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SUPPLEMENTARY MATERIAL **OPEN ACCESS**

Soil Microsclerotial Density Is Strongly Associated with Olive Wilt Severity in Tunisian Orchards

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Table S1. Prospective quality-control checklist for future validation studies.

Quality-control Domain	Prospective Specification
Field blanks	Include sterile field blanks by orchard/sampling round and document results.
Tool decontamination	Clean and disinfect corers/augers between trees and record the procedure.
Sample traceability	Assign unique tree, core/composite, matrix, date, and processing identifiers; retain a chain-of-custody log.
Positive controls	Include soil or culture positive controls with predefined acceptance criteria for plating and molecular assays.
Negative controls	Include media, water/extraction, and no-template controls as appropriate and document all outcomes.
Technical precision	Retain replicate determinations separately and report CV/SE without treating technical replicates as biological units.
Blinded recounts	Blindly recount a prespecified subset of plates and report inter-reader agreement.
Molecular inhibition/recovery	For soil qPCR, include extraction-recovery and inhibition controls and calibrate copy number in representative soil matrices.

Note: Table **S1** is a prospective quality-control checklist. No numerical QC performance values are attributed retrospectively to the archived dataset.

Table S2. Raw microsclerotial plate counts.

Orchard	Disease Stage	Stage Descriptor	Plate Replicate	Ms/g soil
BH	0	Asymptomatic	1	5
BH	0	Asymptomatic	2	7
BH	0	Asymptomatic	3	9
BH	1	Mild	1	33
BH	1	Mild	2	25
BH	1	Mild	3	23
BH	2	Moderate	1	105
BH	2	Moderate	2	97
BH	2	Moderate	3	86
BH	3	Severe/dead	1	366

(Table) contd.....

Orchard	Disease Stage	Stage Descriptor	Plate Replicate	Ms/g soil
BH	3	Severe/dead	2	343
BH	3	Severe/dead	3	302
TG	0	Asymptomatic	1	3
TG	0	Asymptomatic	2	7
TG	0	Asymptomatic	3	5
TG	1	Mild	1	22
TG	1	Mild	2	25
TG	1	Mild	3	31
TG	2	Moderate	1	89
TG	2	Moderate	2	82
TG	2	Moderate	3	78
TG	3	Severe/dead	1	126
TG	3	Severe/dead	2	142
TG	3	Severe/dead	3	137
ZR1	0	Asymptomatic	1	13
ZR1	0	Asymptomatic	2	10
ZR1	0	Asymptomatic	3	10
ZR1	1	Mild	1	29
ZR1	1	Mild	2	35
ZR1	1	Mild	3	32
ZR1	2	Moderate	1	85
ZR1	2	Moderate	2	82
ZR1	2	Moderate	3	70
ZR1	3	Severe/dead	1	122
ZR1	3	Severe/dead	2	114
ZR1	3	Severe/dead	3	109
ZR2	0	Asymptomatic	1	12
ZR2	0	Asymptomatic	2	16
ZR2	0	Asymptomatic	3	11
ZR2	1	Mild	1	28
ZR2	1	Mild	2	35
ZR2	1	Mild	3	24
ZR2	2	Moderate	1	109
ZR2	2	Moderate	2	112
ZR2	2	Moderate	3	121
ZR2	3	Severe/dead	1	110
ZR2	3	Severe/dead	2	115
ZR2	3	Severe/dead	3	111
ZR3	0	Asymptomatic	1	5
ZR3	0	Asymptomatic	2	8
ZR3	0	Asymptomatic	3	5
ZR3	1	Mild	1	32
ZR3	1	Mild	2	36
ZR3	1	Mild	3	37
ZR3	2	Moderate	1	99
ZR3	2	Moderate	2	107
ZR3	2	Moderate	3	109
ZR3	3	Severe/dead	1	153
ZR3	3	Severe/dead	2	148
ZR3	3	Severe/dead	3	134

Table S3. Orchard-stage means and metadata.

Orchard	Disease Stage	Stage Descriptor	mean_MS	sd_MS	sem_MS	Incidence (%)	Irrigation	Previous Crops
BH	0	Asymptomatic	7.00	2.00	1.15	18.00	Drip	Potato + Melon
BH	1	Mild	27.00	5.29	3.06	18.00	Drip	Potato + Melon
BH	2	Moderate	96.00	9.54	5.51	18.00	Drip	Potato + Melon
BH	3	Severe/dead	337.00	32.42	18.72	18.00	Drip	Potato + melon
TG	0	Asymptomatic	5.00	2.00	1.15	5.00	Drip	Potato
TG	1	Mild	26.00	4.58	2.65	5.00	Drip	Potato
TG	2	Moderate	83.00	5.57	3.21	5.00	Drip	Potato
TG	3	Severe/dead	135.00	8.19	4.73	5.00	Drip	Potato
ZR1	0	Asymptomatic	11.00	1.73	1.00	23.50	Drip	Potato
ZR1	1	Mild	32.00	3.00	1.73	23.50	Drip	Potato
ZR1	2	Moderate	79.00	7.94	4.58	23.50	Drip	Potato
ZR1	3	Severe/dead	115.00	6.56	3.79	23.50	Drip	Potato
ZR2	0	Asymptomatic	13.00	2.65	1.53	10.00	Basin	Potato + melon
ZR2	1	Mild	29.00	5.57	3.21	10.00	Basin	Potato + melon
ZR2	2	Moderate	114.00	6.24	3.61	10.00	Basin	Potato + melon
ZR2	3	Severe/dead	112.00	2.65	1.53	10.00	Basin	Potato + melon
ZR3	0	Asymptomatic	6.00	1.73	1.00	7.00	Basin	Potato
ZR3	1	Mild	35.00	2.65	1.53	7.00	Basin	Potato
ZR3	2	Moderate	105.00	5.29	3.06	7.00	Basin	Potato
ZR3	3	Severe/dead	145.00	9.85	5.69	7.00	Basin	Potato

Table S4. qPCR IGS copy-number calibration summaries.

Sample	Mean Ct beta-tubulin	Mean Ct IGS	Delta Ct (beta-tubulin - IGS)	Estimated IGS copies/genome (2^DeltaCt; near-ideal PCR assumption)
Isolate 1	23.86	18.18	5.69	51.63
Isolate 2	22.13	17.16	4.97	31.34
Isolate 3	24.18	19.52	4.66	25.28
Isolate 4	22.49	17.14	5.36	40.93
Isolate 5	24.92	20.52	4.38	20.82
Isolate 6	24.29	19.55	4.74	26.63
Isolate 7	24.59	19.66	4.93	30.48

Note: Displayed mean Ct and ΔCt values are rounded; therefore, the supplied estimated copy-number values may not reproduce exactly from the displayed rounded entries.

Table S5. Pairwise contrasts.

Comparison	Stage a	Stage b	Mean log10 difference (b-a)	t statistic	Raw p	Holm-adjusted p
0 *vs* 1	0	1	0.536	-7.74	0.002	0.005
0 *vs* 2	0	2	1.03	-14.96	<0.001	0.001
0 *vs* 3	0	3	1.24	-9.44	0.001	0.003
1 *vs* 2	1	2	0.494	-14.66	<0.001	0.001
1 *vs* 3	1	3	0.703	-7.15	0.002	0.005
2 *vs* 3	2	3	0.209	-2.30	0.083	0.083

Table S6. Cluster-aware regression and sensitivity analyses.

Analysis	n cells	β (log10 units/stage)	95% CI for β	R^2	Descriptive ρ	Interpretation
Full orchard-fixed-effects trend	20	0.421	0.351-0.491	0.922	0.962	Primary ordered stage trend
Orchard-cluster bootstrap	20	0.421	0.354-0.488	—	—	10,000 resamples of orchards; percentile 95% CI
Excluding BH stage 3	19	0.405	0.331-0.478	0.919	0.961	Influence analysis for the 337 MS/g observation
Leave-one-orchard-out range	16 per fit	0.391-0.444	—	0.914-0.938	—	Five refits, omitting one orchard at a time

Note: The outcome was log10(mean MS/g + 1). The primary model included orchard fixed intercepts. Spearman ρ is reported descriptively because the 20 orchard-stage cells are clustered within five orchards. The cluster bootstrap resampled orchards rather than individual cells.

Table S7. Proposed standardized sampling framework for prospective olive Verticillium wilt validation studies.

Attribute	Proposed prospective specification
Status	Prospective framework only; not used to generate the archived dataset and not a reconstruction of historical sampling.
Independent tree replication	At least 5 independently sampled trees per symptom category per orchard; for pre-symptom prospective cohorts, at least 10 independently retained trees per orchard.
Soil cores per tree	12 cores.
Spatial pattern	Four cardinal directions (N, S, E, W); three cores per direction at 25, 50, and 100 cm from the trunk.
Sampling depth	0-30 cm; prospectively retain 0-15 cm and 15-30 cm strata separately when feasible.
Soil matrices	Retain bulk/core soil and root-adherent rhizosphere soil separately; define rhizosphere as soil firmly adhering to fine roots after gentle shaking. Do not pool the two matrices.
Sampling season	Twice annually: early spring (March-April, before routine irrigation/active growth) and late autumn (October-November, post-harvest).
Compositing	Homogenize cores at the individual-tree level within each soil matrix. Retain tree-level composites independently rather than pooling across trees.
Analytical subsample	Air-dry a 100 g subsample at room temperature (20-25°C) until constant mass, approximately one week.
Storage	Store air-dried samples in sterile, sealed paper bags at 4°C in the dark.
Processing interval	Process within 4-6 weeks of sampling.
Core diameter	Use a standardized soil auger (target diameter approximately 2.5 cm) and record the actual diameter/model.
Tree selection	Screen candidate trees systematically within each orchard; apply prespecified inclusion/exclusion criteria and retain tree identifiers.
Symptom assessment	Use two trained assessors where feasible, record independent scores, and resolve disagreements by consensus.
Location and soil covariates	Record tree coordinates, soil temperature and gravimetric moisture at sampling; characterize soil texture, pH, EC and organic matter for validation strata.

Note: The numerical specifications in Table S6 are proposed design choices for future validation and are not field procedures used to generate the archived orchard-stage data. Illustrative tree-level, seasonal, or quality-control values are excluded from empirical interpretation.

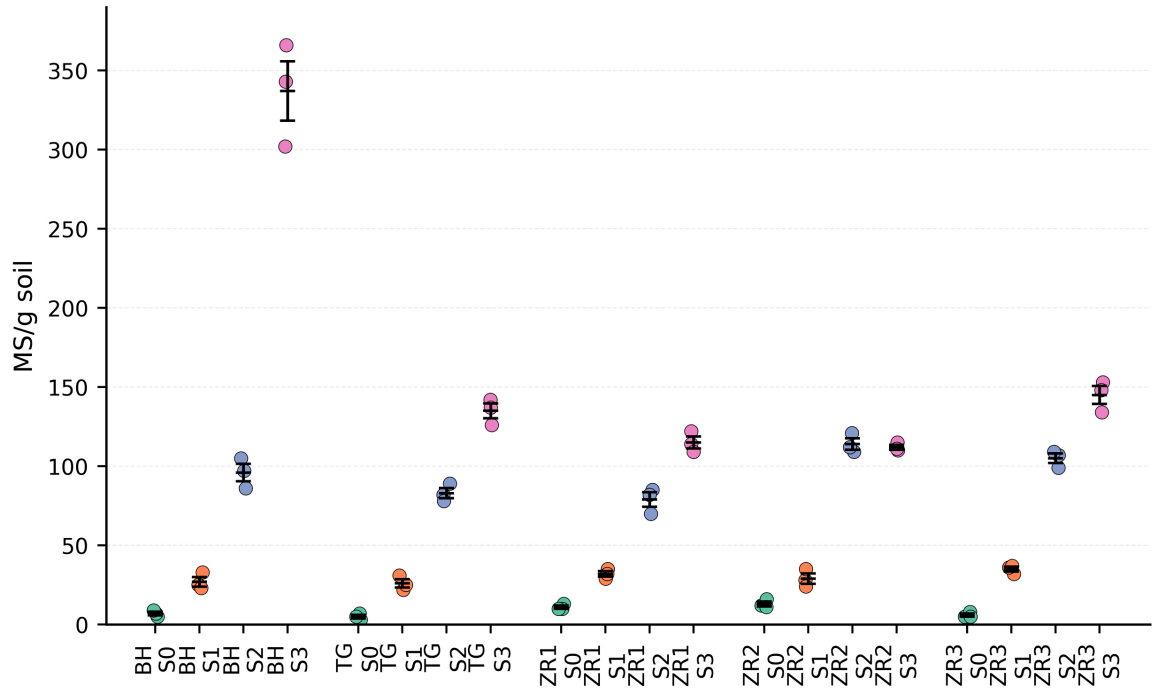


Figure S1. Raw technical plate-replicate dispersion by orchard and disease stage. Points are individual plate counts; the central symbol and error bar summarize the orchard-stage technical mean and dispersion. These replicates quantify laboratory enumeration precision and are not independent field samples.

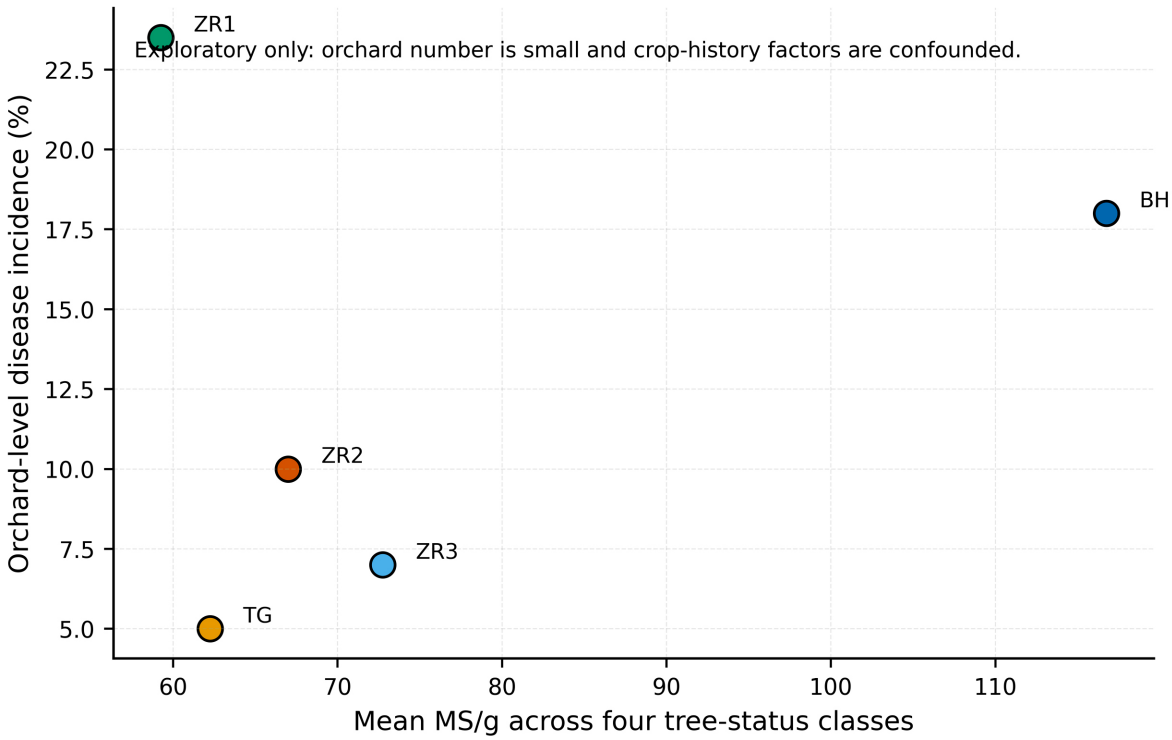


Figure S2. Exploratory orchard-level incidence-inoculum relationship. Each point is one orchard; the x-axis is mean MS/g across the four disease stages and the y-axis is orchard-level disease incidence. This display is descriptive only because n=5 and orchard-level covariates are confounded.