Identification and Pathogenicity of *Fusarium proliferatum* Causing Clove Rot on Garlic

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Introduction

Garlic clove rot, caused by *Fusarium proliferatum* (*Fp*), can result in losses of up to 30% in harvested bulbs during drying, conditioning and storage. Despite the increase in the incidence and severity of clove rot in garlic producing regions all over the world, little is known about the genetic and pathogenic diversity of the pathogen population affecting garlic. The objectives of this research were to: assess the genetic and pathogenic diversity of *Fp* populations associated with garlic.

Pathogen identification

- One hundred and sixteen *Fp-like* isolates associated with garlic accessions in the USDA garlic germplasm collection and symptomatic garlic bulbs in Oregon were used in this study
- DNA was extracted and subjected to PCR to amplify the translation elongation factor-1α (EF-1α) gene (4)
- The sequences of both strands were determined by Sanger sequencing and consensus sequences were compared to those in GenBank using the BLASTn tool

Microsatellite genotyping

- Seventy *Fp* isolates were genotyped using eight SSR markers (3)
- Data analyses were conducted using the "Poppr" package (1, 2) in R
- A minimum spanning network was constructed using the "ape" package (5) in R

Pathogenicity assays

- A total of 25 *Fp* isolates were tested for their virulence on garlic
- Healthy garlic cloves (cv. 'California Early') were surface-sterilized in 3% NaOCl and washed with sterile H_2O
- Surface-sterilized cloves were wounded with a sterile 1-mm-diameter probe and injured cloves were soaked in conidial suspensions (10^5 conidia mL^{-1}) for 12 h
- Cloves were air-dried for 5 h and then incubated at 27ºC for 5 weeks
- Symptom development was assessed according to a 1-5 scale where 1 = no visible symptoms; 2 = less than 20% rotted clove; 3 = 20 - 49% rotted clove; 4 = 50 - 80% rotted clove; and 5 = more than 80% rotted clove

Acknowledgements

Results

- All 116 isolates included in this study were identified as *Fusarium proliferatum* according to partial DNA sequencing of the EF-1α gene (data not shown). Consensus sequences were highly similar (99.3% to 100%) to sequences of *F. proliferatum* already in GenBank.

Summary of the genotypic and genetic diversity statistics from six populations of *Fusarium proliferatum* using eight multilocus markers

Population	n	MLG	H	G	λ	E_5	H_{exp}	I_a	$\bar{r}_d$
Washington	15	13	2.52	11.84	0.92	0.95	0.21	0.58	0.20
New York	10	6	1.64	4.55	0.78	0.85	0.16	0.66	0.33
Oregon	7	7	1.95	7.00	0.86	1.00	0.27	-0.24	-0.08
California	3	3	1.10	3.00	0.67	1.00	0.25	2.00	1.00
China	2	2	0.70	2.00	0.50	1.00	0.25	NA	NA
Unknown	33	29	3.31	25.33	0.96	0.92	0.25	-0.08	-0.01
Total	70	52	3.71	27.84	0.96	0.67	0.25	0.16	0.03

n, number of isolates observed; MLG, multilocus genotypes; H, Shannon-Wiener index of MLG diversity; G, Stoddart and Taylor's index of MLG diversity; λ, Simpson's index; E_5, evenness; H_{exp}, Nei's unbiased gene diversity; I_a, index of association; and $\bar{r}_d$, standardized index of association, which is used to detect linkage disequilibrium.

Minimum spanning network of *Fusarium proliferatum* multilocus genotypes from garlic
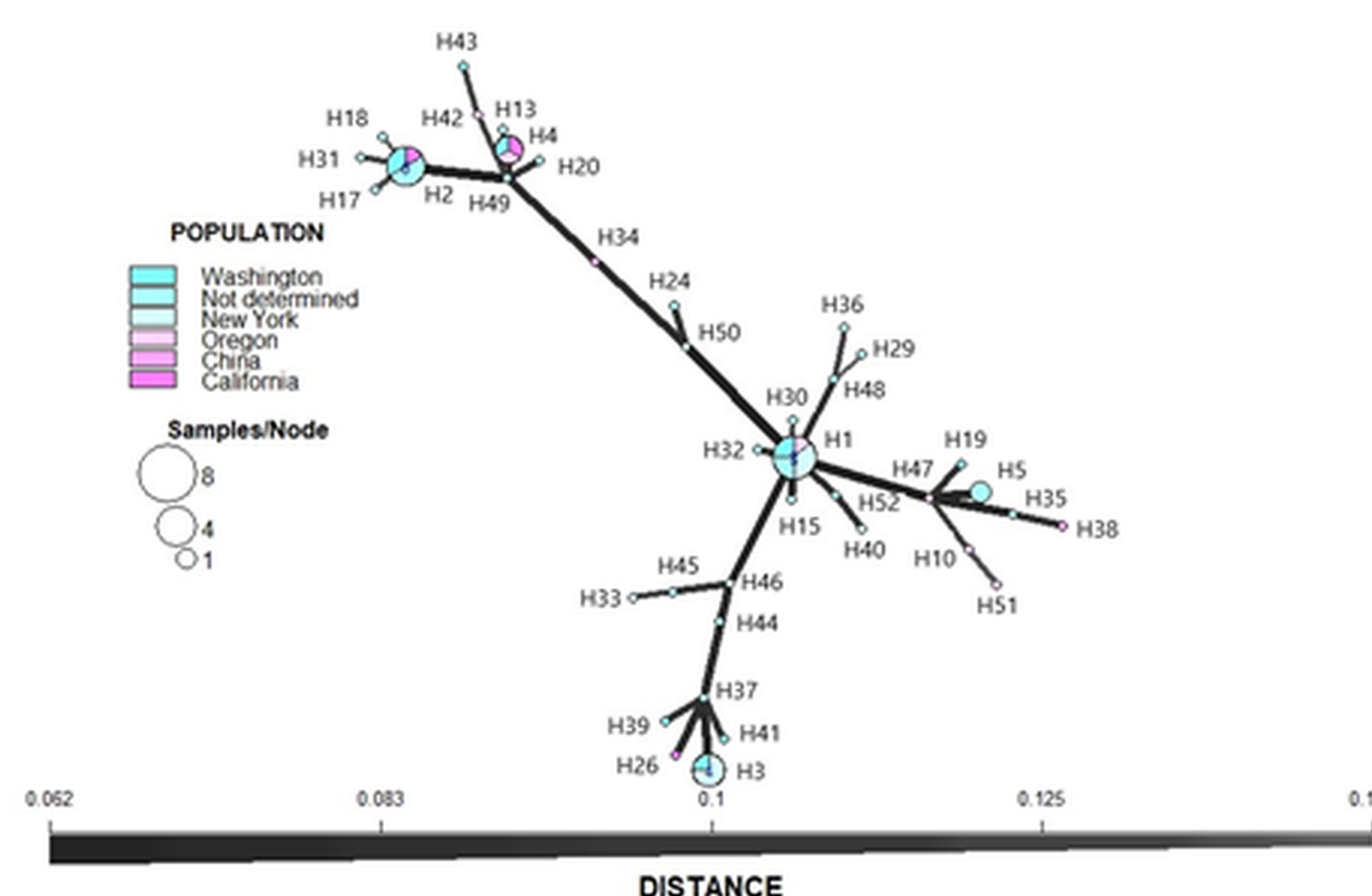
Results cont.
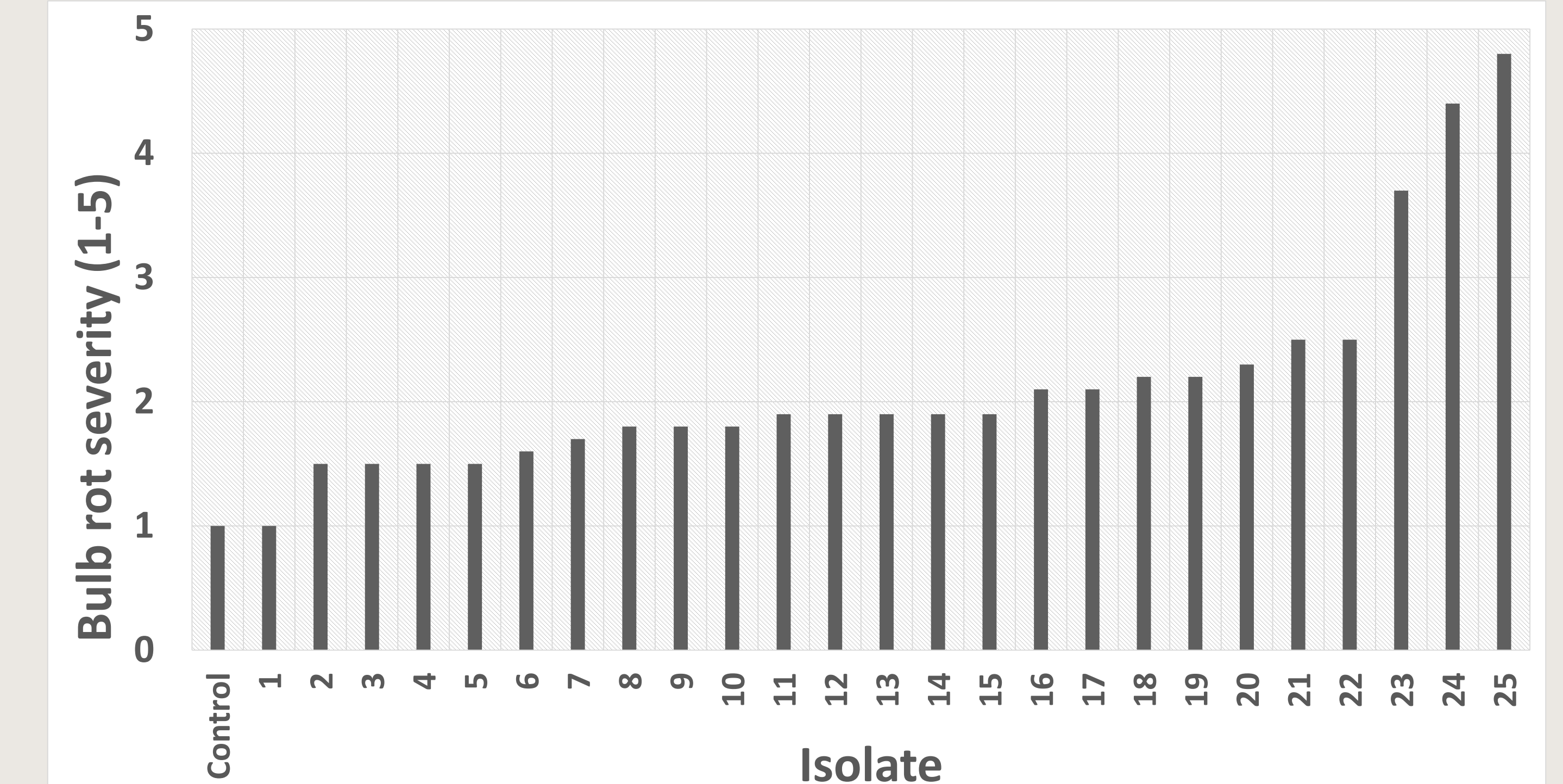
Pathogenicity of *Fusarium proliferatum* isolates on garlic. Symptoms were assessed according to a 1-5 scale where 1 = no visible symptoms; 2 = less than 20% rotted clove; 3 = 20 - 49% rotted clove; 4 = 50 - 80% rotted clove; and 5 = more than 80% rotted clove.

Conclusions

- Fifty-two multilocus genotypes (MLGs) and 18 clones were observed
- Most MLGs observed in this study clustered into one genetic group
- All but one *F. proliferatum* isolates induced rot on garlic cloves
- Knowledge about the genetic diversity and virulence of *F. proliferatum* on garlic will help inform future management strategies for bulb rot

References

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