Improving alfalfa protein content and stability

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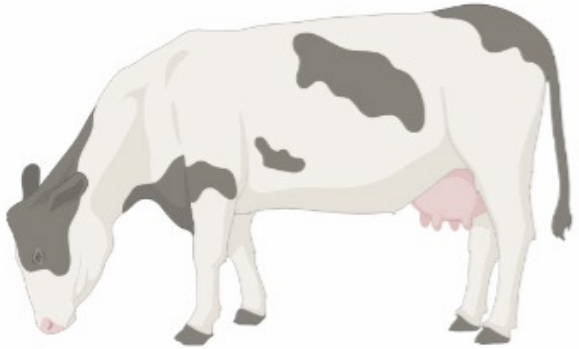
Alfalfa as food



Benefits

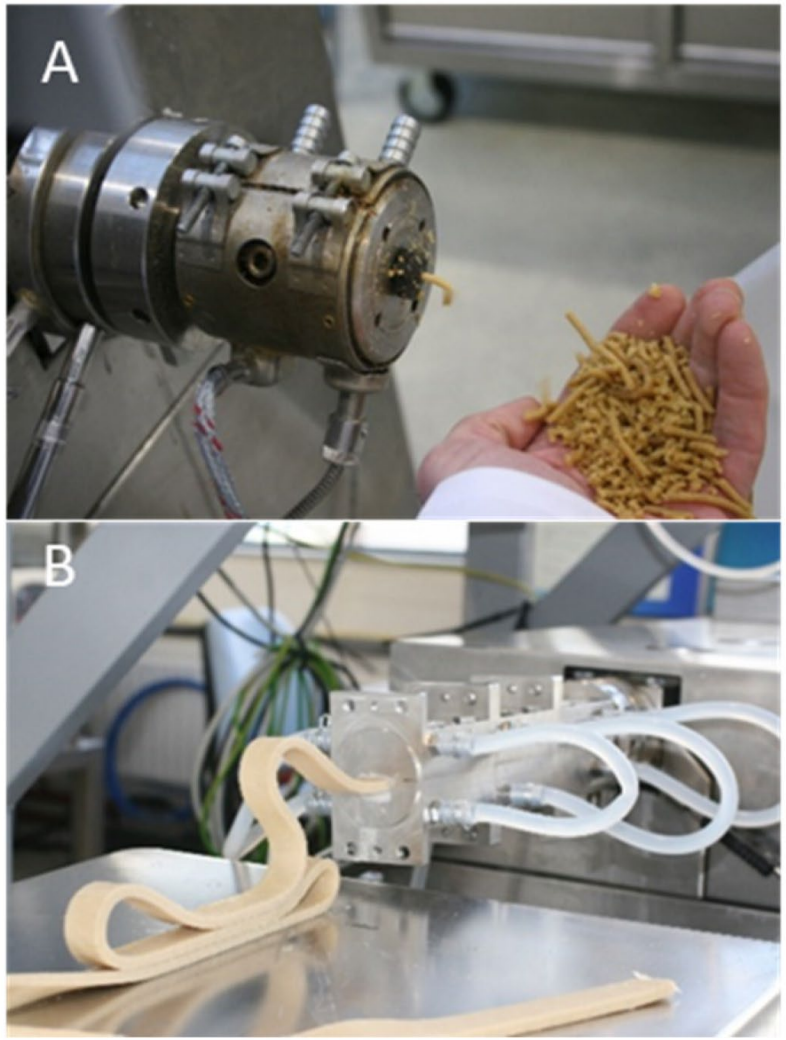
- High protein content
- Good energy source
- Digestible

Drawbacks

- Some amino acids are below optimal levels
- Some problematic specialized metabolites
- Rapid protein degradation after harvest

Alfalfa is a good candidate for protein extraction for human uses

- High leaf protein content
- Relatively low fat content
- Good balance of amino acids relative to pea/ soybean



Kyriakopoulou *et al.*, 2021. *Foods*

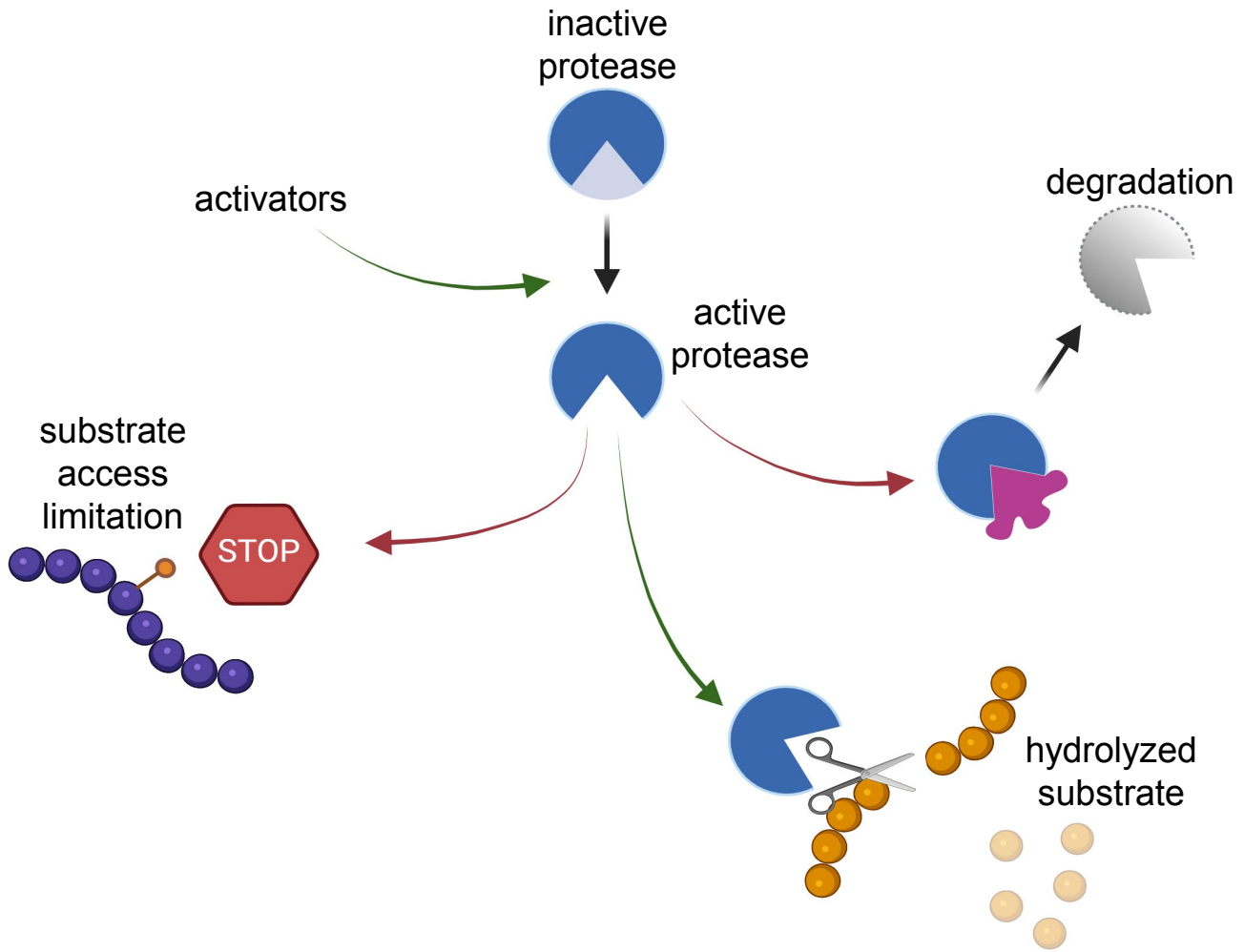
The problem with proteases

Table 4. Soluble nonprotein nitrogen (SNPN) content of the first and second cut (C) herbage of five forage species (S) at three stages of wilting (W) measured prior to ensiling

		SNPN (%TN)†			
Cut	Species	Direct cut	Wilted 6 h	Wilted 24 h	Proteolysis‡ (Δ SNPN)
First	Alfalfa	8.5	16.4	25.2	16.7**
	Red clover	4.1	7.6	11.1	7.0**
	Birdsfoot trefoil	3.8	7.8	16.0	12.1**
	Orchardgrass	7.6	13.4	22.6	15.0**
	Bromegrass	9.1	10.9	23.1	14.0**
Second	Alfalfa	18.3	19.5	28.5	10.2**
	Red clover	8.2	10.9	11.5	3.3*
	Birdsfoot trefoil	13.2	13.0	19.1	5.9**
	Orchardgrass	8.5	17.8	15.7	6.6**
	Bromegrass	10.5	14.7	14.5	3.9*

Papadopoulos and McKersie, 1983. *Canadian Journal of Plant Science*

Protease activity is tightly regulated



Adapted from Fernández-Fernández *et al.* 2023. *Cell Death & Differentiation*

What affects protease activity?

GENETIC VARIANCE OF PROTEOLYTIC ACTIVITY IN ALFALFA HERBAGE

S. R. BOWLEY and B. D. McKERSIE

Department of Crop Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1. *Received 31 Jan. 1986, accepted 26 June 1986.*

BOWLEY, S. R. AND MCKERSIE, B. D. 1987. Genetic variance of proteolytic activity in alfalfa herbage. Can. J. Plant Sci. **67:** 159–165.

13.9% narrow sense heritability

†Significance tests of single degree of freedom comparisons:

Iroquois vs. OAC Minto	**
Cultivars vs. *M.* ssp. *sativa*	NS
Cultivars vs. *M.* ssp. *falcata*	NS
Cultivars vs. *M.* ssp. *varia*	NS
Cultivars vs. *M.* ssp. *praefalcata*	NS
Cultivars vs. *M.* ssp. *glomerata*	*

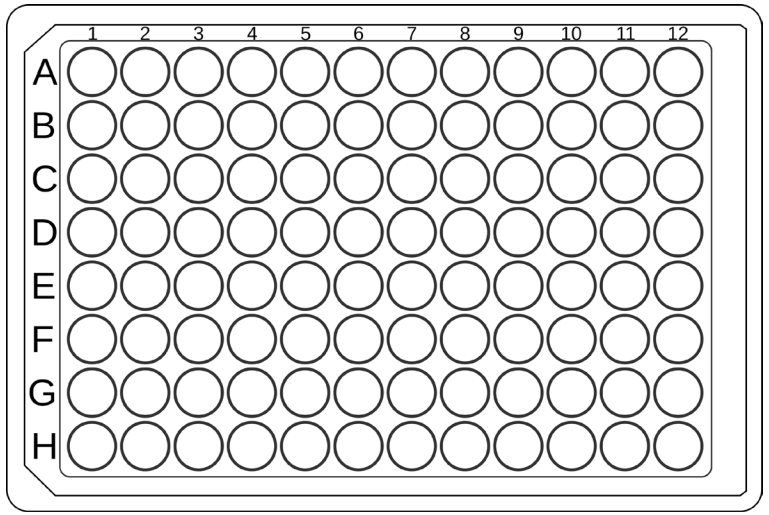
**,* Significant at 0.01 and 0.05 levels, respectively; NS = nonsignificant.
SE of an individual plant mean = 3.06.

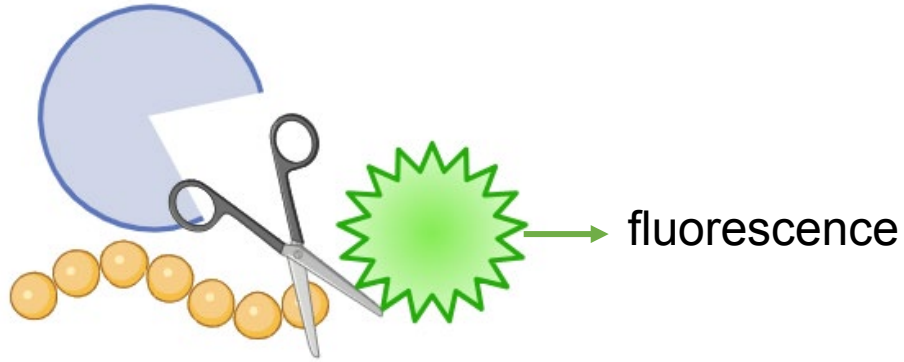
Two main objectives

1. Identify management practices that decrease post-harvest proteolysis in alfalfa

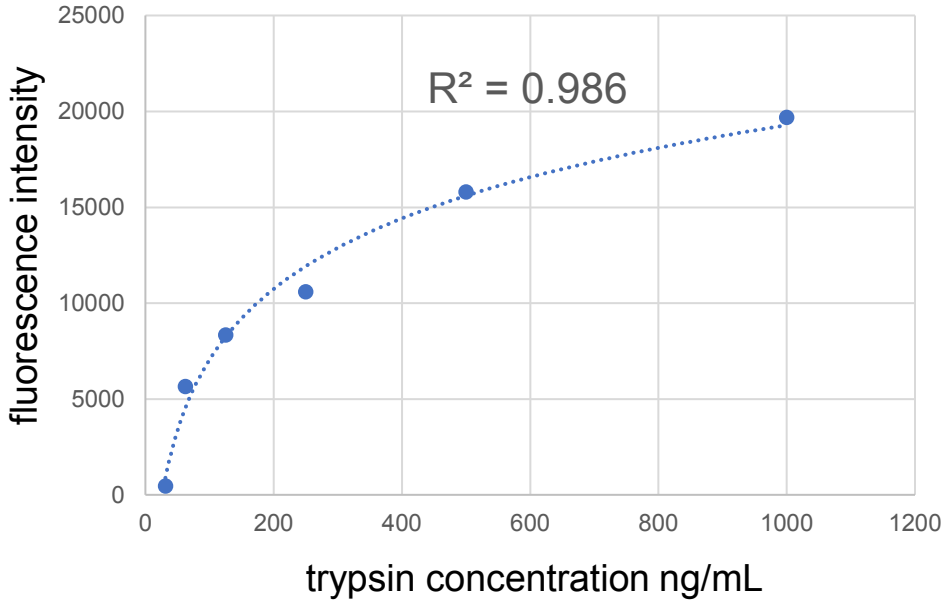
2. Investigate variation in protease activity in different alfalfa varieties to inform breeding/ variety selection

Can we measure protease activity rapidly and sensitively?



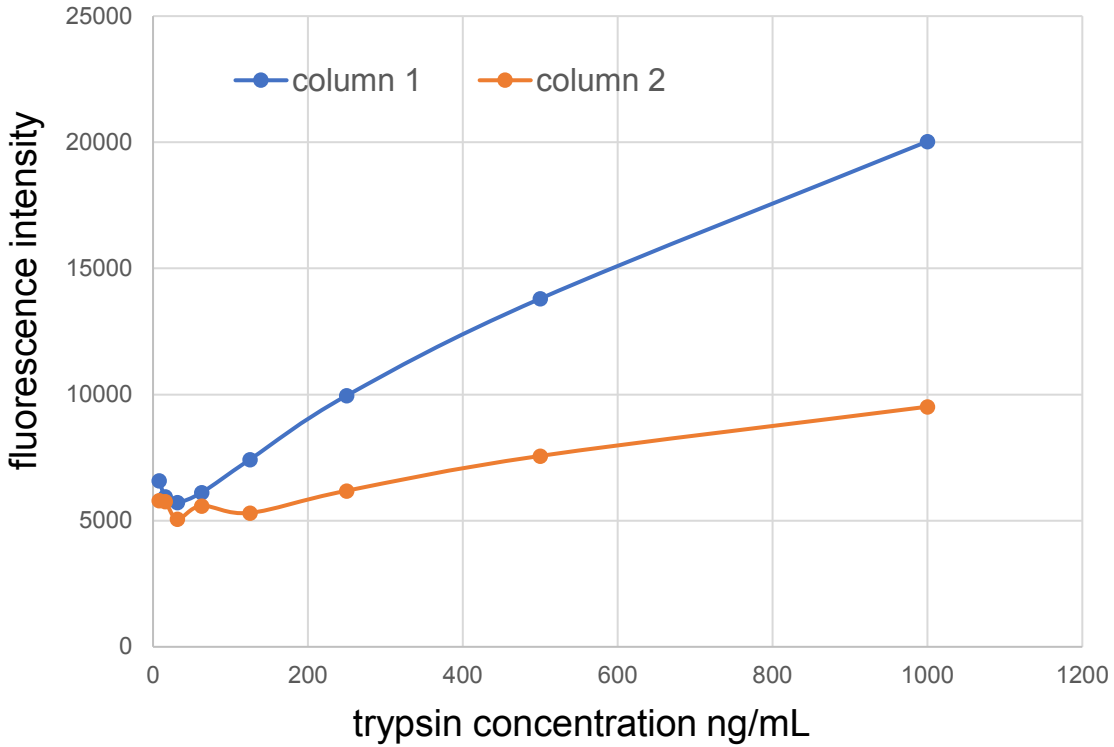


Yes, but…

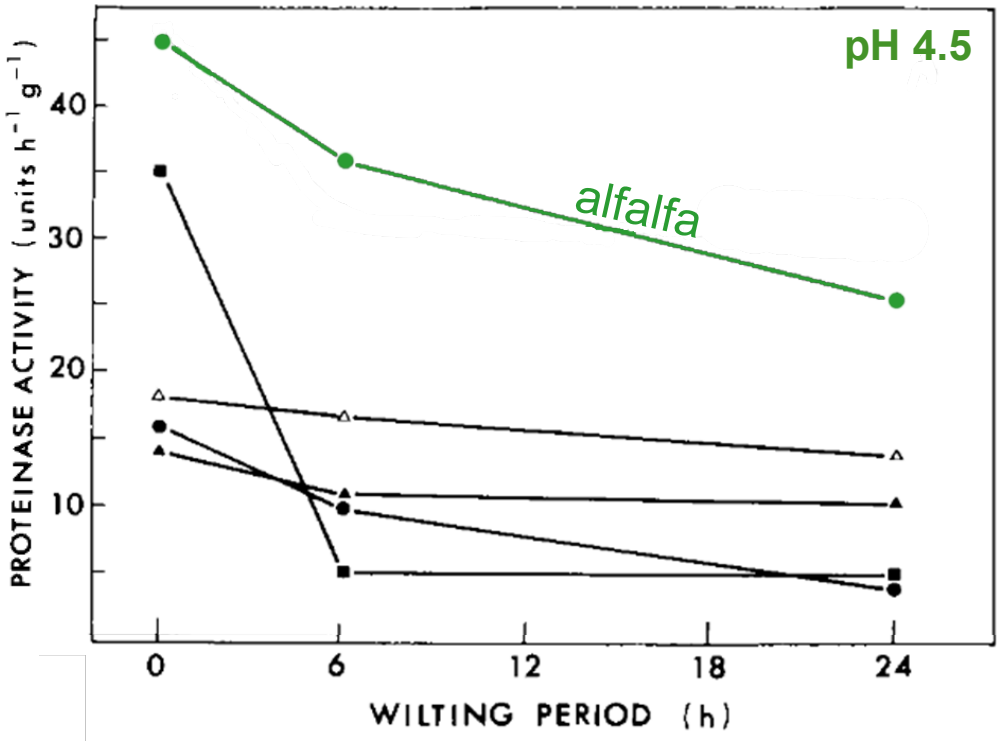


Troubleshooting the protease assay

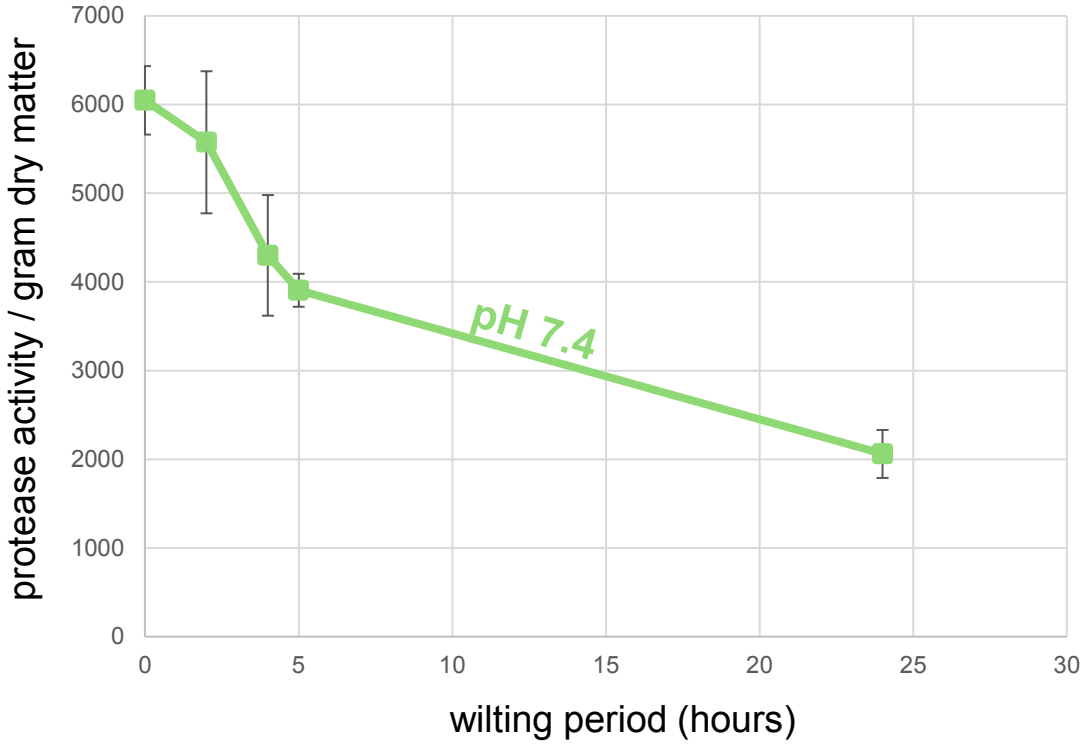
- Choosing the correct dilution of our plant samples is imperative
- Chlorophyll interferes with the fluorescence detection



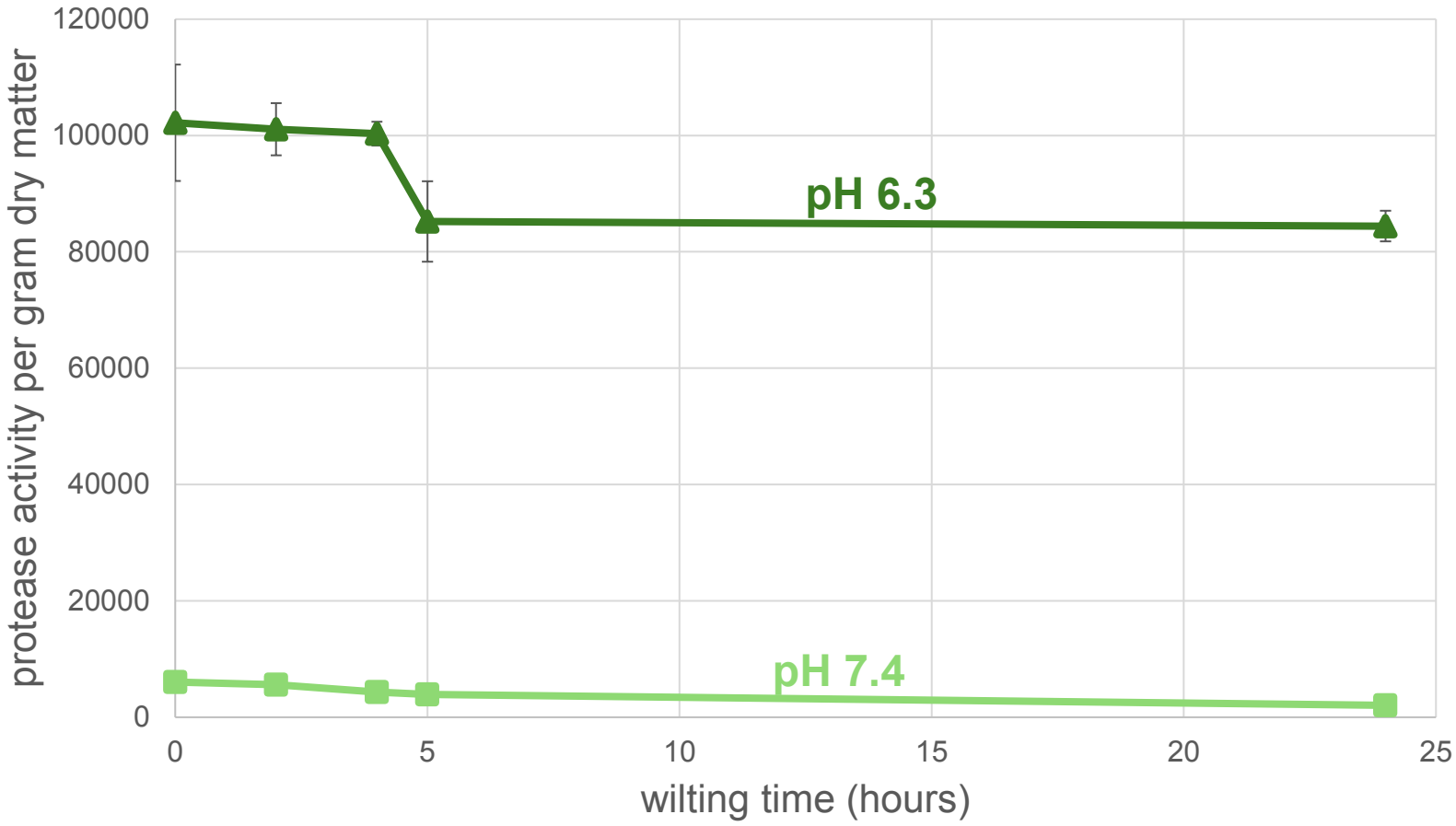
Modified from Papadopoulos and McKersie, 1983. *Canadian Journal of Plant Science*



Alfalfa protease activity during wilting



Choosing a biologically appropriate pH



Next steps

- Understand the effect of management practices on post-harvest protease activity
- Screen alfalfa varieties for variation in protease activity
- Characterize and catalog functional proteases at different stages of development and wilting

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Questions?

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