

Agriculture et Agroalimentaire Canada



The Freezing Tolerance of Alfalfa Nodules Depends on *Sinorhizobium Meliloti* Strains

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Ph.D. project Emmanuelle D'Amours

Winter Survival

change!

Alfalfa

 Single most important factor is the level of freezing tolerance that the plant can achieve

during winter (McKenzie et al. 1988; Castonguay et al. 2006; Bertrand et al. 2014).

• More freeze-thaw episodes with climate



Winter 2024

		WISC	ONSIN STATE FA	RMER			
[No	NEWS Did al	s Editorials Class	ulfieds tegals \odot rvive the polar		S	Search P Profit Tracker Ag Policy Castle Health	Retail Deef 🔠 Subscribe
	Published 4.30 p.m	Spaeth-Bauer nsin State Farmer CT April 23, 2019		Ne	ws Markets	Magazines Weather C	pinion More ~
	When it com a common qu survived the The sooner w be determine decide which lost forage w Dr. David Co Dairy Science Wisconsin-M	es to the "queen of forages," testion flying around at this swhether or not the crop winter. And for good reason. Inter damage to alfalfa can d, the sooner producers can option for replacing that II be best for them. mbb with the Department of at the University of adison, has been getting a		Winter Plains	-Killed Alfalfa	n Prevalent In Northern	
Intario 🕅	lot of questio vortex has af April 17 webi Professional Wisconsin (F how to assess what to do al assessment a	ns about how the polar feeted alfalf corps. In an nar presented by the Dairy Producers of DPW), Combe discussed winter damage to alfalfa, out the crop based off that nd ration adjustments for lastati	Common question that the of parts detailed and the second se	Français			All Decesse in Funds Nor op: All Stream of the Contract Products Cardia Products Prove space Stream of the Contract Products Stream of the Contract Products Prove space Stream of the Contract Products Prove space Stream of the Contract Products Prove space Stream of the Contract Products Stream of the Stream o
IINISTRY OF AGRIC	ULTURE, F	FOOD AND RURAL A	AFFAIRS	Writer-kines attarts (NOSU) Dy ELLEN CRAWS	ORD May 10, 2021	f v in m	Stress In Beef Operations Life/ ego
Home Ab	oout Agriculture	Sec Food Rural Research Public	rch		Ľ	ROUDLY LISEN DECEMBEN	BUILT FOR PROFESSIONAL OXEO.COM
Field Crops Fruit Crops Vegetable Crops Speciality Crops	There was an alfalfa growth returned on on good heal slower regro wilted and di stems have b	early break in alfalfa dormancy in e (up to 6 - 8 inches or more) was fro up to 6 - 8 inches or more) was fro darch 30th (-7°C or colder). Damage thy stands with early, aggressive gro with did not appear as affected. In m did not appear as affected. In did coloured, but plants should comple een frozen to various degrees and g	arly- to mid-March. In some areas, sign st damaged when colder temperature: was most severe in south-western On wth. ironically, fall harvested fields tha Id cases. leaves at the tops of the plan tely recover. In more severe cases, alf rowing points have been destroyed.	ufficant 5 tario. t had ts were alfa	annual e May 2, 2023 08:00 AM Hite Pankin Mana Hays	event Forage Grower	Re See D. Ma YO BAL Pert N Secret
Drgani Soll ani Pest M Use AbonHiszyous A	Caritisticutes en operates instructional operates au-Outline	MAGAZINE DU BULLETIN ; LE NU	MERO DE JUIN EST PRÉT →			STOCK GRAZING MARKETS EQUIPME	VT FARM ISSUES POLL EVENTS
DERNIÈRE 1. Pas facile de f traitements he Eyre 23 herris 2. Météo : besoi douche Her	INFOLETTRE iaire des arbicides in d'une bonne	Un gel meurtrier La température est descendue plusieurs régions	sur les cultures sous le point de congélation dans Public: 18 mai 2023 Actuation, Caliumes	Pils d'Homaile de neileure ster Di L'État des c	Hay, Forage		
3. Il faut être ré devenir méde Il ya 22 borre	silient pour Icin vétérinaire	Radio-Canada Menu v	f y in =	CONSULTER MAINTEN	ready heard som eni me. Although reg	e reports of frost- and freeze-damaged alfalfa t onal, frost or freeze-induced injury to alfalfa se	als spring. More will ems to occur every
4. Ils raffolent de vache Il y a 22 beces 5. Comment ide	e la bouse de ntifier le bon	ICI Manitoba Àlaune Encontinu Arta Sporta	Vidéojournal				
Her Her INSCRIPTION A	UNFOLETTRE	Le manque inquiète les	de couvert neig agriculteurs de	geux es		Le manque de champs inqui	e neige dans les ète
		Prairies ⊙ ®					
		Le champ du fermier Alain Philippet, à Saine D Priorio : Alabos - CAMADA , Voctore Enkolsti	laude, au Manitaba, se retrourio dépournu d'un mantesa n	digeox.	Sarah R. Champagne	Ce texte est tiré du Courrier de la planète. Po cliqueziei.	ir vous abonner,
		Victor Lhoest			LOUIS A REFLICT	X Il faut absolument de la neige avant le prochait m le monde s'entend là-dessus. Sans ce manteau	grand froid. Ça, tout solant, certaines

Symbiosis

Host-Rhizobia Specific

- Crosstalk between the plant and the rhizobia involving root exudation of specific flavonoids
- Optimum temperatures : 25-30°C (Alexandre and Oliveira, 2013).
- **Cold stress** affects both plant and microorganisms and the crosstalk between the two symbiotic partners
- **Cold acclimation process** promotes the accumulation of cryoprotective and reserve metabolites, increase the tolerance to freezing and support spring regrowth







Main objective: To improve alfalfa production under low temperatures by identifying the best associations between alfalfa populations and rhizobial strains in terms of higher tolerance to freezing of both partners and greater crop yield in cold soil, and to study the underlying mechanisms.

- Identifying the best freezing tolerant symbiotic association
- Study the underlying mechanisms of a freezing tolerant symbiotic association

Alfalfa populations A-TF0 et A-TF7

Recurrent selection method performed under controlled conditions (Castonguay et al. 2009).



-2°C -16°C -18°C -20°C -22°C -24°C -26°C -28°C



Sinorhizobium (Ensifer) meliloti strains

Strains isolated from different regions



Commercial strain with high efficiency under various conditions









There will be an additive positive effect on the biomass regrowth after a freezing stress when combining a freezing-tolerant alfalfa population (A-TF7) with freezing tolerant strains of *Sinorhizobium meliloti*

Symbiosis efficiency after freezing stress



Treatments:



Roots and shoot biomass: Alfalfa populations effect

- No effect in growth optimal conditions (NA)
- 16% increase in root biomass with cold acclimation (CA).
- A-TF7 10% (average) root biomass (CA and AFS)
- 3 weeks regrowth after freezing stress
 - **A-TF7** 15% root biomass;
 - **A-TF7** 19% de shoot biomass.



A-TFO

A-TF7



Nodules and shoot biomasses: S. meliloti strain effect

- No effect under optimal growth conditions (NA).
- with cold acclimation (CA):
 - nodule biomass (+68%);
 - differences between strains.
- 48 h after freezing stress (AFS) 22% nodule biomass.
- 3 weeks after freezing stress (RAF) :
 - NRG34 19% nodule biomass than A2;
 - NRG34 46% nodule biomass than the other 4 strains;
 - NRG34 19 % shoot biomass than strains B399 and A2.



5 souches tolérantes

au froid

B399

0

Nodules characterization 3 weeks after freezing: S. meliloti strain effect

3

Shoot dry weight (g)

- Observation of nodule damage caused by freezing on the root system (PH)
- 3 categories:
 - I: No damage, pink nodules;
 - II: Necrotic nodules at the base of the root and showing a pink regeneration zone;
 - III: Completely necrotic nodules.
- Distinct distribution profile between strains:
 - NRG34 85% nodules without damage and showing areas of regeneration.



A2

NRG34

Pink nodules

bc bc

A2634 52,152 1



5 souches tolérantes

au froid

B399

Necrotic nodules + regeneration zones









Necrotic nodules











Results: Nodules characterization 3 weeks after freezing: S. meliloti strain effect

5 souches tolérantes B399 au froid VS



- 4 categories:
 - NRG34 65% palmate-coralloid _ nodules
 - Larger nodules could store more _ resources to ensure the regrowth after freezing and represent a stronger carbon sink to sustain spring regrowth than smaller nodules



Nodules shapes

coralloid nodules





Palmate-coralloid nodules

B399:	30% c
S27:	33% bc
A2:	15% d
RM1521:	40% b
NRG34:	65% a
11:	30% c





Contribution to Knowledge

- ✓ Alfalfa regrowth after freezing is positively linked with the proportion of nodules showing less freezing damages which in turn depends on rhizobial strains.
- ✓ First in vivo observations of variability in nodule freezing damages and of nodules regeneration zones induced by *S. meliloti* strains differing in their level of freezing tolerance.
- ✓ Both the choice of alfalfa populations and of rhizobial strains adapted to stress are complementary to increase the persistence of alfalfa in cold and temperate regions.

Study underlying mechanisms of a freezing tolerant symbiotic association





Cold acclimation induces metabolic and genetic changes in alfalfa overwintering perennial organs and in the flavonoid concentration of root exudates that differ between the most performant and the less performant host/strains associations.

Study underlying mechanisms of a freezing tolerant symbiotic association



Measurements:

Root exudates •



Growth chamber: Fall + Freeze- thaw + Regrowth





[], nature

Soluble sugars





Contribution to Knowledge

- ✓ Nodules play a pivotal role in the acquisition of freezing tolerance and regrowth of alfalfa.
- ✓ New insights into the molecular dialog of alfalfa-rhizobia associations in response to cold acclimation, freezing stress and deacclimation as well as a comprehensive understanding of the contribution of each cold-tolerant partner for a successful symbiosis in alfalfa.



Conclusions:

Investment by the plants in nodules!

Major strain effect observed



Rhizobia and plants play complementary roles to ensure enhanced regrowth after freezing

N storage and remobilization

Importance to include the rhizobial symbiosis in strategies aimed at improving stress tolerance in legumes.



Freezing strain NRG34 has a good potential in cold soil

Need field experiments with other cultivars



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In the long history of humankind (and animal kind, too) those who learned to collaborate and improvise most effectively have prevailed." -Charles Darwin

Thanks for you attention! And thanks to the team:

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Fièrement propulsée par les FRQ



Agriculture and Agri-Food Canada Agriculture et Agroalimentaire Canada

34 metabolites

- 7 sugars
- 21 amino acids
- 6 flavonoids

***Investment in nodules

Concentration increases compared to NA

Concentration decreases compared to NA

		Exuc	lates			Nod	lules			Ro	oots			Cro	owns	
letabolites	NA	CA	AFS	RAF	NA	CA	AFS	RAF	NA	CA	AFS	RAF	NA	CA	AFS	RAF
Sugars		mg/g R	oot DM			mg/g Noo	dules DM			mg/g R	oot DM			mg/g Cr	own DM	
Sucrose	0.514	0.529	0.223	0.129	58.18	158.74	67.64	48.64	49.80	174.25	143.63	73.26	43.70	183.19	124.98	66.08
Glucose	0.361	0.396	0.382	0.691	2.79	3.72	3.47	3.10	2.16	2.13	1.72	1.72	5.22	4.32	2.51	3.72
Stachyose	0.011	0.068	0.038	0.015	0.00	9.12	2.54	0.00	0.22	3.77	2.04	0.27	0.46	4.47	2.16	0.51
Raffinose	0.002	0.082	0.009	0.008	0.01	16.73	3.18	0.00	0.11	4.06	1.12	0.16	0.12	3.05	0.79	0.15
ructose	0.402	0.398	0.401	0.754	0.79	0.77	2.57	2.25	3.68	3.44	2.90	1.86	4.51	4.51	2.76	4.26
Pinitol	0.269	0.123	0.117	0.202	29.54	26.47	12.94	13.08	9.58	8.56	8.64	7.30	10.89	9.16	9.85	7.64
SSTot	1.560	1.597	1.168	1.798	91.24	215.55	92.34	67.06	65.55	196.21	160.05	84.58	64.91	208.70	143.05	82.36
Starch					1.62	4.64	1.48	1.46	321.78	114.05	119.36	209.32	272.32	95.46	101.04	151.24
ISC					89.81	220.19	93.82	68.52	387.33	310.26	279.41	293.90	337.23	304.16	244.10	233.60
Amino Acids		µmol/g f	Root DM			µmol/g No	odule DM			µmol/g F	Root DM			µmol/g C	rown DM	
Əlu	0.181	0.170	0.165	0.193	16.05	18.15	12.95	14.93	5.93	7.87	9.59	5.42	7.50	10.82	13.25	7.95
Əln	0.038	0.005	0.002	0.044	2.45	3.00	3.56	3.73	1.37	0.93	0.78	1.58	1.77	0.87	0.87	2.44
Pro	0.722	0.134	0.128	0.641	25.10	24.73	21.07	22.50	28.83	19.14	21.49	22.63	25.95	22.85	25.10	25.67
Drn	0.017	0.007	0.005	0.019	0.24	2.44	1.03	0.36	0.28	0.49	0.37	0.18	0.34	0.64	0.56	0.42
Arg	0.009	0.011	0.009	0.010	2.15	19.30	22.44	2.54	6.22	11.59	11.47	3.68	8.32	13.92	13.84	4.72
lis	0.011	0.003	0.005	0.010	3.06	7.02	9.11	3.13	1.99	2.68	3.10	0.99	2.83	4.20	4.68	1.68
Asp	0.186	0.174	0.153	0.222	4.99	4.97	4.59	4.28	1.22	2.84	2.28	1.49	3.74	6.63	3.48	4.23
Asn	0.867	0.043	0.004	0.628	416.32	359.93	248.41	278.54	83.01	63.19	62.19	73.56	69.14	44.83	40.54	83.43
Na	0.078	0.042	0.064	0.071	12.89	20.24	15.77	9.87	3.70	3.14	6.51	2.96	2.64	1.64	6.29	2.53
nr	0.039	0.015	0.023	0.036	1.68	1.45	5.33	1.98	1.09	0.82	1.79	0.89	0.89	0.65	1.51	0.97
.ys Act	0.008	0.000	0.008	0.011	0.82	0.84	3.06	0.89	0.08	0.13	0.43	0.08	0.11	0.12	0.37	0.09
het	0.004	0.005	0.005	0.000	0.07	0.08	0.24	0.15	0.03	0.03	0.10	0.03	0.10	0.09	0.00	0.04
ie	0.009	0.000	0.013	0.013	0.72	0.91	0.07	0.95	0.15	0.23	1.17	0.12	0.10	0.20	0.07	0.14
.eu /al	0.011	0.009	0.017	0.014	0.79	1.86	0.74	2.05	0.17	0.22	1.40	0.12	0.10	0.15	1.00	0.15
Ser	0.013	0.012	0.021	0.021	3.47	11.00	9.21	3.00	2.61	4 44	5.93	1.93	2 71	4 38	5.68	2.68
	0.001	0.000	0.017	0.029	2.33	3 34	6.35	2 09	0.39	0.29	0.59	0.37	0.42	0.29	0.61	0.46
GABA	0.175	0.071	0.094	0.137	27.00	25.38	23.51	16.07	5.56	7.50	6.91	3.92	4.25	3.34	3.57	3.61
ABA	0.001	0.000	0.000	0.001	0.39	0.29	0.09	0.10	0.04	0.06	0.05	0.05	0.09	0.13	0.16	0.11
yr	0.004	0.003	0.004	0.006	0.49	1.00	1.71	0.30	0.11	0.31	0.55	0.20	0.11	0.17	0.43	0.21
he	0.009	0.006	0.007	0.010	0.44	0.34	1.73	0.68	0.27	0.27	0.67	0.19	0.30	0.19	0.60	0.23
ATot	2.50	0.77	0.79	2.20	523.42	507.12	414.36	370.26	143.42	126.75	138.94	120.72	131.84	116.63	124.72	142.09
lavonoids	i ds µg/g Root DM					µg/g Nodule DM			µg/g Root DM				log2 Fold changes (Event sampling/NA)			
laringenin	0.038	0.025	0.013	0.020	1.23	1.24	1.82	1.80	0.06	0.57	0.03	0.57	-	decrease	Increase	
uteolin	0.045	0.026	0.027	0.094	1.08	1.43	2.24	2.65	0.00	0.23	0.63	0.44		<	>	
Echinatin	0.236	0.091	0.070	0.363	2.64	5.86	12.34	9.33	0.01	1.39	0.76	1.06		-0.00	0.00	
Coumestrol	0.115	0.077	0.061	0.117	6.14	17.67	21.70	28.49	0.05	0.00	0.01	0.46		-0.25	0.25	
ormononetin	2.044	2.262	1.722	1.237	39.48	22.45	22.61	36.43	84.12	74.04	59.98	24.96		-0.50	0.50	
ledicarpin	0.170	0.504	0.178	0.134	2.48	8.10	5.33	8.55	4.57	10.96	8.33	3.04		-1.00	1.00	
laTot	2.651	2.985	2.070	1.965	51.85	56.76	66.18	87.83	88.81	87.18	69.74	30.55		-2.00	2.00	
														-3.00	3 00	

Alfalfa organs

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Materiel & methods Introduction Chapter 2

Chapter 2: Study the underlying mechanisms of a freezing tolerant symbiotic association





-11°C 10 12 Week 4 events sampling

Biochemical analysis



-10

-15 [0

[], nature

Amino Acids

Collect alfalfa crowns, roots • and nodules





1. There is a variability among *Sinirhizobium meliloti* strains in their ability to nodulate and fix N under low temperatures when associated with alfalfa populations differing in their levels of freezing tolerance.

2.Rhizobial strains selected for their efficiency at low temperatures have the competitive ability to establish an efficient symbiosis with alfalfa in cold soil as compared to natives strains, particularly with freezing-tolerant population (A-TF7).



Introduction	> Materiel & methods	> Chapter 1	> Chapt	-er 2 > C	Chapter 3	Conclusion			
Results: Intera	ction soils x &lfadfia	prostatiaitriens	n Site: Normandin e: Salint-Anne-de- Bellevue	ATFO ATF	7				
	Total nodule num	ber per plant	% осс	upancy	Shoot dry weigh	t (mg) per plant			
_	(# of tagged/ total nodules × 100)								
Effect	Northern soil	Southern soil	Northern soil	Southern soil	Northern soil	Southern soil			
Populations									
A-TF0	9.7°	48.9 ^b	33.5	51.9	37.3°	166.3 ^b			
A-TF7	10.4 ^c	69.4 ^a	31.7	48.4	37.6 ^c	198.4 ^a			
Strains**									
Non inoculated	5.1 ^f	35.9 ^c	Od	Od	25.3 ^d	120.6 ^b			
NRG34	15 ^d	74 ^a	64.7 ^b	79.7 ^a	45.5 ^c	212.9 ^a			
Rm1521	11.3 ^e	60 ^{ab}	32.4 ^c	70.8 ^b	43.56 ^c	187.8ª			
S27	8.9 ^e	55.2 ^b	nd***	nd	35.8 ^{cd}	209.3 ª			



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Chapter '





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Selection of effective and competitive Sinorhizobium meliloti strains that nodulate alfalfa under low temperature

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Contribution to Knowledge

- ✓ Confirmed that strain NRG34, isolated from northern Canada, is cross-adapted to cold and freezing stress and have higher competitive ability to nodulate alfalfa in two soils with different physicochemical and biological properties.
- \checkmark Revealed the potential of that strain to be commercialized as inoculant for alfalfa in northern environments.
- \checkmark Reports that a freezing-tolerant alfalfa population is more effective in the recruitment of rhizobia under low temperature as shown by a larger number of nodules which translated into larger plant biomass compared to a less freezing-tolerant population.

Nodules biomass: Alfalfa populations effect





• No effect under optimal growth conditions (NA).

• **A-TF0** 10% of nodule biomass 48 h after freezing stress (AFS).

