# Preliminary assessment of the effect of sodium chloride on the growth of *Ophiostoma novo-ulmi* isolates

## Preliminarna procjena utjecaja natrijeva klorida na rast izolata *Ophiostoma novo-ulmi*

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## Introduction

The second pandemic of Dutch elm disease (DED) is caused by the highly virulent fungus *Ophiostoma novo-ulmi*, which gradually replaced the first and less aggressive DED pathogen *O. ulmi*. *Ophiostoma novo-ulmi* comprises two subspecies (subsp. *novo-ulmi* and subsp. *americana*) that differ in a range of characteristics[1]. Because reproductive barriers between them are weak, hybridisation frequently occurs in nature, resulting in highly fit and fertile hybrids[2]. Given that the presence of *O. novo-ulmi* has previously been confirmed in Kopački rit and differences among isolates have been detected, the aim of this study was to assess the effects of different sodium chloride (NaCl) concentrations on the mycelial growth of selected isolates.

## Materials and methods

The study included six previously characterized *Ophiostoma novo-ulmi* isolates originating from Kopački rit (Table 1). The isolates were cultivated on malt extract agar (MEA). To evaluate the effect of sodium chloride (NaCl)[3], the media were supplemented with NaCl at concentrations of 0.1 M, 0.25 M, 0.5 M, and 1 M, with MEA without NaCl used as a control. Colony diameter was measured after 2 and 7 days of incubation, and growth rates (mm/day) were calculated and statistically analysed using one-way and factorial ANOVA.

**Table 1.** Characterisation of *Ophiostoma novo-ulmi* isolates from Kopački rit based on analysis of the col1, cu, and MAT type.

| Isolate | *col1* sequence | *cu* sequence | MAT type |
|---|---|---|---|
| **R29-24** | subsp. *americana*-specific | subsp. *novo-ulmi*-specific | MAT-2 |
| **R87-24** | subsp. *novo-ulmi*-specific | subsp. *novo-ulmi*-specific | MAT-1 |
| **R104-25** | subsp. *americana*-specific | subsp. *novo-ulmi*-specific | MAT-1 |
| **R16-24** | subsp. *americana*-specific | subsp. *americana*-specific | MAT-2 |
| **R38-24** | subsp. *novo-ulmi*-specific | subsp. *novo-ulmi*-specific | MAT-1 |
| **R46-25** | subsp. *novo-ulmi*-specific | subsp. *novo-ulmi*-specific | MAT-2 |




**Figure 2.** Growth of *Ophiostoma novo-ulmi* isolate R16-24 on media supplemented with different sodium chloride (NaCl) concentrations: (a) control, (b) 0.1 M, (c) 0.25 M, (d) 0.5 M, and (e) 1 M.

## Conclusion

The results of this study demonstrate that sodium chloride (NaCl) inhibits the mycelial growth of *Ophiostoma novo-ulmi* isolates in a concentration-dependent manner. While the highest concentration (1 M) almost completely inhibited growth, variations in the degree of inhibition were observed among the isolates at lower concentrations. These results suggest that some *O. novo-ulmi* isolates may differ in their response to NaCl, which should be further investigated.

## Results

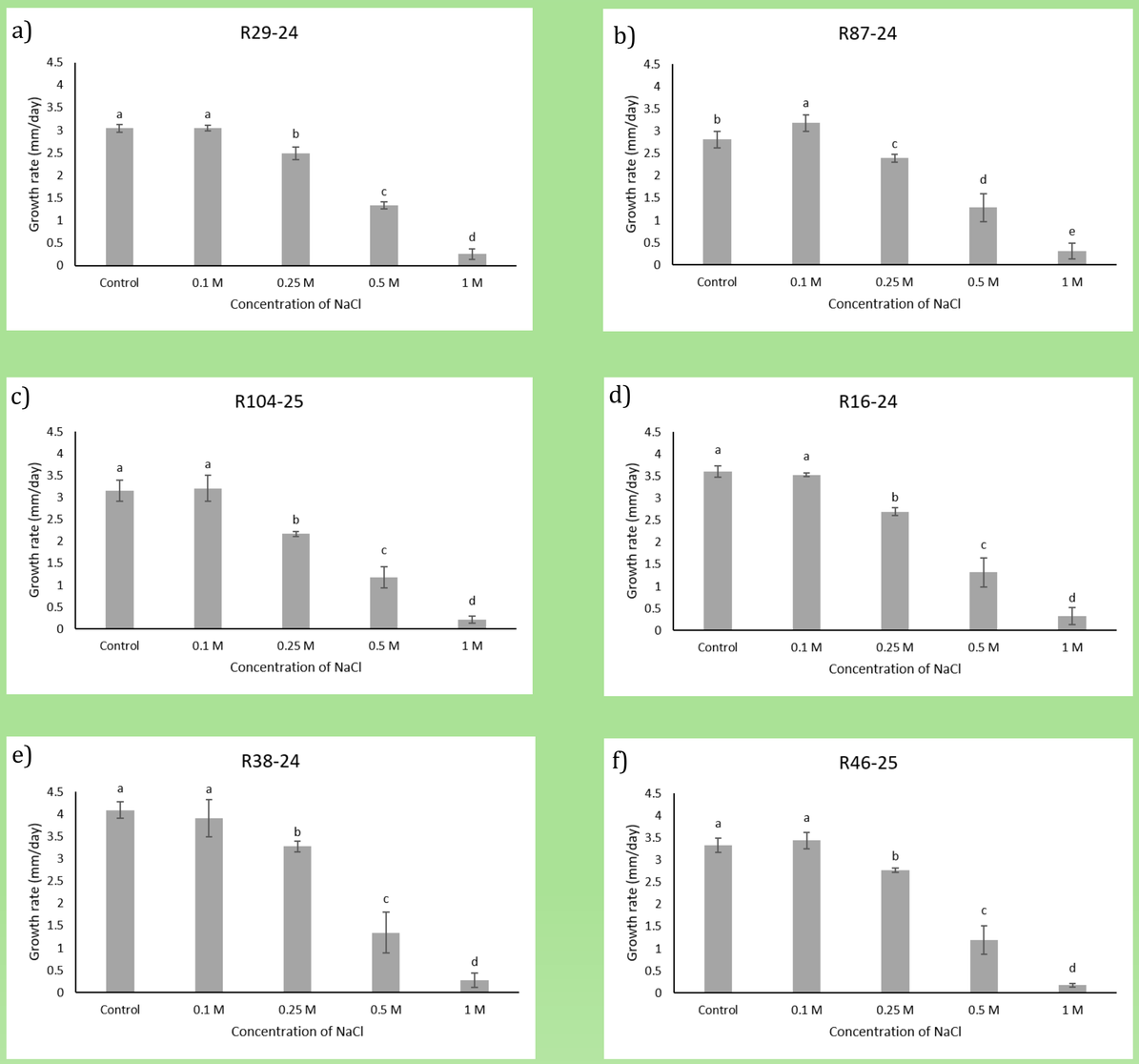



**Figure 1.** Effect of different sodium chloride (NaCl) concentrations on the mycelial growth of *Ophiostoma novo-ulmi* isolates from Kopački rit: (a) R29-24, (b) R87-24, (c) R104-25, (d) R16-24, (e) R38-24, and (f) R46-25. Different letters indicate significant differences among NaCl concentrations within each isolate (one-way ANOVA followed by Fisher's LSD test, $p < 0.05$)

**Table 2.** Relative growth rates of *Ophiostoma novo-ulmi* isolates at different NaCl concentrations, expressed as percentages of the control growth rate.

| | NaCl concentration | | | |
|---|---|---|---|---|
| | 0.1 M | 0.25 M | 0.5 M | 1 M |
| R29-24 | 100,2 | 81,6 | 43,8 | 8,5 |
| R87-24 | 113,3 | 85,1 | 45,7 | 11,0 |
| R104-25 | 101,9 | 68,8 | 37,3 | 6,9 |
| R16-24 | 97,9 | 74,8 | 36,6 | 9,0 |
| R38-24 | 95,6 | 80,1 | 32,8 | 6,7 |
| R46-25 | 103,1 | 83,1 | 36,0 | 5,3 |

Relative growth rate (% of control)
120 100 50 0

Results of factorial ANOVA of log-transformed growth rates, including the control and NaCl concentrations of 0.1–0.5 M, revealed significant effects of isolate and treatment, as well as a significant isolate × treatment interaction ($p < 0.05$).

## References

[1] Brasier, C. M. (1991). Ophiostoma novo-ulmi sp. nov., causative agent of current Dutch elm disease pandemics. Mycopathologia, 115(3), 151–161.

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