The first evidence of gibberellic acid's ability to modulate target species' sensitivity to honeysuckle (*Lonicera maackii*) allelochemicals

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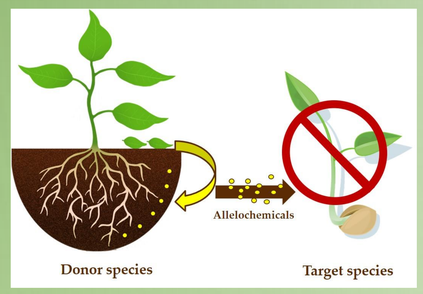
Objective

Invasive species employ competitive strategies such as releasing allelopathic chemicals into the environment that negatively impact native species (**Figure 1**).



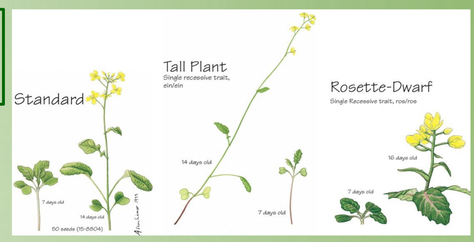
Decomposing Amur honeysuckle (*Lonicera maackii)* leaves leach various allelopathic phenolics into the soil, decreasing the vigor of several native species (**Figure 2**, *source: vTree, Virginia Tech Dendrology*) .

The <u>objective</u> of this study was to evaluate the potentially fundamental role of the seed germination stimulator gibberellic acid (GA_3) in determining target species sensitivity to *L. maackii*, a highly invasive plant species' allelochemicals.

Methodology

<u>Model system</u>: comparative evaluation of the impacts of *L. maackii* leaf metabolites on the germination and growth of control Standard (*Rbr*), GA_3 overproducing (*ein*), and GA_3 deficient (*ros*) *Brassica rapa* varieties (Figure 3, *Wisconsin FastPlants®*).

- *L. maackii* leaf extract preparation;
- Germination and growth of *B. rapa* seeds varieties;
- Treatment of *B. rapa* seed varieties with *L. maackii* leaf extract;
- Combined treatment of *B. rapa* seed varieties with *L. maackii* leaf extract and GA_3;
- Treatment of *B. rapa* seeds (*Rbr*) with the allelochemicals Apigenin (API) and Luteolin (LUT).



Results

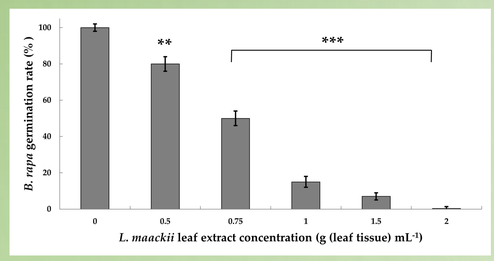


Figure 4. The impact of *L. maackii* leaf extracts on *B. rapa* seed germination. Standard, *Rbr* seeds were treated with *L. maackii* aqueous leaf extracts for 24 h.

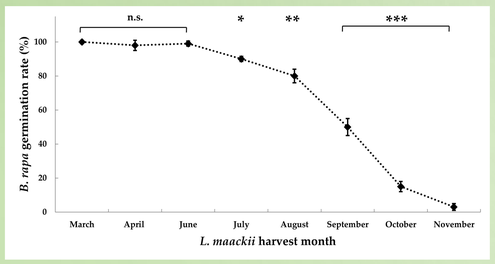
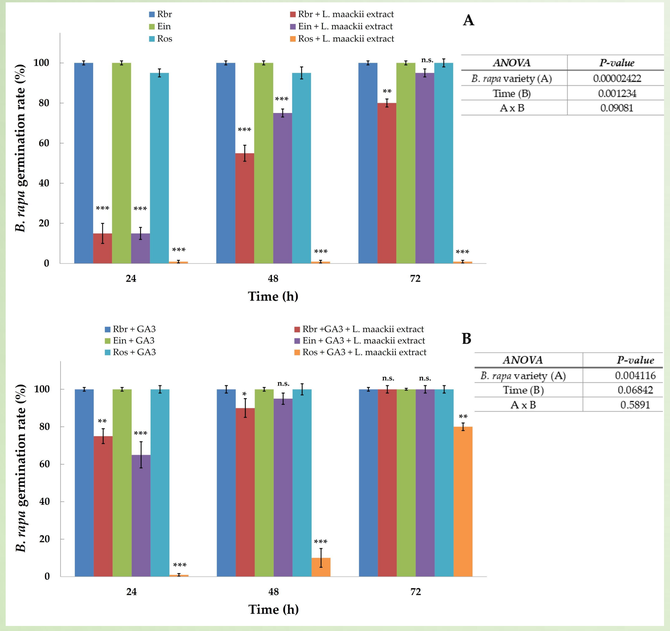


Figure 5. Seasonal variations in the impact of *L. maackii* leaf extracts on *B. rapa* seed germination. *Rbr* Standard *B. rapa* seeds were treated for 24 h with 1 g (leaf tissue) mL^{-1} *L. maackii* extracts prepared from leaves harvested between March – November 2021. Germination rates were compared to *Rbr* germination under control conditions when treated with sterile water. Control germination was 100% after 24 h.



ANOVA	*P-value*
B. rapa variety (A)	0.00002422
Time (B)	0.001234
A x B	0.09081

ANOVA	*P-value*
B. rapa variety (A)	0.004116
Time (B)	0.06842
A x B	0.5891

Figure 6. The impact of endo- and exogenous gibberellin on *B. rapa* seed germination exposed to *L. maackii* leaf extracts. Seeds were treated with 1 g (leaf tissue) mL^{-1} *L. maackii* extracts, without (panel A) or, in the presence of supplemental exogenous GA_3 of 100 µmol (panel B) for 72 h. Corresponding control treatments included seeds imbibed with sterile distilled water (panel A) or sterile distilled water supplemented with 100 µmol GA_3 (panel B).

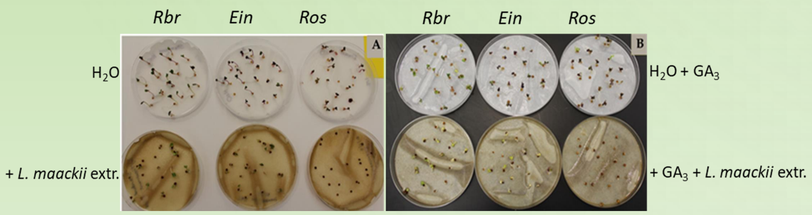
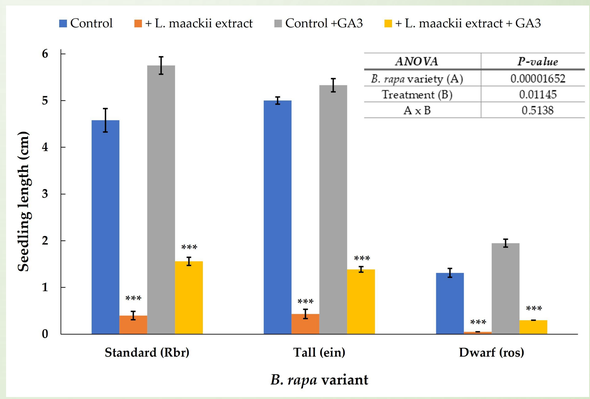


Figure 7. Seed assay plates showing the impact of endo- and exogenous gibberellin on *B. rapa* seed germination exposed to *L. maackii* leaf extracts. *B. rapa* seeds were treated with 1 g (leaf tissue) mL^{-1} *L. maackii* extract, without (panel A) or, in the presence of 100 µmol exogenous GA_3 (panel B) for 72 h. Corresponding control treatments included seeds imbibed with sterile distilled water (panel A) or sterile distilled water supplemented with 100 µmol GA_3 (panel B).



ANOVA	*P-value*
B. rapa variety (A)	0.00001652
Treatment (B)	0.01145
A x B	0.5138

Figure 8. The impact of endo- and exogenous gibberellin on *L. maackii* leaf extract treated *Rbr*, *ein*, and *ros B. rapa* seedling growth. *B. rapa* seeds were treated with 1 g (leaf tissue) mL^{-1} *L. maackii* extract, without (orange bars) or, in the presence of 100 µmol exogenous GA_3 (yellow bars) for 72 h. Corresponding control treatments included seeds imbibed with sterile distilled water (blue bars) or sterile distilled water supplemented with 100 µmol GA_3 (grey bars).

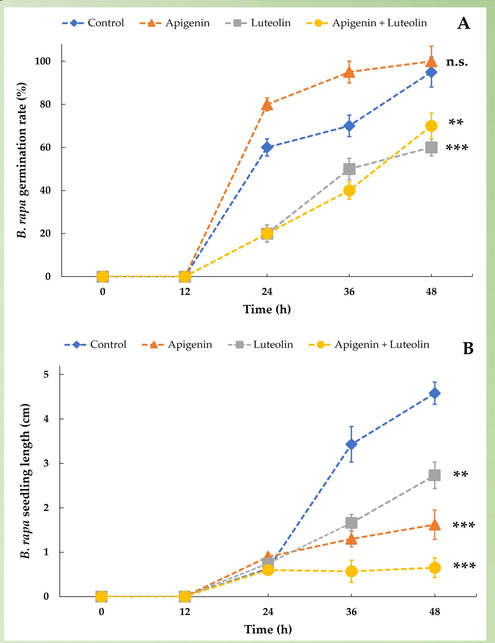


Figure 9. The impact of API, LUT, and their combination on the germination (panel A) and seedling growth (panel B) of *Rbr* (Standard) *B. rapa* seeds and seedlings. Seeds were treated with 50 µg mL^{-1} solutions of API (orange triangles), LUT (grey squares), and the combination of API + LUT (yellow circles) in 0.05% DMSO for 48 hours in Petri-dish assays. Control seeds were treated with an aqueous solution of 0.05% DMSO.

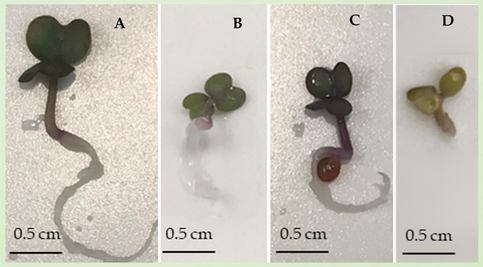


Figure 10. The impact of API, LUT and their combination on *Rbr* (Standard) *Brassica rapa* seedling development. Seeds were treated with 50 µg mL^{-1} solution of API (B), LUT (C), and the combination of API + LUT in 0.05% DMSO for 48 hours. Control seeds (A) were treated with an aqueous solution of 0.05% DMSO.

Conclusions

- **The net allelopathic potential of *L. maackii* leaves is defined by complex chemistry and the interactive effects of multiple metabolites on the target species.**
- **The target's metabolic properties might also influence the net allelopathic impacts of invasives, in addition to environmental factors and the proximity to the source.**
- **High GA_3 concentrations may substantially alleviate the inhibitory effects of *L. maackii* allelochemicals.**

The impact of the study

- **A better understanding of the direct impacts of allelochemicals on target species will contribute to developing novel invasive species control and biodiversity conservation protocols. This knowledge may also contribute to additional applications in other fields—for example, to support applications employing allelopathic species in agriculture, in water recycling, and as substitutes for synthetic herbicides.**

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