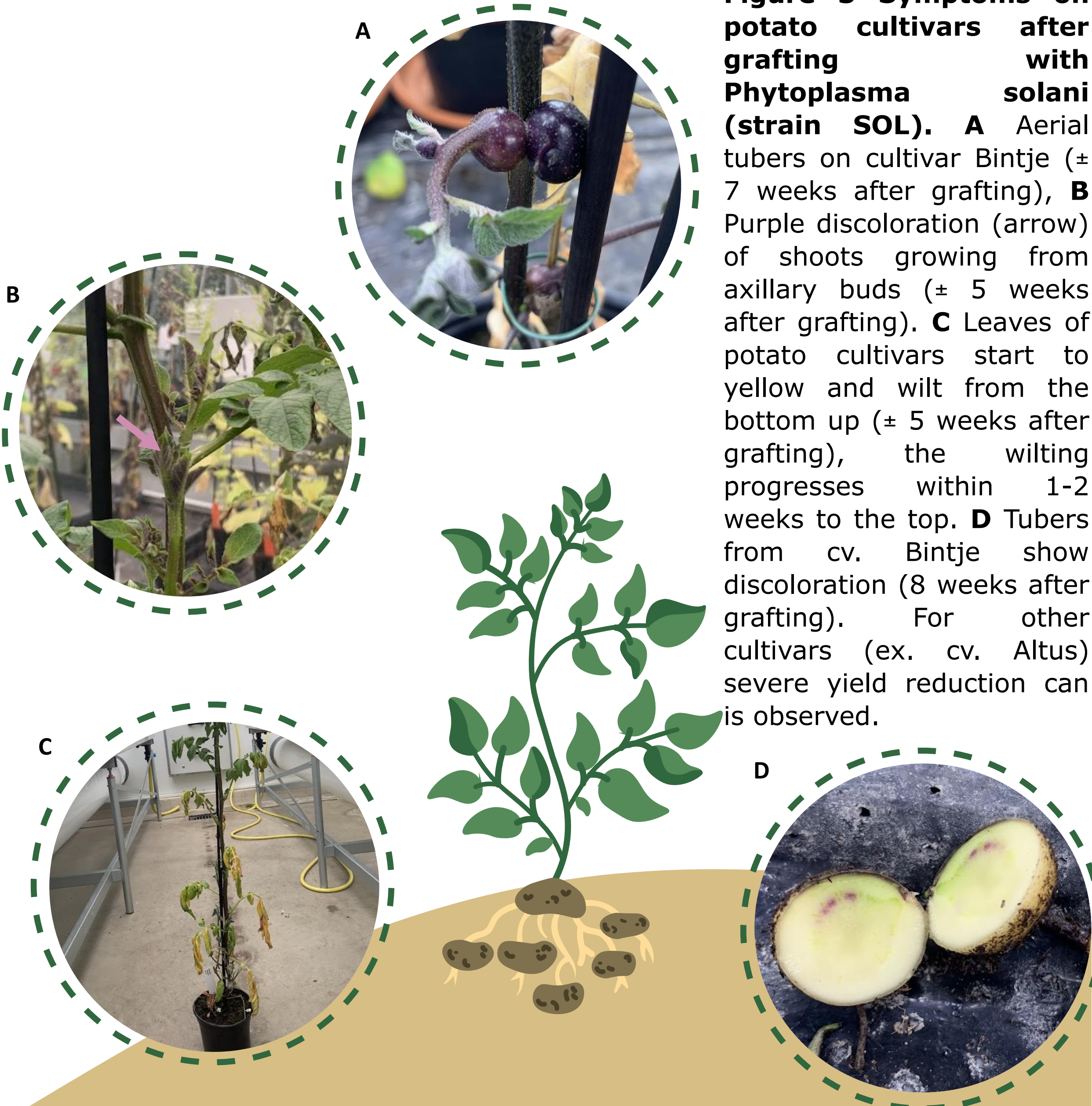
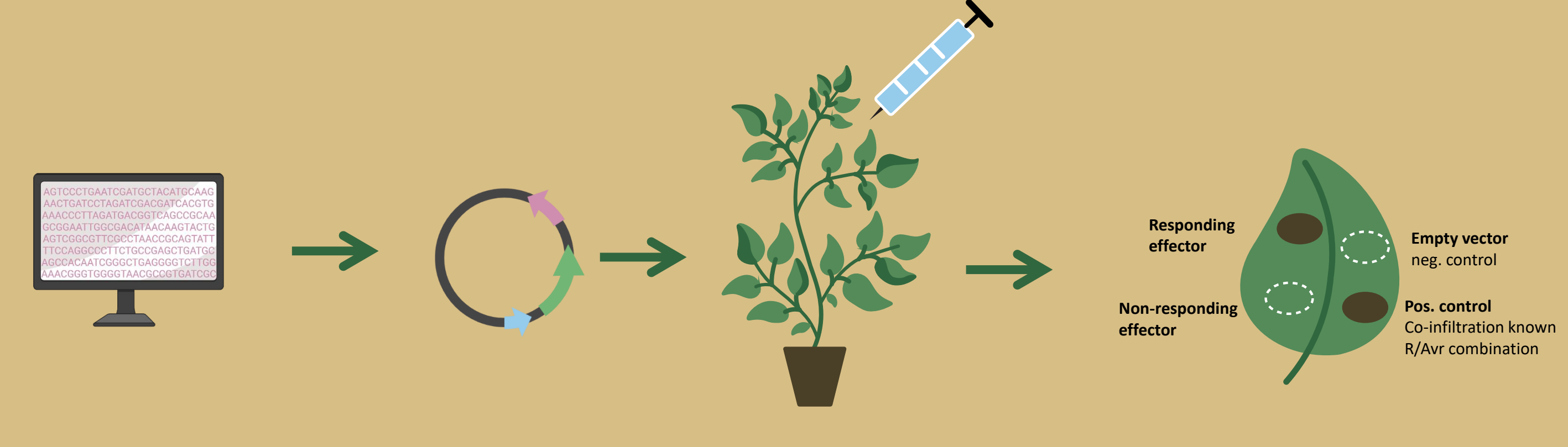
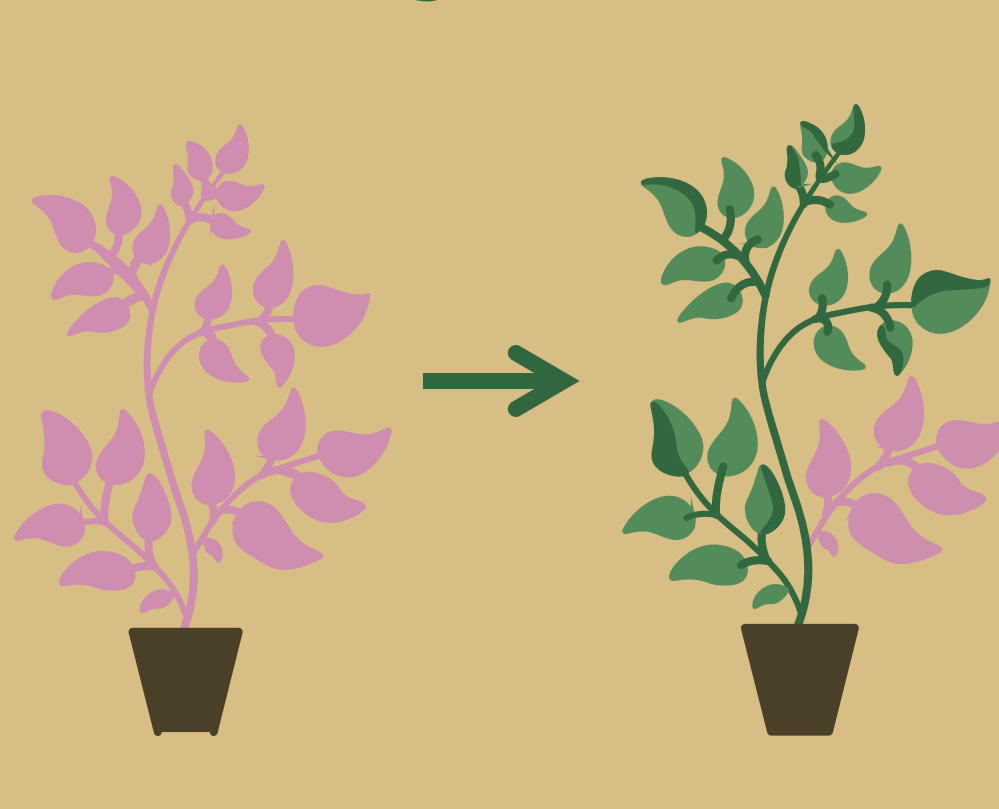


Figure 3 Symptoms on potato cultivars after grafting with *Phytoplasma solani* (strain SOL). **A** Aerial tubers on cultivar Bintje (± 7 weeks after grafting), **B** Purple discoloration (arrow) of shoots growing from axillary buds (± 5 weeks after grafting), **C** Leaves of potato cultivars start to yellow and wilt from the bottom up (± 5 weeks after grafting), the wilting progresses within 1-2 weeks to the top. **D** Tubers from cv. Bintje show discoloration (8 weeks after grafting). For other cultivars (ex. cv. Altus) severe yield reduction can be observed.

A Effectoromics



B Grafting



C Insects



Figure 2 Workflow of effectoromics-grafting-insect approach. **A** Effector candidates are predicted or identified from literature. Effectors are then cloned into *Agrobacterium tumefaciens* and transiently expressed in plants by PVX agroinfection or agroinfiltration¹. **B** Healthy responding plants (green) are grafted with bacteria-infected shoots (purple) to see if disease symptoms develop. **C** If plants are resistant in the grafting experiment they are tested with the bacteria-infected insects

Recognition of effectors by *Solanum* genotypes

Effector screens show that diverse wild *Solanum* genotypes mount defence responses to effectors (Figure 4), which suggest the presence of immune receptors.

Genotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
HPE1A	13	51	24	42	22	3	100	3	3	0	8	0	21	0	3	2	28
HPE1B	13	78	40	36	6	0	100	0	0	3	14	0	14	4	0	0	0
HPE3 ▲	29	100	17	31	0	0	100	11	0	3	8	0	14	0	0	0	6
HPE7	29	26	15	33	0	0	0	0	20	0	3	0	14	11	6	0	0
HPE17	29	97	13	26	44	0	100	69	0	28	6	0	0	25	25	0	0
HPE27 ▲	29	100	13	21	16	0	100	63	0	17	6	0	0	17	0	0	0
HPE33 ▲	25	15	8	15	0	0	0	0	0	0	8	0	0	0	0	0	0
HPE74 ▲	97	100	94	53	100	100	100	88	88	44	33	60	100	89	100	30	8
Empty vector	30	6	17	31	0	3	0	0	5	0	0	5	0	0	0	4	0
Crn2	72	60	90	94	20	39	90	34	57	44	100	77	77	30	88	72	54

Figure 4 PVX agroinfection of *Ca. Liberibacter* effectors. Heat-plot of 17 wild *Solanum* genotypes PVX agroinfected with 8 effectors, symptoms range from no response (0) to confluent cell death (100), Crn2 from *P. infestans* and the empty vector were used as positive and negative controls, respectively. Triangles represent expression of effector in insect (▲) vs plant (▲), no triangle means they are equally expressed³.



References
1 Du, J. and Vleeshouwers, V. G. A. A. (2014) The Do's and Don'ts of Effectoromics. In: Plant-Pathogen Interactions - Methods Molecular Biology. 257-268
2 Reyes Caldas PA(2022) Mol Plant Microbe Interact. 2022 Dec. 35(12). 1067-1080
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