Assessing Plant Root Viability:
Method Comparison and Development

Morgan Luce
Alexii Cornell & Ylva Lekberg
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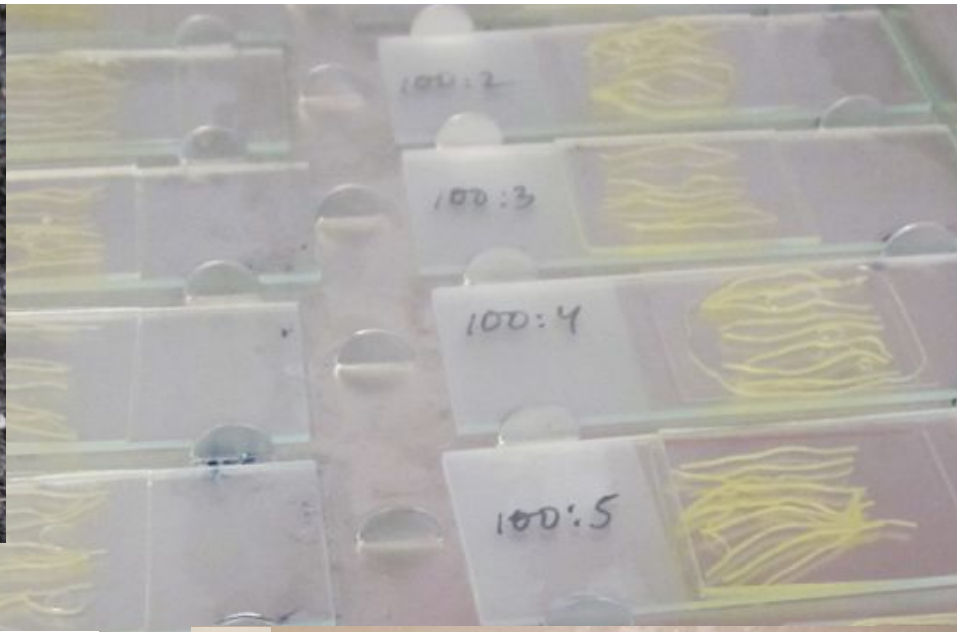
We use measurements of root viability to asses overall plant vigor and the extent of pathogen or chemical damage. In the context of invasion and restoration – we can use root viability to better understand the efficacy of weed management practices and restoration treatments.

In this experiment we compared two methods used to assess root viability; TTC reduction and FDA fluorescence microscopy. We adapted the latter to create a high-throughput, inexpensive, reliable method for use with the plate reader. We then tested the three methods with apple, fescue (bunch grass), and knapweed roots collected from MPG.

a.



Methodology:

1. Grew pea plants in sand.
2. Harvested roots.
3. Killed a portion of roots by boiling.
4. Prepared samples consisting of live and dead roots with following proportions: **100**, **75**, **50**, **25** and **0%** viable.
5. Used these mixtures to compare TTC, FDA Microscopy, FDA Plate Reader methods.

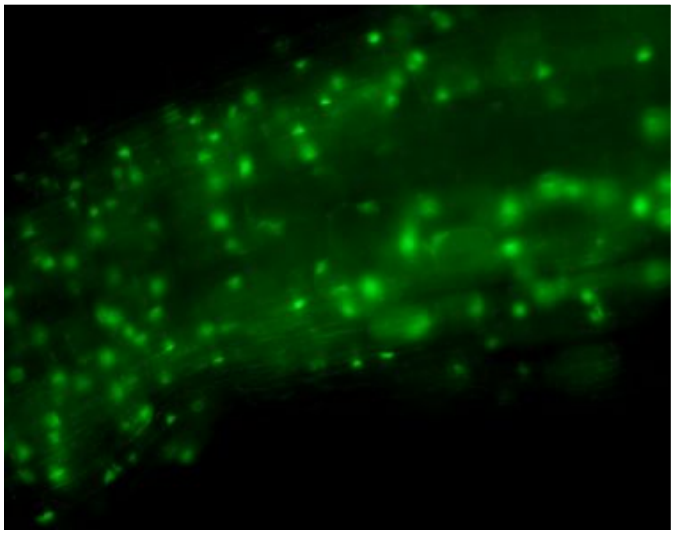
b.

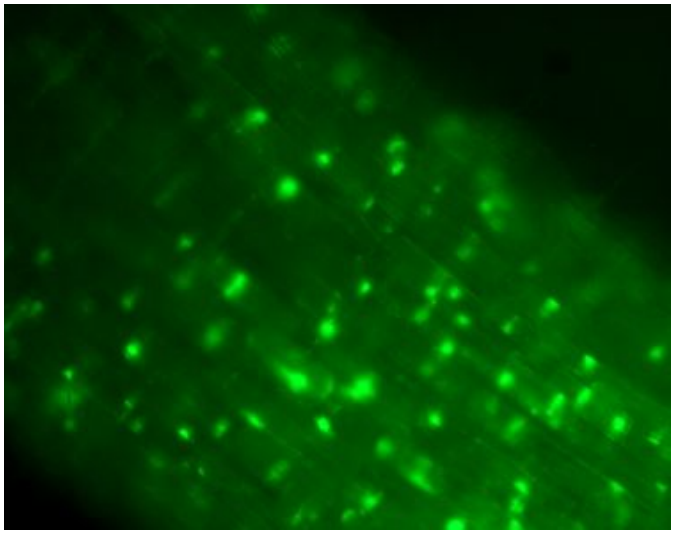
a. *Garden pea – methods were compared using pea roots grown in sand.*
b. *Alexii transfers pea roots into tubes with TTC solution.*
Background. *Pea roots stained with FDA and mounted for microscopy.*

TTC – Colorless triphenyl tetrazolium chloride (TTC) is reduced by living roots to red formazan (TF). Following a method of Comas (*et al.* 2000), we incubated roots in TTC for 20 hours and then extracted TF from the roots using ethanol. We then measured TF concentration.

FDA Microscopy – Fluorescein diacetate (FDA) is membrane-permeable. Once inside a living cell, intracellular esterases hydrolyze FDA producing the fluorescent compound fluorescein. We stained pea roots with FDA (Noland (*et al.* 1997), mounted them on slides, and used a fluorescence microscope to score % viability based on the proportion of intercepts that fluoresced (*top and center right*).

FDA Plate Reader – We reviewed the literature and adapted the microscopy method above for use with our plate reader. FDA stained roots were loaded into multi-well plates (*bottom left*) and compared to known fluorescein concentrations (*bottom right*).





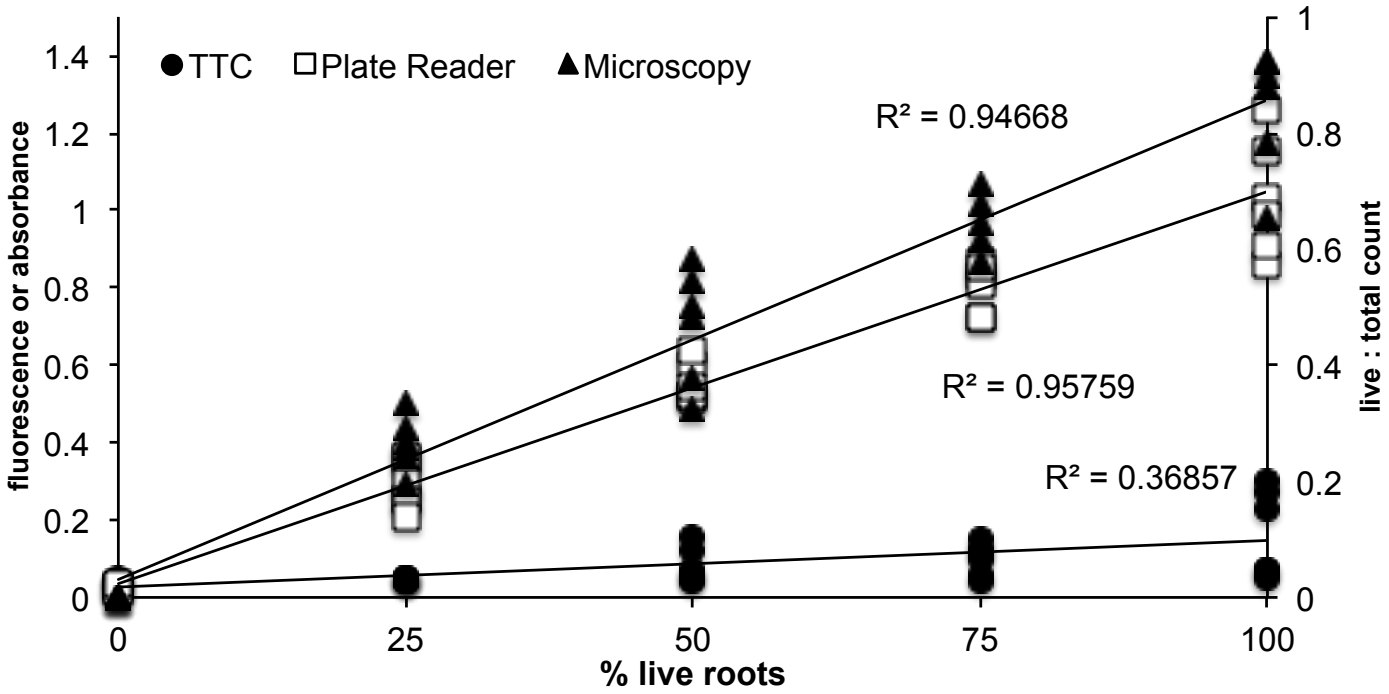


Figure 1. Viability measures of pea roots of known viability. The two FDA methods were more accurate and sensitive than the TTC method as indicated by the higher R^2 values (see figure) and slopes (Plate reader: y = 0.0101x; Microscopy: y = 0.0083x; TTC: y = 0.0012x).

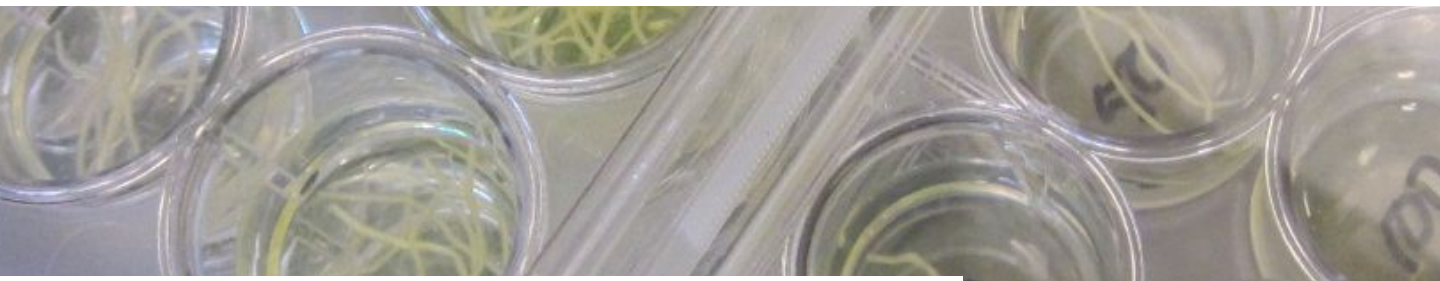
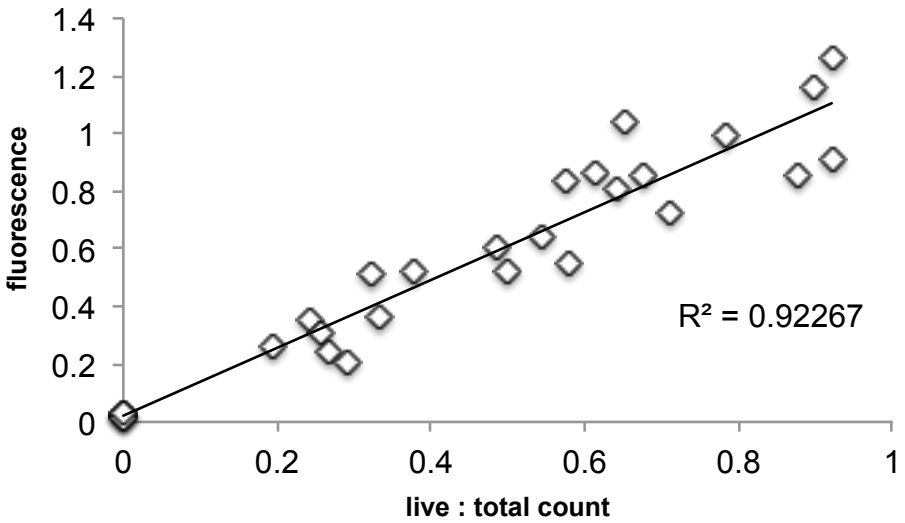


Figure 2. Root viability values correlated well between the microscopy and plate reader method, suggesting that measuring fluorescence with the plate reader accurately reflects root viability.



We also compared the time and monetary investments per sample among the three methods**. Our novel plate reader method provided accurate root viability measures in half the amount of time at a similar cost.**

Pea Roots – Time and Cost Comparison

	TTC	Plate Reader	Microscopy
Root Preparation	60	60	60
Additional Preparation	300	60	64
Incubation	*1080	30	30
Reading	2	12	144
Total Time (30 samples)	362	162	298
Time / Sample	**12**	**5**	**10**
Cost / Sample	**$0.22**	**$0.29**	**$0.34**

*not included in time/sample calculation

To determine how these methods compare under more realistic conditions, we collected apple, fescue, and knapweed roots from the field and measured root viability using the three methods.

Left. *Freshly harvested fescue*
Center. *Washed knapweed roots - new roots are finer and lighter in color than the old roots.*
Right. *Knapweed(K) and fescue(C) roots, stained and mounted for FDA-microscopy.*

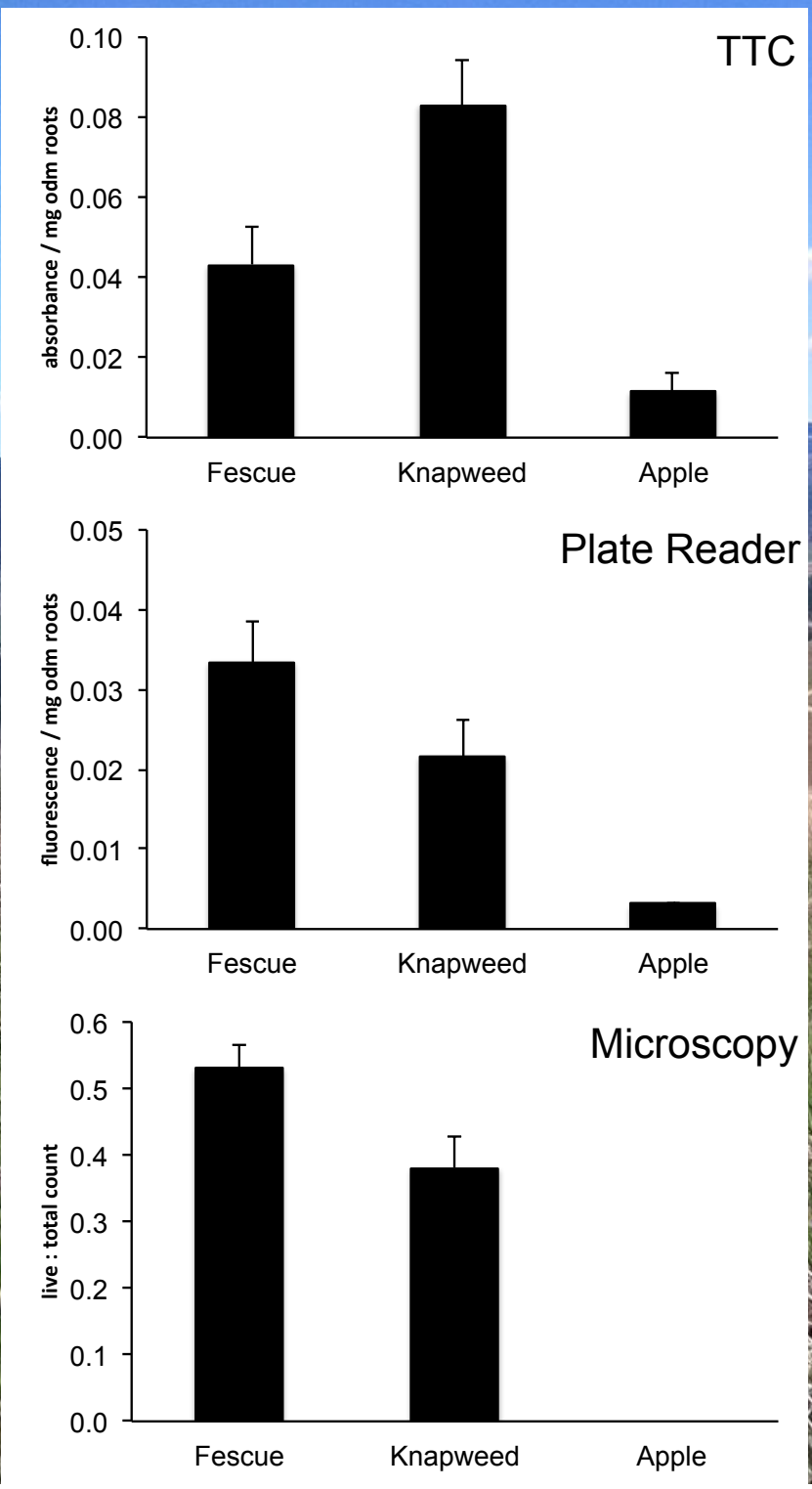


Figure 3. Fine root viability of field harvested fescue, knapweed, and apple roots. The plate reader and microscopy method were in agreement and showed that fescue roots were more viable than knapweed roots, whereas TTC showed the opposite. This is problematic given that both TTC and FDA methods are frequently used to assess root viability. Apple roots had the lowest viability using both the plate reader and TTC, and because roots were too thick, no measurements were made using microscopy.

Background: *Field collection site for knapweed and fescue roots.*

Final Thought:
Our novel FDA Plate Reader method is cost effective, reliable, and saves time. In the near future, we plan to use this method to evaluate the direct effects of herbicide on roots as well as AM mediated uptake and transfer of herbicide to plant roots.

References

Comas, Louise H., David M. Eissenstat, and Alan N. Lakso. "Assessing root death and root system dynamics in a study of grape canopy pruning." *New Phytologist*

Noland, Thomas L., and Gina H. Mohammed. "Fluorescein diacetate as a viability stain for tree roots and seeds." *New forests* 14.3 (1997): 221-232.