

Introduction:

Strawberries are a key fruit crop for Ontario. Since 2020 *Neopestalotiopsis* spp. have been identified across Ontario via the use of an annual survey carried out with the help of OMAFRA and Department of Plant Agriculture. We developed a visual infiltration bioassay based on the research [2]. to compare aggressiveness/virulence of *N. Rosae* and *N spp* (aggressive strain). at the concentrations of 8.94×10^4 - 9.4×10^4 and symptoms and signs were observed at 24 hrs, 48hrs, and 72 hrs.



Fig. 1 shows a field in 2020 devastated by *N Sp*. In Southern Ontario. 40% of plants showed symptoms which appeared as light brown necrotic lesions with darker borders on leaves, severe wilting in crowns and 5% plant death.

Methods:

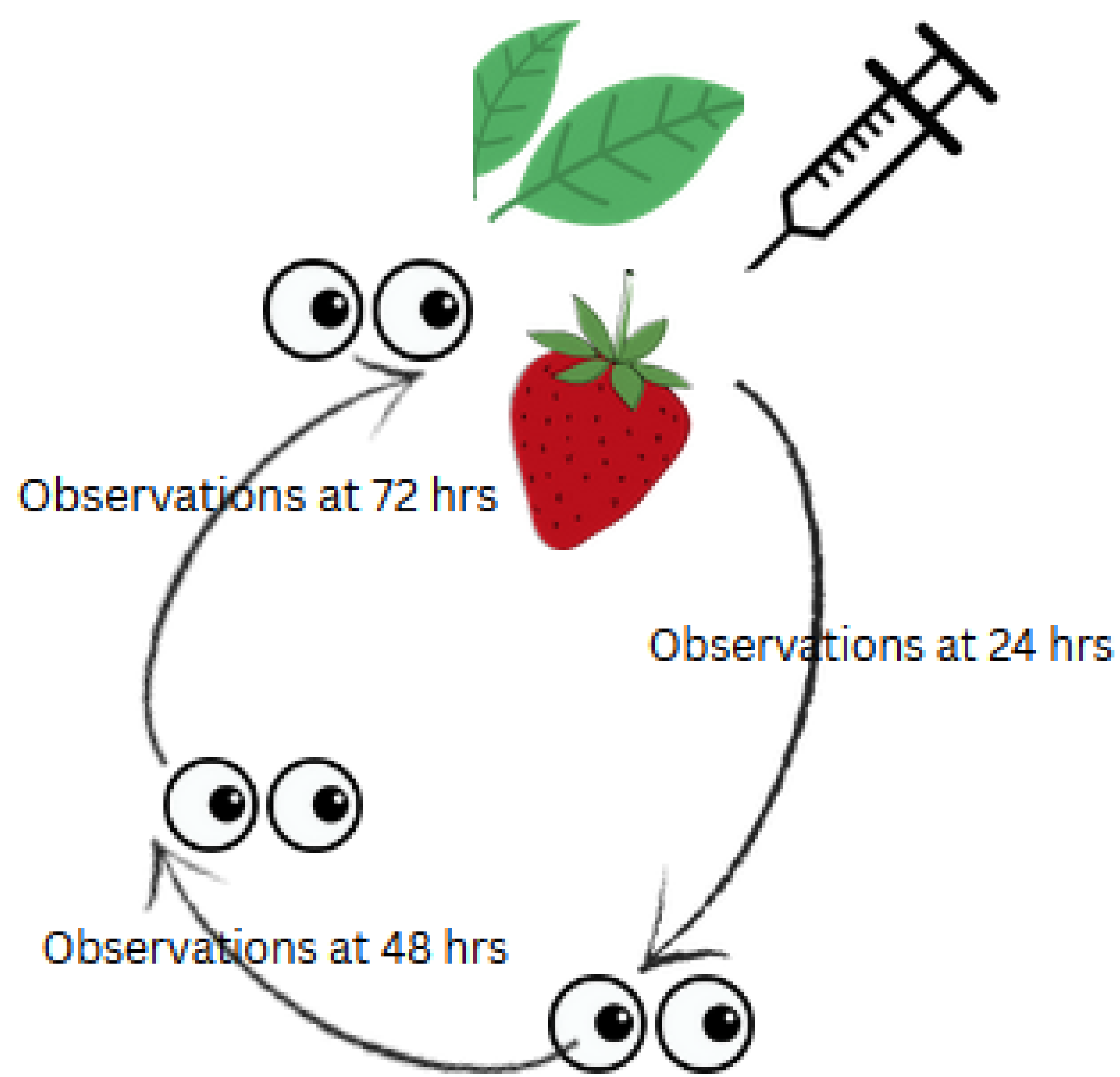


Fig. 2 shows how the infiltrations were carried out in the lab. Separate infiltrations were run for both fruit and leaves. Observations were made 24,48, and 72 hours.

Pathogen Survey:

Specimens were collected throughout the province from 2020 to present, DNA was extracted using DNAeasy kits and the primers used for identification were found on the beta tubulin Loci of *Bt2a* and *Bt2b* [1]. The DNA was sent to Advanced Analysis center for sequencing.

Sample number	Cultivar	Result	Id
20_01	Albion	-	
20_02	Albion	+	<i>N sp.</i>
20_03	Albion	+	<i>N sp.</i>
20_04	Albion	+	<i>N rosae.</i>
20_05	Albion	+	<i>N sp.</i>
20_06	Albion	-	
21_01	Albion + San Andreas	-	
21_02	Albion	-	
21_03	Albion	-	
21_04	Albion	-	
21_05	Albion	-	
21_06	Albion	+	<i>N sp.</i>
21_07	Albion	-	
21_08	Albion	-	
21_09	Albion	+	<i>N sp.</i>
21_10	Albion	+	<i>N sp.</i>
21_11	Jewel	-	
21_12	Jewel	+	<i>N sp.</i>
21_13	Albion	-	
21_14	Albion	-	
21_15	Albion	+	<i>N sp.</i>
21_16	Albion	+	<i>N sp.</i>
21_17	Jewel	-	
21_18	Albion	+	<i>N sp.</i>
21_19	San Andreas	-	
21_20	Albion	+	<i>N sp.</i>
21_21	Albion	+	<i>N rosae</i>
21_22	Variety X	+	<i>N sp.</i>
21_23	Jewel	-	
21_24	Albion	-	
21_25	Albion	-	
22-01	Jewel	+	<i>N sp.</i>
	Jewel	-	
	Jewel	-	
	Jewel	-	
22-02	Albion	+	<i>N sp.</i>
22-03	Albion	+	<i>N sp.</i>
	Monterey	-	
	Jewel	-	
	Jewel	-	
22-04	Albion	+	<i>N sp.</i>
	Albion	-	
	Jewel	-	

Table 1: *N. spp.* field survey in Ontario. Of 44 field showing symptoms of *N.spp.*, only 15 tested positive for *N. sp.* and 2 tested positive for *N. rosae*. With total infection rate of 39%.

Results

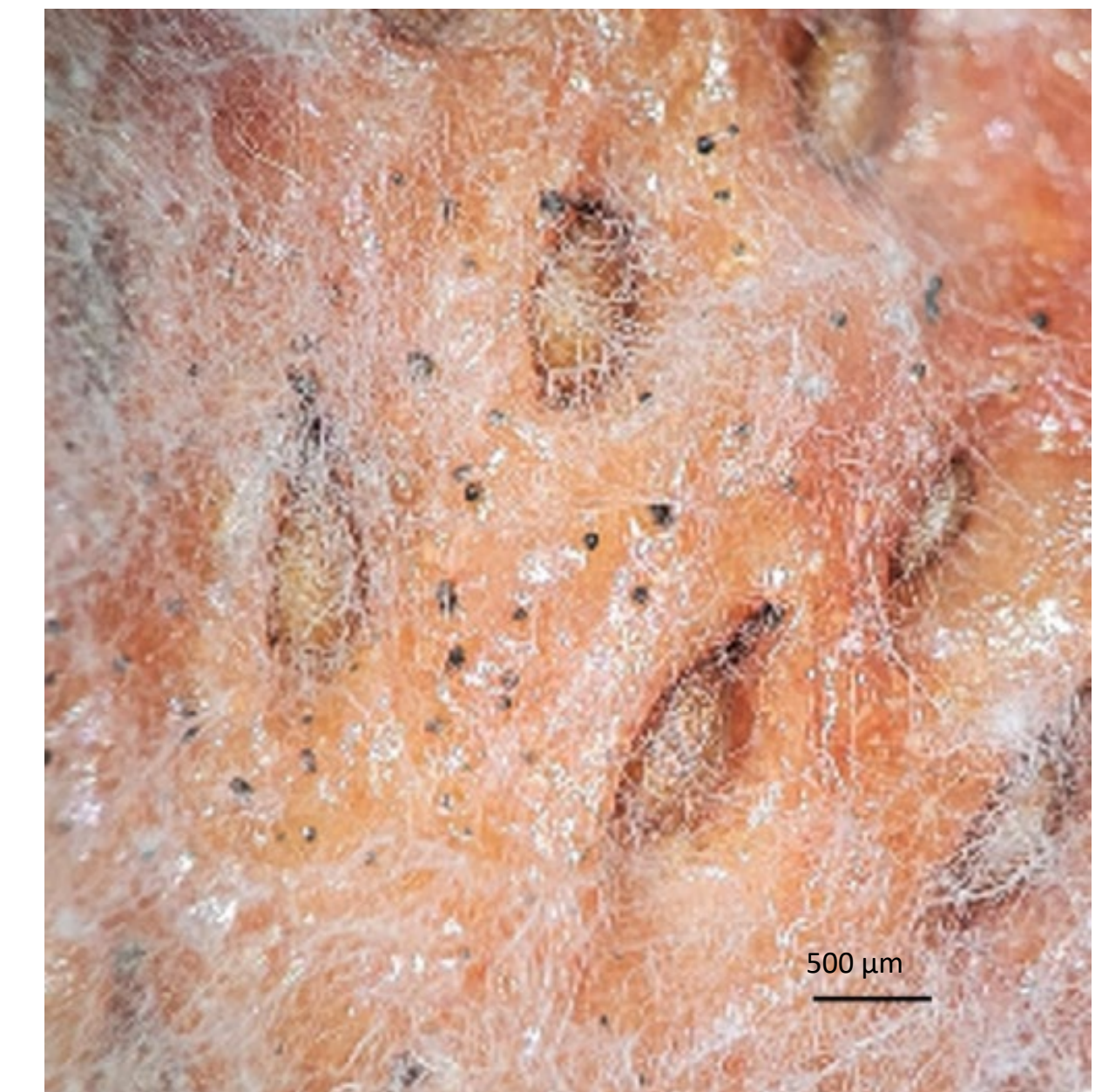


Fig. 3 Aggressiveness/virulence infiltration assay on leaf and berry tissue. Symptoms on leaf tissue include necrotic lesions and brown staining on the bottom of the leaf. Acervuli were visible after 72 hours post infiltration

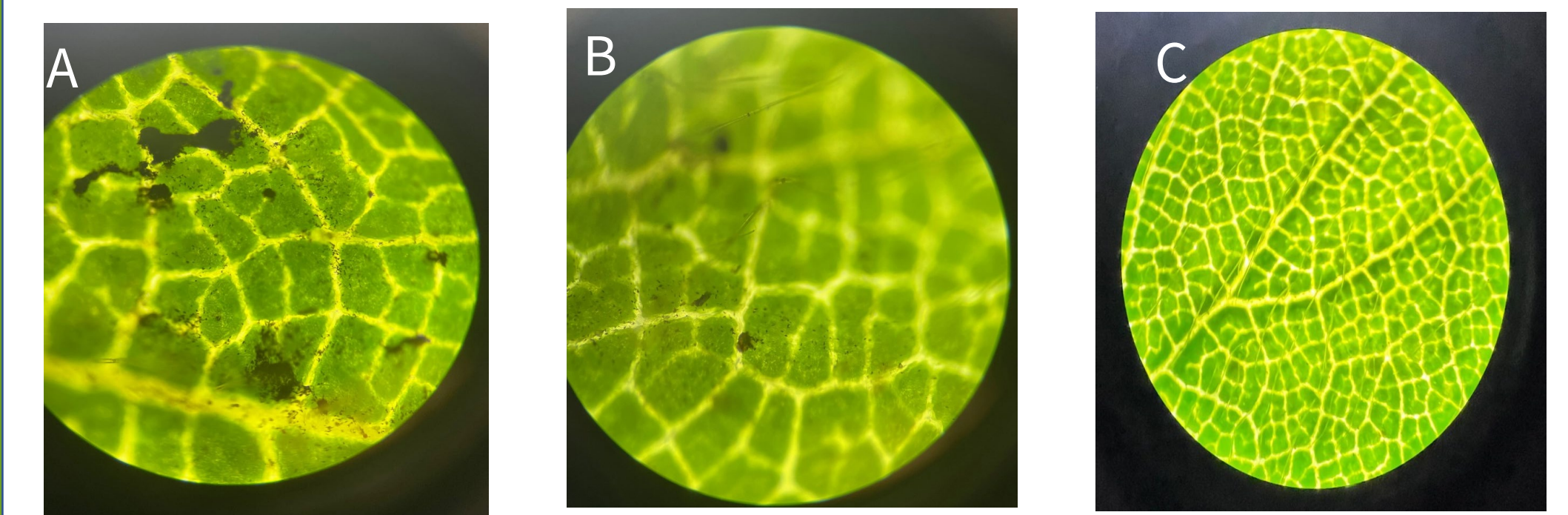


Fig.4 Microscopic images of *Fragaria x ananassa* cv. Albion using (A) *N.sp* (aggressive)(B) *N. rosae* (less aggressive) and (C) sterile distilled water negative control . (A and B at 100x, C at 50 x)

Future Work

Future work will include optimizing the aggressiveness/virulence infiltration assay to compare *N. sp.* and *N. rosae*. Derived cleaved polymorphic sequences (DCAPs) and recombinase polymerase (RPA) assays. Will be developed to identify and differentiate between the two current species in Ontario. Field monitoring will continue and more work is needed to advance our understanding of this potentially detrimental disease complex.

Acknowledgements:

Thanks to the Berry Growers (Grant 055219) of Ontario and Ontario Ministry of Agriculture, Food and Rural Affairs for supporting our work and special thanks to Rural Affairs our berry consultants for helping with sampling.

References

- [1]Baggio J.S., et al. 2021. Plant Dis, 105:305-315
- [2]Miller- Butler., et al. 2018. Plant Dis, 102:2112-2119