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Arnica montana; Le Prese, Valposchiavo (GR)

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Plantes médicinales et aromatiques
Medizinal- und Aromapflanzen

Auteurs
Claude-Alain Carron, José Vouillamoz, Catherine Baroffio

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Renseignements:	catherine.baroffio@agroscope.admin.ch
Rédaction:	C.-A Carron, J. Vouillamoz, C Baroffio
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Equipe / Team

Agroscope
Institut des sciences en Production Végétale IPV
Groupe PMA - Plantes Médicinales et Aromatiques
Centre de recherche Conthey
Route des Vergers 18, CH-1964 Conthey (VS)
Tél.: +41 (0)58 481 35 11 – Fax.: +41 (0)27 346 30 17
Site internet: www.agroscope.ch

Agroscope,
Institut für Pflanzenbauwissenschaften IPB
Forschungsgruppe Medizinal- und Aromapflanzen
Forschungszentrum Conthey
Route des Vergers 18, CH-1964 Conthey (VS)
Tel.: +41 (0)58 481 35 11 – Fax.: +41 (0)27 346 30 17
Webseite: www.agroscope.ch

Responsables / Verantwortliche

Catherine Baroffio
Biologiste, cheffe de groupe Baies et PMA
Biologin, Leiterin Forschungsgruppe Beeren, Medizinal- und Aromapflanzen
catherine.baroffio@agroscope.admin.ch

Dr José Vouillamoz,
Biologiste, domestication, sélection
Biologe, Domestikation, Züchtung
jose.vouillamoz@agroscope.admin.ch

Collaborateurs / Mitarbeiter

Claude-Alain Carron
Technicien *Techniker*
Sélection, technique de culture
Züchtung, Anbautechnik
claude-alain.carron@
agroscope.admin.ch

Dr Vincent Michel
Agronome *Agronom*
Protection des végétaux maladies
Pflanzenschutz Krankheiten
vincent.michel@
agroscope.admin.ch

Charly Mittaz
Technicien *Techniker*
Protection des végétaux
Ravageurs
Pflanzenschutz Schädlinge
charly.mittaz@
agroscope.admin.ch

Bénédicte Bruttin
Auxiliaire technique
Technische Mitarbeiterin
Laboratoire
Labor
benedicte.bruttin@
agroscope.admin.ch

David Farquet
Apprenti horticulteur « plantes vivaces »
Gartenbaulehrling « mehrjährige Pflanzen »
david.farquet@agroscope.admin.ch

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- Emilie Walbaum, auxiliaire, t*emporäre Mitarbeiterin*
- Aline Délèze, auxiliaire, programme PONTE, *temporäre Mitarbeiterin*
- Mélanie Delasoie, *temporäre Mitarbeiterin*

Liste des publications et colloques / Liste der Publikationen und Vorträge

Publications / *Publikationen*

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- Carlen C. (2014). Influence of postharvest treatments on the artemisinin content of leaves of *Artemisia annua*. XXIX International Horticultural Congress: IHC2014. août, Ed. ISHS, Brisbane, Australia.
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- Carlen C. , Baroffio C.A., Vouillamoz J.F., Carron C.-A (2014). Einfluss der Agrotextilabdeckung auf den Ertrag an Blättern, ätherischem Öl und Rosmarinsäure. 7. Tagung Arznei-und Gewürzpflanzenforschung, Wien - Innovation entlang der Produktionskette. 14-17.9.
- Vouillamoz J.F., Carron C.-A., Baroffio C.A., Carlen C. (2014). 'Mattmark', a high yielding *Rhodiola rosea* cultivar launched in Switzerland. Book of abstract; Phytochemicals in Medicine and Pharmacognosy, Piatra-Neamt, Romania, 27-30.4.

Domaines / Betriebe

Domaine des Fougères

Situation: altitude 480 m
Latitude: 46.12 N, longitude 7.18 E
Sol: alluvions d'origine glaciaire, teneurs en calcaire moyennes (2 à 20 % de $CaCO_3$ tot., pH 7-8) granulométrie: légère à moyenne, teneur en cailloux faible à moyenne, matière organique: 1,5 à 2%.

Les nuances suivantes sont à relever selon les domaines:
Fougères: sol léger à moyen, caillouteux, calcaire
Epines: sol très léger, limoneux, absence de cailloux
Irrigation: par aspersion (Fougères et Epines)

Lage: 480 m über Meer
Breitengrad: 46.12 N, Längengrad 7.18 E
Boden: Gletscherablagerungen, mittlerer Kalkgehalt (tot. 2 bis 20 % $CaCO_3$, pH 7-8) Granulometrie: leicht bis mittel, Kiesvorkommen schwach bis mittel, organische Substanz: 1,5 bis 2%.

Je nach Betrieb treten folgende Besonderheiten auf:
Fougères: leichter bis mittelschwerer Boden, kies- und kalkhaltig
Epines: sehr leichter Boden, schlammig, kein Kies
Bewässerung: Beregnung (Fougères und Epines)

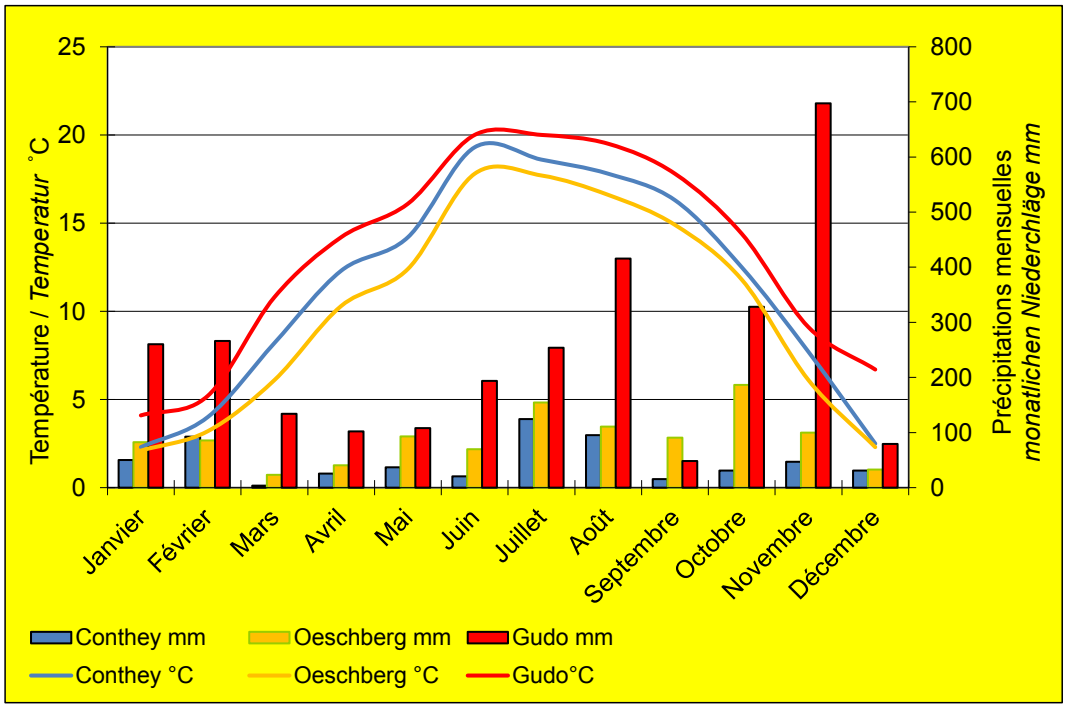
Domaine de Bruson

Situation: altitude 1060 m
Latitude: 46.04 N, longitude 7.14 E
Sol: plateau morainique, au sol moyennement léger et caillouteux, riche en matière organique (> 3,5 %) et légèrement acide (pH 6,5).
Exposition: nord-est
Pente: ± 10%
Irrigation: par aspersion

Lage: 1060 m über Meer
Breitengrad: 46.04 N, Längengrad 7.14 E
Boden: Moränengelände, Boden mässig leicht und kieshaltig, reich an organischer Substanz (> 3,5 %) und leicht sauer (pH 6,5).
Exposition: Nordost
Neigung: ± 10%
Bewässerung: Beregnung

Parcelle de sélection de sauge sur le domaine de Bruson. Juin 2014.
Parzelle für die Züchtung von Salbei auf dem Betrieb Bruson. Juni 2014.

La météorologie / Meteorologie



Courbes de températures et sommes mensuelles des précipitations à Conthey (VS), Oeschberg (BE) et Gudo (TI) en 2014

Verlauf der monatlichen Temperaturen und Niederschläge in Conthey (VS), Oeschberg (BE) und Gudo (TI) im 2014. [Daten : www.agrometeo.ch]

2014 L'année des extrêmes [source: meteosuisse]

La température annuelle en Suisse en 2014 a souvent été entre 1.0 et 1.4 degré au-dessus de la norme 1981-2010. Au Sud des Alpes et en Engadine, l'écart à la norme a été d'environ 1.0 degré. Moyenné sur l'ensemble de la Suisse et d'après la projection d'ici la fin de l'année, l'excédent thermique atteindra 1.3 degré, dépassant légèrement le record annuel de température relevé en 2011.

Les précipitations annuelles ont été normales ou légèrement déficitaires sur la plupart des régions du pays du Nord des Alpes. En revanche, au Sud des Alpes et en Engadine, l'année a été nettement trop humide. À Lugano et à Locarno-Monti, avec précipitations correspondant à 150 à 160% de la norme, l'année 2014 a été la troisième la plus humide depuis le début des mesures il y a plus de 100 ans. Il faut remonter en 1960 pour retrouver une année encore plus humide avec l'équivalent de 160% de la norme 1981-2010.

L'ensoleillement s'est fréquemment situé dans la norme 1981-2010. Au Tessin et dans les Grisons, il a été massivement déficitaire. C'est même l'année la moins ensoleillée en Haute-Engadine et la deuxième ou la troisième la plus sombre au Tessin. Les mesures homogénéisées d'ensoleillement existent depuis 1959.

2014 Das Jahr der Witterungsextreme [Quelle: Meteoschweiz]

Die Jahrestemperatur 2014 lag in der Schweiz verbreitet 1.0 bis 1.4 Grad über der Norm 1981–2010. Auf der Alpensüdseite und im Engadin betrug die Abweichung von der Norm rund 1.0 Grad. Über die ganze Schweiz gemittelt erreicht der Überschuss, berechnet bis zum Jahresende, 1.3 Grad, womit die bisherige Rekordwärme des Jahres 2011 minim übertroffen wird.

Der Jahresniederschlag erreichte in den meisten Regionen nördlich der Alpen normale oder leicht unterdurchschnittliche Werte. Auf der Alpensüdseite und im Engadin war das Jahr deutlich zu nass. In Lugano und Locarno-Monti wurde mit 150 bis 160 % der Norm das dritt nasseste Jahr in den weit über 100-jährigen Messreihen aufgezeichnet. Etwas mehr Niederschlag brachte hier letztmals das Jahr 1960 mit über 160 % der Norm 1981–2010.

Die Sonnenscheindauer bewegte sich verbreitet im Bereich der Norm 1981–2010, in Graubünden und im Tessin blieb sie zum Teil massiv unterdurchschnittlich. Im Oberengadin war es das deutlich sonnenärmste, im Tessin das zweit- oder dritt sonnenärmste Jahr. Homogene Messreihen zur Sonnenscheindauer liegen seit 1959 vor.

Introduction / Einleitung

Le présent rapport relate l'activité du groupe PMA plantes médicinales et aromatiques d'Agroscope IPV durant l'année 2014. Axés sur les interrogations et les soucis des praticiens, nos travaux tentent d'apporter des indications et des renseignements précis sur les espèces qui présentent des difficultés variétales ou culturales.

Des recherches sur la qualité des plantes, les techniques culturales et la comparaison variétale ont été réalisées en parallèle avec la domestication de nouvelles espèces et la sélection. La priorité de ces travaux est discutée dans un réseau de compétence (Forum Plantamont) constitué par la production suisse, l'industrie de transformation et la recherche. Que tous les acteurs de la filière des PMA trouvent ici l'expression de notre reconnaissance pour l'excellent esprit de collaboration dont ils nous gratifient.

Bonne lecture !

Der vorliegende Bericht beschreibt die Tätigkeiten der Forschungsgruppe Medizinal- und Aromapflanzen vom IPB von Agroscope im Jahr 2014. Unsere Arbeiten sind auf Fragestellungen der Praxis ausgerichtet und haben zum Ziel, Informationen und gezieltes Wissen zu Pflanzenarten zu erarbeiten, die bezüglich Anbau und Sorteneigenschaften besondere Herausforderungen darstellen.

Nebst Forschungsarbeiten zu Sortenqualität und Anbau sowie Sortenvergleichen wurden auch die Domestikation und Züchtung neuer Arten durchgeführt. Die Schwerpunkte dieser Tätigkeiten werden im Kompetenz-Netzwerk (Forum Plantamont) bestehend aus Schweizer Produzenten, Vertretern der Verarbeitungsindustrie und der Forschung diskutiert. Wir danken hiermit allen Akteuren des Medizinal- und Aromapflanzensektors für die hervorragende Zusammenarbeit und freuen uns auf die weiteren gemeinsamen Aktivitäten.

Wir wünschen viel Vergnügen beim Lesen!

Journées d'information chez Reto Raselli au val Poschiavo (Le Prese, GR), les 21 et 22 août 2014.
Informationstage bei Reto Raselli im Val Poschiavo (Le Prese, GR), 21. und 22. August 2014.

Comparaison clonale *Mentha* x *piperita* / Klon-Vergleich von *Mentha* x *piperita*

But de l'essai

Rechercher pour la production en zone de montagne (marché suisse) un nouveau clone de menthe poivrée satisfaisant au niveau agronomique et sensoriel.
A la demande de T. Aeschlimann, tester les clones allemands développés au LFL de Bayern.
Contactée par email, le Dr Heidi Heuberger, qui est responsable du Arbeitsgruppe Heil- und Gewürzpflanzen au Bayerische Landesanstalt für Landwirtschaft (LfL), nous confirme que les clones BLBP sont libres de droits et peuvent être utilisés pour la production en Suisse.
Critères : Vigueur, rendement, % de feuilles, teneur en HE, teneur en menthol (et profil aromatique), sensibilité à la rouille

Ziel des Versuchs

Einen neuen Pfefferminze-Klon für die Bergregion suchen (Schweizer Markt), welcher die sensorischen und agronomischen Anforderungen erfüllt.
Im Auftrag von T. Aeschlimann, die von der LFL Bayern entwickelten Klone testen.
Dr. Heidi Heuberger, Verantwortliche der Arbeitsgruppe Heil- und Gewürzpflanzen an der Bayerischen Landesanstalt für Landwirtschaft (LfL), bestätigte uns per Mail, dass für die BLBP-Klone ein Recht auf freie Verwendung besteht und sie für die Produktion in der Schweiz verwendet werden dürfen.
Kriterien: Wuchskraft, Ertrag, Blattanteil, Gehalt an ätherischem Öl, Gehalt an Menthol (und aromatisches Profil), Rost-Empfindlichkeit.

Matériel et méthodes/Material und Methoden

Informations générales de l'essai		**Allgemeine Versuchsdaten**	
Site	Bruson (Agroscope)	Standort	Bruson (Agroscope)
Variété / Sorte Origine/Herkunft	Type Dunkel : BLBP 35, BLBP 47 et BLBP 56 (LFL) ; 'Mary Mitcham' et 'Multimentha' (Jardin des Senteurs , NE) Type Grün : BLBP 02 et BLBP 04 (LFL) ; '541' (Valplantes)		
Irrigation	Aspersion (env. 30mm/semaine)	Bewässerung	Beregnung (ca. 30mm/Woche)
Fumure	normes Agridea	Düngung	Agridea Normen
Données culturales pour l'essai 2014		**Versuchsdaten 2014**	
repetitions	4 de 3.2 m^2 Plate-bande de 4 lignes: (3 x 40cm –passage 80cm) x 2m	Wiederholungen	4 à 3.2 m^2 Beet mit 4 Reihen (3x 40cm –Zwischengang 80cm) x 2m
Parametres	Rendements en matière sèche Pourcentage de feuilles résistance à la rouille Rapport poids frais/poids sec Teneur en huile essentielle Composition de l'huile essentielle	Parameter	Ertrag an Trockensubstanz Blattanteil Rostresistenz Verhältnis Frischgewicht/Trockengewicht Gehalt an ätherischen Ölen Zusammensetzung der ätherischen Öle
Analyses 2014	32 HE + 8 GC	Analysen 2014	32 ÄÖ + 8 GC

Date/Datum	**Travaux**	**Arbeiten**
Mai 2013	Labour, fumure	Pflügen, Düngung
Juin 2013	Hersage	Eggen
2 juillet 2013	Plantation	Auspflanzen
Récoltes/Ernten	3 OCTOBRE 2013 26 JUIN 2014 5 septembre 2014	3. Oktober 2013 26. Juni 2014 5. September 2014

Résultats / Ergebnisse 2014

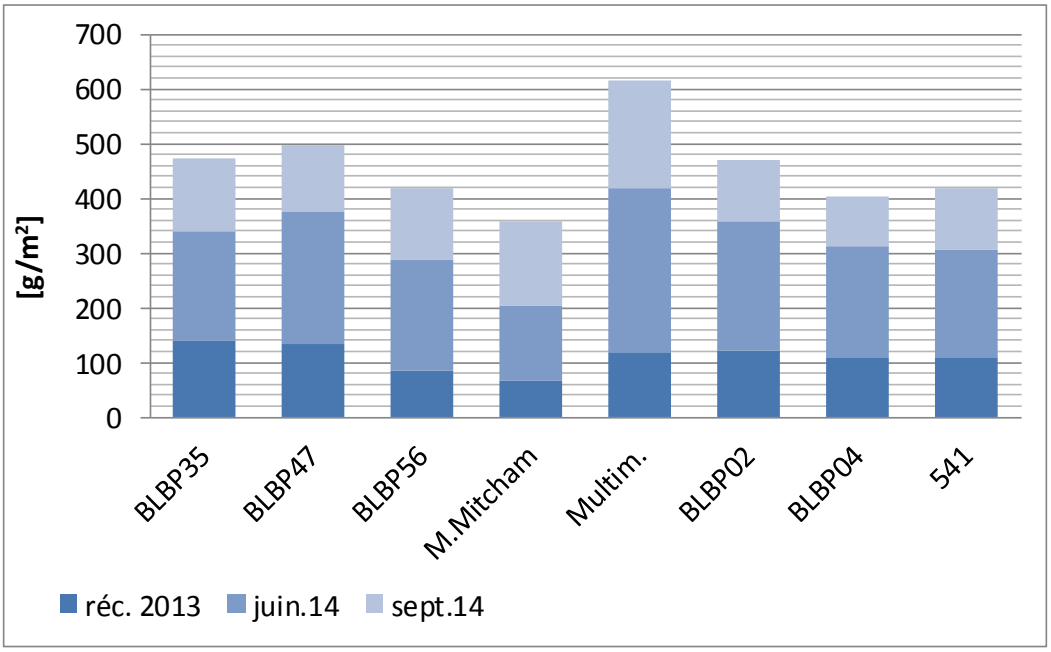


Figure 1. Rendement en matière sèche en g/m^2. Cumul des deux récoltes. Moyenne de quatre répétitions.
Abbildung 1. Ertrag an Trockensubstanz in g/m^2. Summe der beiden Ernten. Mittelwert der vier Wiederholungen.

Tableau 1. Rendement en matière sèche, % de feuilles et teneur en huile essentielle en 2013 et 2014. Moyenne de 4 répétitions.
Tabelle 1. Ertrag an Trockensubstanz, % Blätter und Gehalt an ätherischen Ölen im 2013 und 2014. Mittelwert der 4 Wiederholungen.

Clones	Rendement [g/m^2]					% feuilles				% huile essentielle			
	2013	juin.14	sept.14	2014	Total	2013	juin.14	sept.14	moy.	2013	juin.14	sept.14	moy.
BLBP35	139^{a}	200^{ab}	134^{bc}	334^{ab}	473^{ab}	73.6	69.4	68.8^{abc}	68.2	2.66^{bc}	2.32^{b}	2.54^{b}	2.40^{de}
BLBP47	133^{ab}	241^{ab}	121^{bc}	362^{ab}	496^{ab}	75.1	65.2	65.9^{abc}	65.4	2.54^{c}	2.20^{b}	2.52^{b}	2.28^{e}
BLBP56	85^{ab}	204^{ab}	130^{bc}	334^{ab}	419^{ab}	74.9	70.8	67.7^{abc}	69.6	2.29^{c}	2.38^{b}	2.60^{b}	2.45^{cde}
M.Mitcham	68^{b}	135^{b}	154^{ab}	289^{b}	357^{b}	74.8	68.6	60.4^{c}	64.2	2.49^{c}	2.40^{b}	3.13^{ab}	2.77^{bc}
Multim.	120^{ab}	298^{a}	198^{a}	496^{a}	616^{a}	72	66.9	63.4^{bc}	65.6	2.48^{c}	2.31^{b}	3.13^{ab}	2.62^{cd}
BLBP02	122^{ab}	235^{ab}	112^{bc}	347^{ab}	470^{ab}	77.7	67.1	70.4^{ab}	68.2	3.10^{a}	3.28^{a}	3.37^{a}	3.28^{a}
BLBP04	110^{ab}	201^{ab}	93^{c}	294^{b}	404^{ab}	76.5	73.6	72.3^{ab}	72.9	2.99^{ab}	3.12^{a}	2.91^{ab}	3.02^{ab}
541	109^{ab}	198^{ab}	111^{bc}	309^{b}	418^{ab}	77.8	74.1	74.4^{a}	74.2	3.33^{a}	3.36^{a}	2.95^{ab}	3.17^{a}

Tukey test : Les lettres différentes indiquent les différences significatives. / Die verschiedenen Buchstaben zeigen die signifikanten Unterschiede.

Discussion/Diskussion

Au cumul du rendement des deux années, le clone 'Multimentha' se distingue par sa bonne vigueur, alors que le clone 'Mary Mitcham' présente le moins bon potentiel de production. Les autres clones ont eu une production statistiquement similaire.

Les types Grün (BLBP 02; BLBP 04 et '541') ont montré significativement une plus haute teneur en huile essentielle. L'analyse de la composition de l'huile essentielle n'a pas encore été effectuée.

Tendanciellement '541' et BLBP 04 ont obtenu un meilleur taux de feuilles, mais la seule différence significative a été mesurée sur la seconde récolte en 2014.

Globalement, la pression de rouille est restée faible les deux années. Aucune différence de comportement clonal n'a été observée. En revanche, la pression des *Longitarsus* a été très forte en 2014 et a prétérité le rendement de la seconde récolte de septembre (fig. 2). Les clones type Dunkel semblent être un peu moins apprécié par ce coléoptère.

Kumuliert man die Erträge der beiden Jahre, so zeigt sich, dass sich der Klon 'Multimentha' durch seine gute Wuchskraft auszeichnet während der Klon 'Mary Mitcham' das geringste Potential aufweist. Die anderen Klone zeigten statistisch ähnliche Resultate.

Die Typen Grün (BLBP 02 ; BLBP 04 und '541') wiesen einen signifikant höheren Gehalt an ätherischen Ölen auf. Die Zusammensetzung der ätherischen Öle wurde noch nicht analysiert.

Tendenziell erreichten '541' und BLBP 04 einen höheren Blattanteil, wobei die Differenz nur in der zweiten Ernte 2014 signifikant war.

Insgesamt war der Rostbefall in beiden Jahren niedrig. Im Verhalten der Klone wurde kein Unterschied beobachtet. Der *Longitarsus*-Befall war jedoch im Jahr 2014 sehr stark und beeinträchtigte den Ernteertrag im September (Abb. 2). Die Klone des Typs Dunkel scheinen bei diesem Käfer etwas weniger beliebt zu sein.

Perspectives/Perspektiven 2015

- Mise en place d'un nouvel essai à Bruson afin de choisir 3-4 clones qui seront testé 'on farm' en 2016-2017.
- Observation du développement de la rouille et suivi de la pression des Longitarsus sur la parcelle 2013-2014 (pas de récolte / contrôle hebdomadaire de l'état du feuillage).
- Durchführung eines neuen Versuchs in Bruson, um 3-4 Klone für die 'on farm'-Untersuchung im 2016-2017 auszuwählen.
- Beobachtung der Rost-Entwicklung sowie des Longitarsus Befalls auf der Parzelle 2013-2014 (keine Ernte / wöchentliche Kontrolle des Blattzustandes).

Figure 2. Dégâts de *Longitarsus sp.* sur clone de *Mentha* x *piperita* '541'. Une forte pression de ce coléoptère nuit sensiblement au rendement et diminue la qualité intrinsèque des feuilles. Des travaux visant une meilleure connaissance de ce ravageur et des essais de lutte sont en cours.

Abbildung 2. Durch *Longitarsus sp* verursachte Schäden auf dem Klon *Mentha* x *piperita* '541'. Ein starker Befall durch diesen Schädling führt zu einem deutlichen Ertragsrückgang und verringert die Qualität der Blätter. Die Forschungsarbeiten haben zum Ziel, diesen Schädling besser kennen zu lernen und Versuche zur dessen Bekämpfung durchzuführen

Amélioration variétale *Primual veris* / *Primula veris* : Sortenverbesserung

But de l'essai

Amélioration variétale pour obtenir des tiges plus hautes et une meilleure floribondité. Choix des meilleurs individus pour un polycross : Sélection Bruson 2012 et Bruson 2013.

Ziel des Versuchs

Sortenverbesserung für höhere Stängel und mehr Blumen. Auswahl der besten Individuen und Polycross -> Züchtung Bruson 2012 und Bruson 2013.

Matériel et méthodes/Material und Methoden

Informations générales de l'essai		**Allgemeine Versuchsdaten**	
Site	Bruson (Agroscope)	Standort	Bruson (Agroscope)
Variété / Sorte Origine/Herkunft	1) Comparaison de Bruson 2012 avec trois provenances (Carillo, Hofer, Ruhleman's) 2) Vergleich von Bruson 2012 mit Bruson 2013, und Vergleich beide Bruson mit den Nachkommen der 17 besten Pflanzen aus den Provenienzen «Hofer» und «Wies» 1) Vergleich von Bruson 2012 mit drei Provenienzen (Carillo, Hofer, Ruhleman's) 2) Vergleich von Bruson 2012 mit Bruson 2013, und Vergleich beide Bruson mit den Nachkommen der 17 besten Pflanzen aus den Provenienzen «Hofer» und «Wies»		
Irrigation	Aspersion (env. 30mm/semaine)	Bewässerung	Beregnung (ca. 30mm/Woche)
Fumure	normes Agridea	Düngung	Agridea Normen
Données culturales pour l'essai 2014		**Versuchsdaten 2014**	
Plantations	Semis 17 février, 1 mois de vernalisation. Repiquage en mottes pressées (120) en avril. Répétitions : 3 de 32 plantes (8x4), 3 m^2 Distances de plantation: plate-bande de 4 lignes (25 cm x 25 cm)	Pflanzung	Aussaat 17 Februar, 1 Monat vernalisation. Umpflanzung im Topfen (12) im April. Wiederholungen : 3 von 32 Pflanzen (8x4), 3 m2 Pflanzabstände: Beet mit 4 Reihen (25 cm x 25 cm)
Paramètres	Hauteur Nombre de hampes florales Mortalité Poids sec des fleurs Nombre de plantes en fleurs Régularité et vigueur	Parameter	Höhe Anzahl Blumenspitzen Sterblichkeit Blumentrockengewicht Anzahl blühenden Pflanzen Regularität und Vitalität

Résultats / Ergebnisse 2014

Tableau 1 : Comparaison de Bruson 2012 avec trois provenances.
Tabelle 1 : Vergleich von Bruson 2012 mit drei Provenienzen.

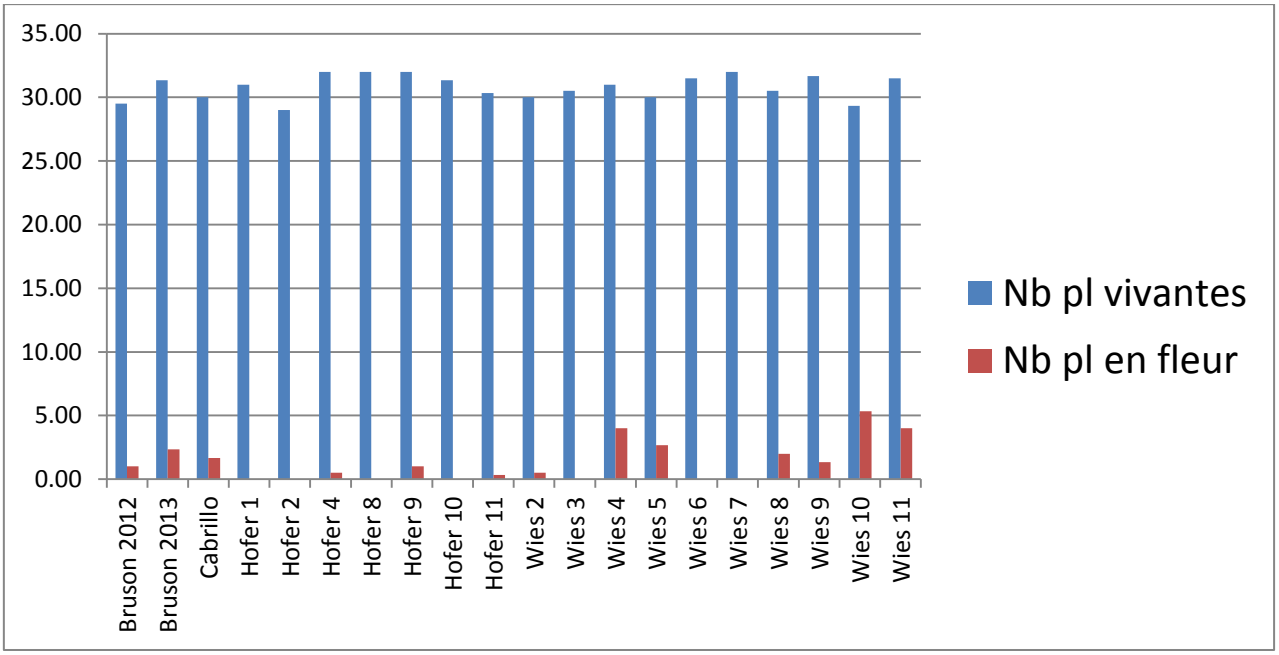
Provenance/Herkunft	**Hauteur/Höhe (cm)**		**Nombre de hampes florales/ Anzahl Blumenspitzen**	
	1. récolte/Ernte	2. récolte/Ernte	1. récolte/Ernte	2. récolte/Ernte
Bruson 2012	10.55 ^{ab}	16.35 ^{ab}	3.58 ^{b}	6.34 ^{a}
Carillo	8.63 ^{b}	13.57 ^{b}	3.21 ^{b}	5.98 ^{a}
Hofer	8.53 ^{b}	17.62 ^{a}	2.88 ^{b}	5.82 ^{a}
Ruhleman's	12.86 ^{a}	15.07 ^{ab}	6.17 ^{a}	4.39 ^{a}

Provenance/ Herkunft	Mortalité/ Sterblichkeit %	Poids sec des fleurs/Blumentrockengewicht				
		1. récolte/ Ernte [g/bloc]	2. récolte/ Ernte [g/bloc]	Total/ Gesamtsumme [g/bloc]	Par plante plantée/ Pro Pflanze gepflanzt [g]	Par plante récoltée/ Pro Pflanze geerntet [g]
Bruson 2012	25.8 a	7.6 b	16.6 ab	24.1 ab	0.75	1.00
Carillo	12.5 a	6.8 bc	13.1 b	19.9 b	0.62	0.71
Hofer	9.4 a	4.2 c	23.9 a	28.1 ab	0.88	0.96
Ruhleman's	9.4 a	15.8 a	15.8 ab	**31.6 a**	0.99	1.09

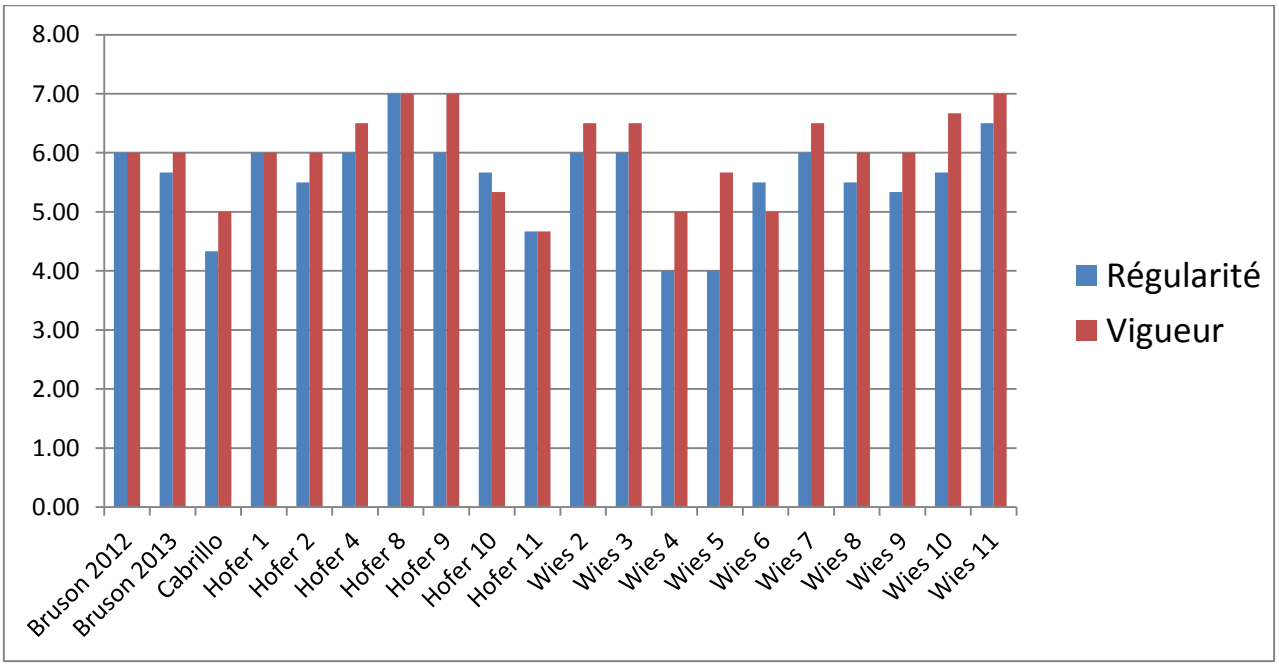
Tukey test : Les lettres différentes indiquent les différences significatives. / Die verschiedenen Buchstaben zeigen die signifikanten Unterschiede.

Remarque : La sélection Bruson 2012 est similaire aux provenances Hofer et Ruhleman's mais très hétérogène.
Bemerkung: Die Züchtung Bruson 2012 ist ähnlich wie die Provenienzen Hofer und Ruhleman's, aber sehr heterogen.

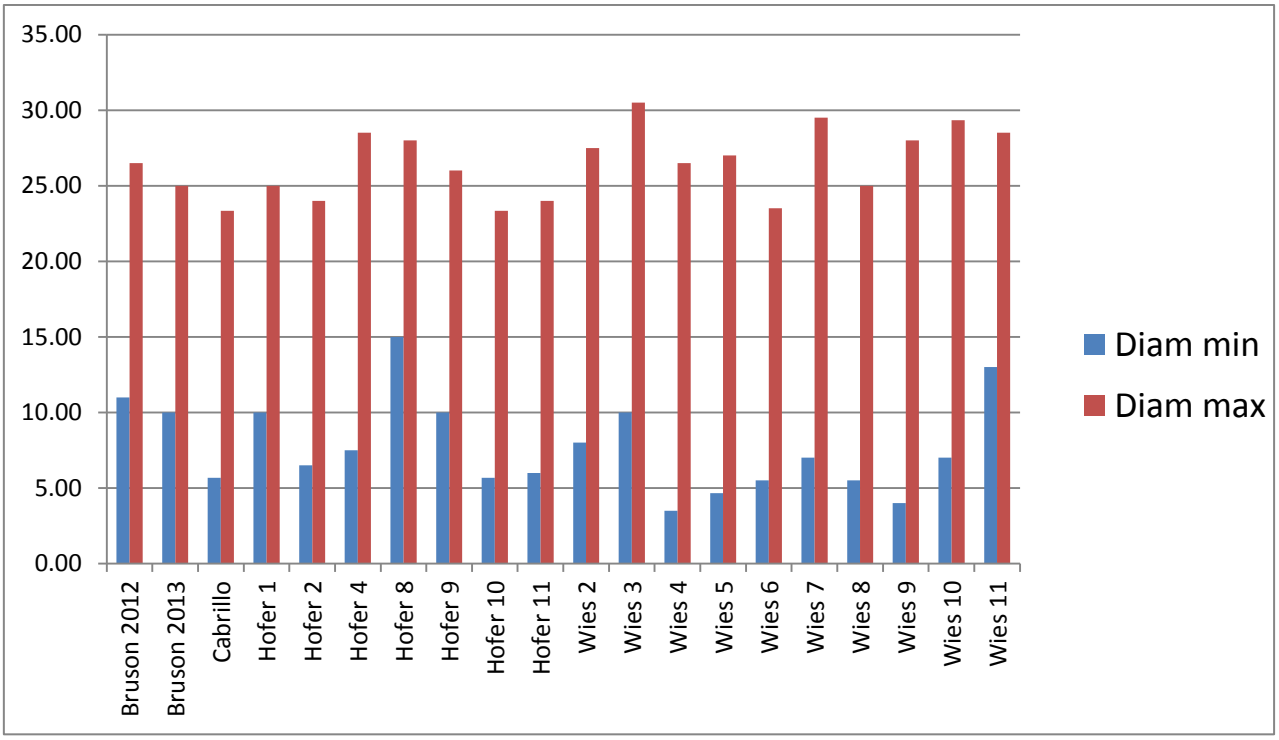
Figure 1 :
Comparaison de Bruson 2012 avec Bruson 2013 et avec 17 descendants des meilleures plantes de Hofer et Wies.
Vergleich von Bruson 2012 mit Bruson 2013 und mit 17 Nachkommen von den besten Pflanzen aus Hofer und Wies.



Remarque : Aucune différence dans le nombre de plante vivantes, dans l'hétérogénéité et les plantes en fleurs.
Bemerkung: Kein Unterschied bei der Anzahl lebender Pflanzen, betreffend Heterogenität und blühenden Pflanzen.



Remarque : Bruson 2012 et 2013 sont similaires avec des valeur intermédiaires pour la régularité et la vigueur, Hofer 8, 9 et Wies 2, 3, 7, 10 et 11 paraissent un peu meilleures.
Bemerkung: Bruson 2012 und 2013 sehr ähnlich mit Zwischenwerten für die Regularität und die Vitalität, Hofer 8, 9 und Wies 2, 3, 7, 10 und 11 ein bisschen besser.



Remarque : Bruson 2012 et 2013 sont similaires avec des valeurs intermédiaires pour le diamètre des rosettes, Hofer 8 et Wies 3, 11 paraissent un peu meilleurs.
Bemerkung: Bruson 2012 und 2013 sehr ähnlich mit Zwischenwerten für die Durchmesser, Hofer 8 und Wies 3, 11 ein bisschen besser.

Discussion/Diskussion

Les sélections Bruson 2012 et 2013 sont pour le moment très semblables. En perspectives, une sélection de lignées parentales en 2015-6, avec premières semences en 2017-8, puis comparaison avec les variété standards.

Die Züchtungen Bruson 2012 und 2013 im Moment sehr ähnlich. Im Perspektiven, Selektion der Elternlinien im 2015-6, erstes Saatgut 2017-8, Vergleich mit Standardsorten.

Salvia officinalis : sélection d'une nouvelle variété / *Salvia officinalis : Züchtung einer neue Sorte*

But de l'essai

La variété 'Regula', qui ne produit pas assez de semences (0,5 g / m2 sur trois ans!), devrait être remplacée. Nous avons mis sur pied un nouveau programme de sélection.

Ziel des Versuchs

Die Sorte 'Regula', die nicht genug Samen produziert (0,5 g / m2 über drei Jahre!), sollte ersetzt werden. Wir haben ein neues Züchtungsprogramm aufgestellt.

Résultats / Ergebnisse 2014

Choix des meilleurs parents/Auswahl der besten Eltern

Critères:

- [HE] > 2.0%, composition similaire à 'Regula'
- Fertilité, homogénéité et résistance au gel hivernal
- Production de semences régulière
- Viabilité du pollen

Kriterien:

- [ÄÖ] > 2.0%, Zusammensetzung ähnlich wie 'Regula'
- Ergiebigkeit, Homogenität und Frostbeständigkeit
- Regelmässige und hohe Samenproduktion
- Lebensfähigkeit der Pollen

Choix de 10 mâles fertiles (MF) avec optimum de floraison synchrone, meilleur poids de semences, viabilité du pollen, hauteur et teneur en HE /
Auswahl von 10 männliche Fertil (MF) mit optimal synchrone Blütezeit, Samengewicht, Pollen Lebensfähigkeit, Höhe und ÄÖ Gehalt

Matricule	Provenance/ Herkunft	Nom/ Name	Hauteur/ Höhe [cm]	N fleurs/tiges N Blüten/Stengel	Poids semences/ Samengewicht [g]	Viabilité pollen/ Pollen Lebensfähigkeit [%]	Teneur HE/ÄÖ Gehalt [%]
7-3-MF	LFL	Julius Wagner	60	24.0	5.9	55	1.775
10-9-MF	LFL	B.S.V	60	38.5	3.2	56	1.803
13-1-MF	Corvinus	Poznan	86	42.0	2.6	-	1.883
14-7-MF	Corvinus	Nantes	72	31.5	5.2	65	1.535
14-9-MF	Corvinus	Nantes	68	37.5	9.0	65	1.535
17-6-MF	Corvinus	Napoca	55	82.5	5.9	-	1.703
17-7-MF	Corvinus	Napoca	61	81.0	5.0	-	1.703
19-7-MF	Corvinus	Regensburg	68	48.0	4.7	80	1.790
21-8-MF	Corvinus	Fäldesi	80	43.5	5.1	83	1.663
29-2-MF	Hem Zaden	-	60	37.5	8.9	-	2.055
29-8-MF	Hem Zaden	-	70	36.0	8.3	-	2.055
28 (Ø)	Agroscope	Regula			2.2		1.413

2015: Poly-cross avec ces 10 MF/Poly-cross mit diesen 10 MF

Melolontha melolontha: Essai de lutte contre les hannetons / Versuch zur Bekämpfung des Maikäfers

Contexte

En 2013, une parcelle de thym vulgaire de 2 ha a été plantée aux Grisons, le Prese, *alt. 971 m (Coordonnées (m) 803543 ;130648)*. Vu l'ampleur des dégâts occasionnés par les hannetons communs (*Melolontha melolontha*), la culture a été labourée. Au printemps 2014, une nouvelle plantation de thym a été effectuée. Le sol a été hersé 2 fois afin de détruire mécaniquement le maximum de larves de hannetons et de diminuer la pression des ravageurs.
Etant donné que la lutte à l'aide de champignons entomopathogènes (*Beauveria brongniartii*) n'était pas indiquée en 2014, elle doit être pratiquée au printemps suivant le vol (vol en 2015).
Agroscope, en collaboration avec Andermatt Biocontrol et la firme Consagros, a mis en place un essai afin de tester l'efficacité d'autres produits phytosanitaires compatibles avec la production biologique des plantes aromatiques et médicinales.

But

Test préliminaire d'efficacité de 4 produits phytosanitaires en arrosage contre les hannetons : Galanem et Naturalis-L (Biocontrol) ; Ravastop et Ecofort Repulse (Seydoux).

Melolontha melolontha au stade larvaire L3.
Melolontha melolontha, Larvenstadium L3

Détermination

Les larves collectées ont été identifiées par M. G. Grabenweger Agroscope Reckenholz : stade larvaire L3, vol prévu printemps 2015.

Kontext

Im Jahr 2013 wurde in Le Prese, Graubünden, 971 m.ü.M. eine Parzelle von 2 ha mit Thymian bepflanzt (Koordinaten (m) 803.543; 130.648). Aufgrund der durch Maikäfer, (*Melolontha melolontha*) verursachten Schäden, musste die Kultur umgepflügt werden. Im Frühjahr 2014 wurde erneut Thymian angepflanzt. Der Boden wurde zweimal geeggt, um möglichst viele Engerlinge mechanisch zu eliminieren und den Schädlingsdruck zu reduzieren.
Da die Bekämpfung mit entomopathogenen Pilzen (*Beauveria brongniartii*) im Jahr 2014 nicht angezeigt war, muss diese im Frühling nach dem Flug durchgeführt werden (Flugjahr 2015).
Agroscope führt in Zusammenarbeit mit Andermatt Biocontrol und der Firma Consagros einen Versuch durch, um die Wirksamkeit von anderen Pflanzenschutzmitteln zu untersuchen, die die Anforderungen des biologischen Anbaus von Aroma- und Medizinalpflanzen erfüllen.

Ziel

Vorversuch zur Wirksamkeit von 4 Pflanzenschutzmitteln gegen Maikäfer, Anwendung mittels Giesskanne: Galanem und Naturalis-L (Biocontrol) ; Ravastop und Ecofort Repulse (Seydoux)

Bestimmung

Die gesammelten Larven wurden von M. G. Grabenweger Agroscope Reckenholz identifiziert: Larvenstadium L3, Flug im Frühling 2015.

DONNEES GENERALES POUR L'ESSAI		*ALLGEMEINE VERSUCHSDATEN*	
Site	LE PRESE (R.RASELLI)	Standort	LE PRESE (R.RASELLI)
Détermination	M. G. Grabenweger Agroscope Reckenholz	Bestimmung	M. G. Grabenweger Agroscope Reckenholz
Stratégie de lutte	Essais de traitement Naturalis L, Galanem, Ravastop, Ecofort Repulse	Bekämpfung	Behandlungsversuche Naturalis L, Galanem, Ravastop, Ecofort Repulse

Matériel et méthode / Material und Methoden

Parcelles / Parzelle	
Site / Standort	Le Prese, 970m alt.
VARIÉTÉS Sorten	*Thymus vulgaris* 'Varico 3'; culture de 1^{e} année *Thymus vulgaris* 'Varico 3'; 1. Jahr
Surface / Fläche	300 m^2 / 12.5m^2 par blocs ; in Blöcken
Densité / Dichte	6.7 pl. /m^2 ; 30 cm x 50 cm
Répétitions / Wiederh.	4 de 12.5 m^2 (5 lignes x 5 m) ; 4 zu 12.5 m^2 (5 Linien x 5 m)
Variantes de traitements Behandlungsvarianten	T : Témoin non traité ; nicht behandelte Vergleichsfläche G1: Galanem (Biocontrol), 1 mio/m^2 / 2l d'eau (Wasser) /m^2 G2: Galanem (Biocontrol), 2 mio/m^2 / 2l d'eau (Wasser) /m^2 N : Naturalis-L (Biocontrol), 0.5 % / 2l d'eau (Wasser) /m^2 R : Ravastop (Seydoux), 0.003l/m^2 / 2l d'eau (Wasser) /m^2 E : Ecofort Repulse (Seydoux) 0.002l/m^2 / 2l d'eau(Wasser) /m^2
Application /Anwendung	Arrosoir 200 l/a / Giesskanne 200 l/a
Date de traitements / Datum der Behandlung	6 juin 2014 6. Juni 2014
Dates des contrôles Kontrolldaten	15 juillet et 9 octobre 15. Juli und 9. Oktober
Methodologie / Methodologie	Comptage des plantes vivantes; contrôle et notation de l'état racinaire sur une ligne centrale; capture des larves d'hannetons et de taupins. Auszählung der mehrjährige Pflanzen, Kontrolle und Beurteilung des Wurzelzustands auf der Mittellinie, Einsammeln der Larven der Maikäfer und Schnellkäfer.

Contrôle et notation de l'état racinaire sur une ligne centrale, le 15 juillet 2014.
Kontrolle und Beurteilung des Wurzelzustands auf der Mittellinie am 15. Juli 2014.

Commentaires et résultats

La plantation de thym a eu lieu les premiers jours de juin. Les traitements ont été effectués le 6 juin sur un sol sec, à l'arrosoir à la dose 200 litres d'eau/are, selon les recommandations de M. Gentizon/Biocontrol. L'idée étant d'obtenir une bonne percolation du sol afin d'atteindre le maximum de larves.

Lors du premier contrôle , le 15 juillet, une perte de plantes d'environ15% était notée avec des différences notables entre les procédés. Les causes de cette diminution sont probablement multiples (sarclage, mauvaise reprise ou hanneton). Les mesures sur l'état sanitaires des racines n'ont pas montré de différences significatives. Cependant, les racines des plantes traitées au Naturalis-L et à l'Ecofort Repulse étaient les mieux notées (tabl. 1). Lors de ce contrôle, seules quelques larves d'hannetons ont été capturées, la majorité de la population étant supposée être déjà plus profondément enfouie dans le sol.

Lors du second contrôle, le 9 octobre, une petite diminution supplémentaire du nombre de plantes vivantes était observée. Il apparaissait assez clairement qu'au moins une partie de cette perte était imputable au sarclage mécanique. Cependant, la tendance positive du système racinaire des parcelles traitées au Naturalis-L et à l'Ecofort Repulse était confirmée (tabl. 1).

Kommentare und Resultate

Der Thymian wurde in den ersten Junitagen gepflanzt. Die Behandlungen wurden am 6. Juni auf trockenen Boden mittels Giesskanne mit einer Aufwandmenge von 200 Liter/a durchgeführt, entsprechend den Empfehlungen von Herrn Gentizon/Biocontrol. Ziel war eine gute Versickerung im Boden, um möglichst viele Larven zu erreichen.

Bei der ersten Kontrolle am 15. Juli wurde ein Verlust von ca. 15 % der Pflanzen festgestellt mit beträchtlichen Unterschieden zwischen den Verfahren. Die Verluste sind vermutlich auf mehrere Ursachen (Hacken, schlechtes Anwachsen oder Engerlinge) zurückzuführen. Die Untersuchungen des Wurzelzustands zeigten keine signifikanten Unterschiede, wobei allerdings die mit Naturalis-L und Ecofort Repulse behandelten Pflanzenwurzeln besser bewertet wurden (Tab. 1). Bei dieser Kontrolle wurden nur wenige Maikäferengerlinge erfasst. Wahrscheinlich hatte sich die Mehrheit der Population bereits tiefer in den Boden eingegraben.

Anlässlich der zweiten Kontrolle am 9. Oktober wurde ein weiterer Verlust an lebenden Pflanzen beobachtet. Es wurde klar, dass mindestens ein Teil dieser Verluste auf die mechanische Unkrautbekämpfung zurückzuführen waren. Allerdings bestätigte sich die eher positive Entwicklung des Wurzelsystems der mit Naturalis-L und Ecofort Repulse behandelten Parzellen (Tab. 1).

Tableau 1. Test de quatre insecticides biologiques contre les hannetons. Nombre de plantes de thym vivantes, état sanitaire des racines et perte de plantes entre les deux contrôles. Moyenne de quatre répétitions.
Tabelle 1. Test von vier biologischen Insektiziden gegen Maikäfer. Anzahl der lebenden Thymian-Pflanzen, Wurzelzustand und Verluste zwischen den beiden Kontrollen. Mittelwert der vier Wiederholungen.

Traitement Behandlung	**Erste Kontrolle 15.07.2014**		**Zweite Kontrolle 09.10.2014**		**Perte de plantes 2e vs 1e contrôle [%] Verluste zw 1. u 2. Kontrolle [%]**
	Nb plantes vivantes 1) Anz. lebende Pflanzen	Etat sanitaire des racines 2) Wurzel-zustand	Nb plantes vivantes 1) Anz. lebende Pflanzen	Etat sanitaire des racines 2) Wurzel-zustand	
Ohne	42.9 bc	1.26 a	31.6 c	1.44 ab	26.4%
Galanem 1 Mio	43.1 bc	1.19 a	31.7 bc	1.17 ab	26.3%
Galanem 2 Mio	38.7 d	1.29 a	32.0 bc	1.13 b	17.3%
Naturalis-L	45.6 a	1.41 a	35.3 ab	1.63 ab	22.7%
Ravastop	42.4 bc	1.30 a	35.0 abc	1.35 ab	17.4%
Ecofort repulse	40.9 cd	1.42 a	36.4 a	1.75 a	11.0%

1) Nb de plantes sur 7.5 m^2 / Anz. Pflanzen auf 7.5 m^2
2) Etat sanitaire des racines : notes de 0-2; 0 = très mauvais; 2 = 100 % très bon gut
Wurzelzustand: Bewertung von 0-2; 0 = sehr schlecht; 2 = 100 % sehr gut

.

Perspectives

- Tester en boîte de Pétri l'efficacité des insecticides.
- Nouvel essais contre les hannetons au Poschiavo

Possibilités de luttes

- Préventif prophylactiques: éviter les plantations après prairies dans les zones à risques.
- Mécanique : dans les régions à risques, herser 2-3 fois les parcelles
- Mécanique : pose de filets après pour empêcher la ponte l'année du vol http://www.arrigoniagriculture.net/fra/biological_control.htm

Biologique : application de champignons enthomopathogènes (*Beauveria brongniartii*, Beaupro chez Biocontrol) au printemps l'année succédant au vol.

Ausblick

- Untersuchung der Wirkungseffizienz der Insektizide in der Petrischale.
- Neuer Versuch zur Bekämpfung der Maikäfer in Poschiavo

Bekämpfungsmöglichkeiten

- Prophylaktische Massnahmen: Vermeidung von Anpflanzung nach Wiesen in gefährdeten Gebieten.
- Mechanisch: Parzellen in gefährdeten Gebieten 2-3 mal eggen.
- Mechanisch: Anbringen von Netzen, um in den Flugjahren die Eiablage zu verhindern. http://www.arrigoniagriculture.net/fra/biological_control.htm
- Biologisch: Anwendung von entomopathogenen Pilzen (*Beauveria brongniartii*, Beaupro in Biocontrol) im Frühling nach dem Flugjahr.

Pimpinella peregrina

Résumé du rapport de Sára Kindlovits, PhD, Université de Corvinus, Budapest, Hongrie
Zusammenfassung des Berichts von Sára Kindlovits, PhD, Corvinus Universität, Budapest, Ungarn

But de l'essai

1. Evaluer l'effet de la densité de semis sur la production en racines fraîches et sèches de la pimprenelle boucage.
2. Suivre la formation de la biomasse en racines.
3. Définir la fenêtre optimale de récolte en fonction du rendement en racines et de la perte en eau de séchage

Ziel des Versuchs

1. Einfluss der Saatdichte auf die Bildung von frischen und trockenen Wurzeln der grossen Bibernelle beurteilen.
2. Bildung von Wurzelbiomasse beobachten.
4. Bestimmung des optimalen Ernteperiode abhängig von Wurzelertrag und Wasserverlust beim Trocknen.

Matériel et méthode / Material und Methode

Informations générales de l'essai		**Allgemeine Versuchsdaten**	
SITE	Conthey (Agroscope)	STANDORT	Conthey (Agroscope)
VARIETE / SORTE	'Licora'		
IRRIGATION	Aspersion (env. 30mm/semaine)	BEWÄSSERUNG	Beregnung (ca. 30mm/Woche)
FUMURE	normes Agridea	DÜNGUNG	Agridea Norm
Données culturales pour l'essai 2014		**Daten zum Versuch 2014**	
REPETITIONS	4 de 9 m^2 Plate-bande de 4 lignes: (4 x 25cm –passage 75cm) x 6m	WIEDERHOLUNGEN	4 à 9 m^2 Saatbeet mit 4 Reihen (4 x 25cm – Reihenabstand 75cm) x 6m
PARAMETRES	Germination Poids en feuilles Rendements en racines Nombre de racines/m^2 Longueur et diamètre des racines Poids du feuillage Rapport poids frais/poids sec Perte en eau au séchage Rapport feuilles/racines Teneur en huile essentielle Teneur en matière sèche soluble (Brix %)	PARAMETER	Keimung Gewicht der Blätter Wurzelertrag Anzahl Wurzeln/ m^2 Länge und Durchmesser der Wurzeln Gewicht der Blätter Verhältnis Frischgewicht/Trockengewicht Wasserverlust beim Trocknen Verhältnis Blätter/Wurzeln Gehalt an ätherischen Ölen Gehalt an löslicher Trockensubstanz (Brix %)
DENSITE	Semis : 6g, 12g, 18g, 24g, 30g/are Env. 120-600 semences viables/m^2	DICHTE	Saat : 6g, 12g, 18g, 24g, 30g/Are ungef. 120-600 lebensfähige Samen/m^2

Date/Datum	**Travaux**	**Arbeiten**
Mars 2014	Labour, fumure	Pflügen, Düngung
Avril 2014	Hersage	Eggen
Mai 2014	Semis : 12 mai	Aussaat: 12. Mai
Septembre-octobre 2014	8 récoltes hebdomadaires du 2 septembre au 21 octobre	8 wöchentliche Ernten vom 2. September bis 21. Oktober

Résultats / Discussions

Poids des feuilles

Les récoltes ont été effectuées chaque semaine entre le 2 septembre et le 21 octobre. Tendanciellement, les rendements en feuilles fraîches et sèches par mètre carré diminuent au fil de l'avancement de la saison. Cette diminution est particulièrement observable lors de la dernière récolte (tableau 1 et 2). Les valeurs les plus basses ont été mesurées pour la densité de semis de 6 g alors que les plus élevées ont été mesurées à celle de 12 g. Au-delà de cette densité de semis, la surface foliaire semble atteindre son optimum et n'augmente plus .

Resultate / Erläuterungen

Blättergewicht

Die Ernten wurden zwischen 2. September und 21. Oktober wöchentlich durchgeführt. Mit fortschreitender Erntesaison geht der Ertrag an frischen und getrockneten Blättern pro Quadratmeter tendenziell zurück. Dieser Rückgang ist insbesondere bei der letzten Ernte (Tabelle 1 und 2) zu beobachten. Die Saatdichte von 6 g ergab die niedrigsten Erträge, während die höchsten Erträge bei einer Saatdichte von 12 g gemessen wurden. Bei dieser Saatdichte scheint die Blattfläche ihr Optimum erreicht zu haben und nimmt bei höherer Saatdichte nicht mehr zu.

Tableau 1. Rendement en feuilles fraîches (g/m^2). Moyenne des répétitions.
Tabelle 1. Ertrag an frischen Blättern (g/m^2). Mittelwert der Wiederholungen.

Densité Dichte	1 (2.09.14)	2 (9.09.14)	3 (16.09.14)	4 (23.09.14)	5 (30.09.14)	6 (7.10.14)	7 (14.10.14)	8 (21.10.14)
6g	2746.2	3361.3	3448.0	3627.1	2583.1	3614.7	3222.7	1964.0
12g	4313.3	4018.7	4273.8	4930.2	3774.2	3038.2	4412.4	2303.6
18g	3588.4	5067.1	3933.8	4297.8	3824.4	3261.8	4181.8	2066.2
24g	3473.8	4172.0	4300.0	4375.1	3781.8	2674.2	3712.4	2000.0
30g	3695.1	4432.4	3072.4	4251.1	3263.1	3272.0	4001.3	2016.9

Tableau 2. Rendement en feuilles fraîches (g/m^2). Moyenne des répétitions.
Tabelle 2. Ertrag an frischen Blättern (g/m^2). Mittelwert der Wiederholungen.

Densité Dichte	1 (2.09.14)	2 (9.09.14)	3 (16.09.14)	4 (23.09.14)	5 (30.09.14)	6 (7.10.14)	7 (14.10.14)	8 (21.10.14)
6g	2746.2	3361.3	3448.0	3627.1	2583.1	3614.7	3222.7	1964.0
12g	4313.3	4018.7	4273.8	4930.2	3774.2	3038.2	4412.4	2303.6
18g	3588.4	5067.1	3933.8	4297.8	3824.4	3261.8	4181.8	2066.2
24g	3473.8	4172.0	4300.0	4375.1	3781.8	2674.2	3712.4	2000.0
30g	3695.1	4432.4	3072.4	4251.1	3263.1	3272.0	4001.3	2016.9

Rendement en racines

Concernant la production en racines, plusieurs tendances sont observées: en moyenne, les rendements augmentent au fil des semaines, mais se stabilisent à la mi-septembre, à partir du quatrième prélèvement (tabl. 3-4 ; fig 1.). La légère diminution de rendement mesurée lors de la dernière récolte est attribuée au plus petit nombre de racines récoltées à cette date. Même si les différences de rendements en racines n'ont été significatives que lors de la 4^e et de la 8^e récolte (tabl. 4), des tendances assez claires se profilent. A l'instar du feuillage, la densité 6 g/100 m^2 a produit la plus faible biomasse, tandis que la plus haute production en racines a été obtenue à une densité de 30 g/100m^2. Cette densité permet d'atteindre dès la mi-septembre un rendement moyen supérieur à 200g/m^2. Le pourcentage de racines sèches (MS) après séchage est demeuré stable en cours de saison (20.7-25.7 % de MS) (Table 5). Aucune corrélation entre le volume du feuillage et les racines n'a pu être établie. Cependant le ratio de racines par rapport à la biomasse totale (racine + feuillage) augmente au fil de la saison de 18.5 % to 33.0 %.

Wurzelertrag

Bei der Wurzelernte sind mehrere Trends zu beobachten: die durchschnittlichen Erträge nehmen im Laufe der Wochen zu, stabilisieren sich aber Mitte September nach der vierten Probenahme (Tabelle 3-4; Abb 1.). Der leichte Ertragsrückgang bei der letzten Ernte ist auf die geringere Anzahl geernteter Wurzeln zurückzuführen. Obwohl die Unterschiede im Wurzelertrag nur in der 4. und 8. Ernte (Tab. 4) signifikant sind, zeichnen sich ziemlich klare Tendenzen ab. Wie beim Laub ergab die Saatdichte von 6 g/100 m^2 die geringste Biomasse, während der grösste Wurzelmasse bei einer Saatdichte von 30 g/100m^2 erreicht wurde. Mit dieser Saatdichte kann ab Mitte September ein durchschnittlicher Ertrag von mehr als 200 g/m^2 erreicht werden. Der Anteil an trockenen Wurzeln (TS) nach der Trocknung blieb im Laufe der Saison stabil (20.7 bis 25.7% TS) (Tabelle 5). Es konnte keine Korrelation zwischen dem Blättervolumen und den Wurzeln hergestellt werden. Das Verhältnis der Wurzeln zur gesamten Biomasse (Wurzeln + Blätter) steigt jedoch im Laufe der Saison von 18.5% auf 33.0%.

Tableau 3. Rendements en racines fraîches (g/m^2). Moyenne des répétitions
Tabelle 3. Ertrag an frischen Wurzeln (g/m^2). Mittelwert der Wiederholungen

Densité Dichte	1 (2.09.14)	2 (9.09.14)	3 (16.09.14)	4 (23.09.14)	5 (30.09.14)	6 (7.10.14)	7 (14.10.14)	8 (21.10.14)
6g	417.8	499.6	509.8	561.3	597.3	753.8	683.6	584.0
12g	764.0	671.1	738.2	861.3	822.7	787.6	856.4	825.3
18g	636.4	936.9	732.4	808.4	842.7	839.1	899.1	734.2
24g	602.2	798.2	898.7	929.3	1016.4	673.3	965.8	784.0
30g	694.2	946.7	800.4	1020.0	902.2	1052.4	1053.3	930.7

Tableau 4. Rendements en racines sèches (g/m^2). Moyenne des répétitions
Tabelle 4. Ertrag an trockenen Wurzeln (g/m^2). Mittelwert der Wiederholungen

Densité Dichte	1 (2.09.14)	2 (9.09.14)	3 (16.09.14)	4 (23.09.14)	5 (30.09.14)	6 (7.10.14)	7 (14.10.14)	8 (21.10.14)
6g	91.1	113.8	109.8	120.4^{b}	137.8	171.6	150.2	123.6^{b}
12g	159.1	149.3	169.3	192.4^{ab}	181.8	165.8	182.7	179.1^{ab}
18g	141.8	228.4	171.6	208.9^{ab}	201.3	188.0	199.6	164.0^{ab}
24g	129.8	185.3	212.0	219.1^{ab}	237.3	150.2	205.3	174.2^{ab}
30g	153.3	218.7	188.4	240.9^{a}	203.6	236.9	236.4	210.7^{b}

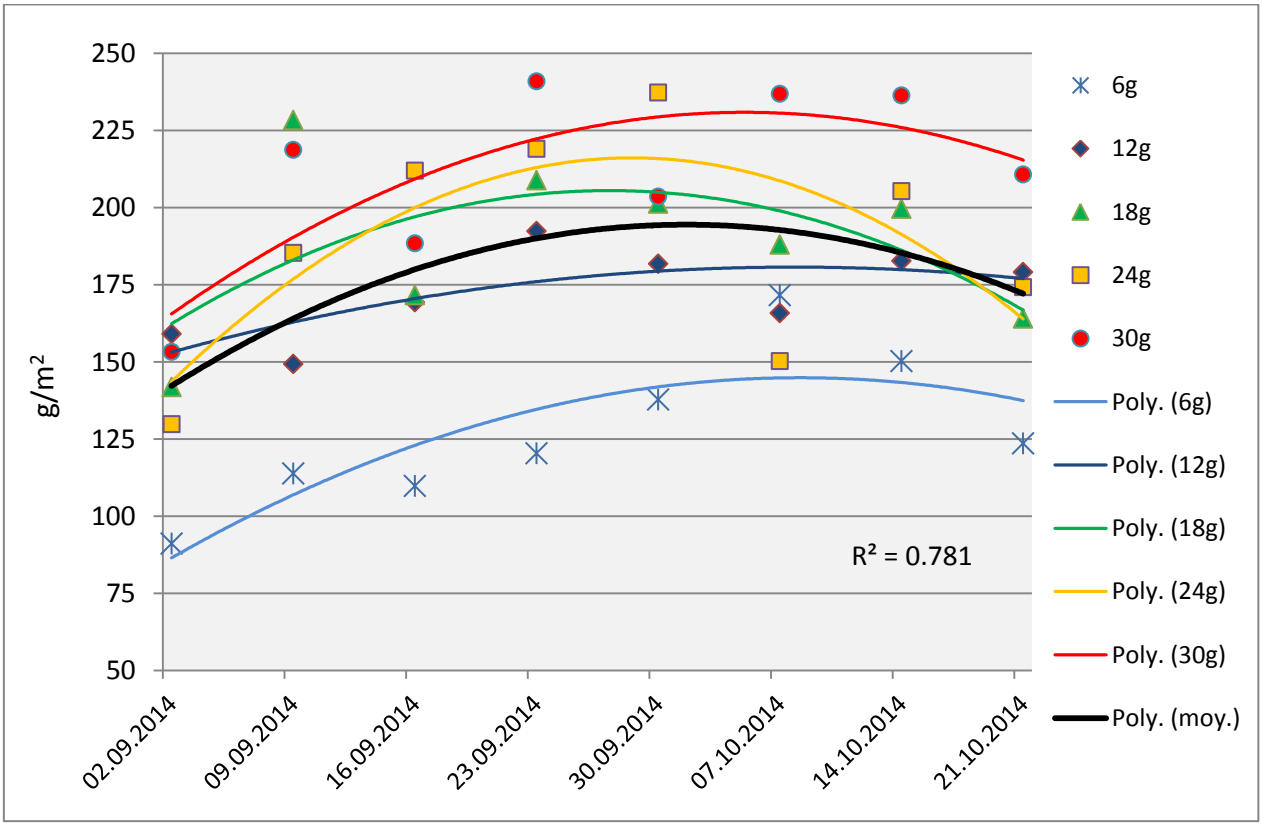


Figure 1. Rendements en racines sèches [g/m^2], avec les courbes de tendance polynomiale. (Moyenne des répétitions).
Abbildung 1. Ertrag an trockenen Wurzeln [g/m^2], mit polynomialer Trendkurve (2). (Mittelwert der Wiederholungen).

Tableau 5. Pourcentage de racines sèches après le séchage (%).Moyenne des répétitions
Tabelle 5. Prozentanteil an trockenen Wurzeln nach der Trocknung (%).Mittelwert der Wiederholungen

Densité Dichte	1 (2.09.14)	2 (9.09.14)	3 (16.09.14)	4 (23.09.14)	5 (30.09.14)	6 (7.10.14)	7 (14.10.14)	8 (21.10.14)
6g	21.7	22.4	21.4	21.5	22.4	22.4	22.0	21.2
12g	21.0	22.2	22.7	22.1	22.1	21.0	20.7	21.5
18g	22.3	24.2	23.5	25.7	23.8	22.4	22.1	22.4
24g	21.6	23.2	23.5	23.6	23.3	22.3	21.3	22.2
30g	22.1	23.0	23.1	23.6	22.6	22.4	22.5	22.8

Les caractéristiques morphologiques des racines ont également montré des tendances claires. Le nombre des racines récoltées augmente logiquement en fonction de la densité de semis (tabl. 6) en dépit de la germination quelque peu irrégulière. La longueur moyenne des racines n'a été influencée ni par la date de récolte, ni par la densité de semis (tabl. 7), en revanche le diamètre des racines a été clairement corrélé avec ces deux paramètres (tabl. 8). La relation entre le poids moyen des racines et la densité des semis est évidente. Les plus grosses racines ont été récoltées à la plus faible densité de semis (tableau 9). Le rendement n'a pas beaucoup été affectée par la densité de semis. Le poids moyens des racines a pratiquement doublé dans toutes les densités de semis durant les huit semaines d'expérimentation.

Auch die morphologischen Eigenschaften der Wurzeln zeigten klare Tendenzen. Die Anzahl der geernteten Wurzeln steigt entsprechend der Saatdichte (Tab. 6), trotz der etwas unregelmässigen Keimung. Die durchschnittliche Wurzellänge wurde weder vom Zeitpunkt der Ernte, noch von der Saatdichte beeinflusst (Tab. 7). Der Wurzeldurchmesser hingegen korreliert deutlich mit beiden Parametern (Tab. 8). Das Verhältnis zwischen Durchschnittsgewicht der Wurzeln und Saatdichte ist offensichtlich. Die grössten Wurzeln wurden bei der niedrigsten Saatdichte (Tabelle 9) geerntet. Der Ertrag wurde kaum durch die Saatdichte beeinflusst. Das durchschnittliche Wurzelgewicht hat sich bei fast allen Saatdichten während des achtwöchigen Versuchs verdoppelt.

Tableau 6. Nombre de racines récoltées par m^2. Moyenne des répétitions.
Tabelle 6. Anzahl der pro m^2 geernteten Wurzeln. Mittelwert der Wiederholungen.

Densité Dichte	1 (2.09.14)	2 (9.09.14)	3 (16.09.14)	4 (23.09.14)	5 (30.09.14)	6 (7.10.14)	7 (14.10.14)	8 (21.10.14)
6g	32.3	34.3	29.3	31.3	31.7	38.3	28.0	21.3
12g	92.7	84.3	56.0	70.7	65.3	54.3	63.3	61.3
18g	97.3	113.7	68.3	84.0	77.0	84.0	82.0	62.3
24g	135.7	140.7	123.0	145.0	152.3	94.3	136.3	89.3
30g	180.0	186.3	123.7	137.7	154.7	160.7	154.3	113.0

Tableau 7. Longueur moyenne des racines (cm). Moyenne des répétitions.
Tabelle 7. Mittlere Wurzellänge (cm). Mittelwert der Wiederholungen.

Densité Dichte	1 (2.09.14)	2 (9.09.14)	3 (16.09.14)	4 (23.09.14)	5 (30.09.14)	6 (7.10.14)	7 (14.10.14)	8 (21.10.14)
6g	23.40	22.73	21.60	25.50	22.93	24.17	23.10	23.33
12g	23.73	24.33	25.63	25.37	23.20	24.70	23.03	23.57
18g	23.07	23.70	24.07	25.63	23.57	24.27	23.23	23.77
24g	20.63	23.30	23.70	24.90	23.37	23.47	23.83	24.27
30g	22.37	23.83	24.93	26.30	23.87	23.27	24.17	23.87

Tableau 8. Diamètre moyen des racines (cm). Moyenne des répétitions.
Tabelle 8. Mittlerer Durchmesser der Wurzeln (cm). Mittelwert der Wiederholungen.

Densité Dichte	1 (2.09.14)	2 (9.09.14)	3 (16.09.14)	4 (23.09.14)	5 (30.09.14)	6 (7.10.14)	7 (14.10.14)	8 (21.10.14)
6g	1.19	1.07	1.19	1.27	1.13	1.10	1.29	1.25
12g	0.91	0.97	0.93	1.05	0.93	0.98	0.86	1.05
18g	0.76	0.94	0.81	0.91	0.86	0.85	0.86	0.92
24g	0.63	0.77	0.68	0.79	0.81	0.74	0.75	0.83
30g	0.63	0.76	0.67	0.70	0.72	0.63	0.69	0.79

Tableau 9. Poids moyens des racines (g). Moyenne des répétitions.
Tabelle 9. Mittleres Wurzelgewicht (g). Mittelwert der Wiederholungen.

Densité Dichte	1 (2.09.14)	2 (9.09.14)	3 (16.09.14)	4 (23.09.14)	5 (30.09.14)	6 (7.10.14)	7 (14.10.14)	8 (21.10.14)
6g	11.0	10.5	13.4	13.6	14.6	15.2	18.4	21.0
12g	7.0	7.2	10.9	9.7	9.6	11.2	10.1	10.1
18g	4.9	6.4	8.2	7.4	8.2	7.5	8.3	9.1
24g	3.4	4.3	5.5	5.1	5.3	5.4	5.3	6.8
30g	3.0	3.9	4.9	5.6	4.4	5.0	5.2	6.3

Les teneurs en huile essentielle sont toujours demeurées en dessous des exigences de la Pharmacopée Helv. (0.2%).
L'effet de la densité de semis ou de la date de récolte sur la formation de l'huile essentielle n'est pas claire. En moyenne, le pourcentage d'huile augmente très légèrement, et de manière non significative, jusqu'en octobre, puis tend à diminuer au fil de la saison. Les analyses effectuées en décembre confirme cette tendance (tabl. 11 et 12). Les faibles densités des semis (6g/100 m^2 et 12g/100 m^2) semblent être légèrement favorable à la teneur en huile essentielle.
Les différentes parties de la racines (a. tissus vascularisés centraux ; b. périderme et cortex ; c. racines latérales et fines (Ø < 0.3 cm)) ont été analysées séparément. L'huile essentielle se localise presqu'exclusivement dans le cortex et le périderme (tabl. 11). Cela explique la faible différence entre les variantes. En effet, dans les faibles densités, la teneur en huile essentielle est pénalisée par les grosses racines qui contiennent une proportion plus élevée de tissus centraux vascularisés (env. 18-20% de la biomasse), alors que les hautes densités de semis favorisent davantage de racines fines pauvre en huile essentielle (tabl.11).
Des mesures effectuées sur les parties aériennes ont montré que les feuilles contiennent un quantité minime d'huile essentielle (0.046-0.070 ml/100 g).

Der Gehalt an ätherischem Öl blieb immer unter den Anforderungen des Helv. Arzneibuches (0,2%).
Der Einfluss der Saatdichte oder des Erntezeitpunkts auf die Bildung von ätherischem Öl ist nicht bekannt. Im Durchschnitt steigt der prozentuale Anteil des Öls bis Oktober sehr langsam und nicht signifikant und nimmt dann bis zum Ende der Saison tendenziell ab. Die im Dezember durchgeführten Analysen bestätigen diesen Verlauf (Tab. 11 und 12). Eine niedrige Saatdichte (6 g/100 m^2 und 12 g/100 m^2) scheint den Gehalt an ätherischen Ölen zu begünstigen.
Die verschiedenen Teile der Wurzeln (a. vaskularisiertes zentrales Gewebe; b. Periderm und Cortex, c. Seiten- und Feinwurzeln (Ø <0,3 cm)) wurden separat analysiert. Das ätherische Öl befindet sich fast ausschliesslich im Cortex und Periderm (Tab. 11). Dies erklärt die geringe Differenz zwischen den Varianten. Bei niedrigen Saatdichten beeinträchtigen die grossen Wurzeln den Gehalt an ätherischem Öl, denn sie enthalten einen höheren Anteil an vaskularisiertem zentralem Gewebe (ca. 18 bis 20% der Biomasse), bei hohen Aussaatdichten hingegen entwickeln sich die Feinwurzeln, die arm an ätherischem Öl sind (Tab. 11), stärker.
Messungen an den oberirdischen Pflanzenteilen zeigten, dass die Blätter wenig ätherisches Öl (0.046-0.070 ml/100 g) enthalten.

Tableau 10. Teneur en huile essentielle dans les racines sèches (ml/100 g MS). Moyennes des répétitions.
Tabelle 10. Gehalt an ätherischen Ölen in den trockenen Wurzeln (ml/100 g MS). Mittelwert der Wiederholungen.

Densité Dichte	1 (2.09.14)	2 (9.09.14)	3 (16.09.14)	4 (23.09.14)	5 (30.09.14)	6 (7.10.14)	7 (14.10.14)	8 (21.10.14)
6g	0.131	0.162	0.146	0.147	0.162	0.178	0.137	0.128
12g	0.136	0.139	0.137	0.155	0.145	0.156	0.147	0.141
18g	0.152	0.145	0.116	0.137	0.137	0.159	0.161	0.103
24g	0.130	0.137	0.132	0.137	0.137	0.151	0.158	0.088
30g	0.138	0.105	0.129	0.139	0.150	0.145	0.142	0.128

La matière sèche soluble exprimée par la teneur Brix (%) est significativement plus élevée (9.7 %) dans les racines dont le diamètre est compris entre 1.5-2 cm par rapport aux deux autres classes de diamètre de racines (1-1.5 ou < 1.0). Des différences significatives ont aussi été mesurées dans les différentes parties de la racines (a. tissus vascularisés centraux ; b. périderme et cortex ; c. racines latérales et fines (Ø < 0.25 cm). Les tissus vascularisés du centre de la racine contiennent plus de matière sèche soluble (tabl. 12). Cela signifie que si la teneur Brix est retenue comme critère pertinent de qualité, il faut privilégier la formation de grosses racines par la densité de semis.

Die lösliche Trockensubstanz, ausgedrückt als Brix-Gehalt (%), ist bei den Wurzeln mit einem Durchmesser zwischen 1.5-2 cm im Vergleich zu den beiden anderen Kategorien von Wurzeldurchmessern (1-1.5 oder <1.0) deutlich höher (9,7%). Signifikante Unterschiede wurden auch in verschiedenen Teilen der Wurzeln (a. zentrales vaskularisiertes Gewebe; b. Periderm und Cortex, c. Seiten- und Feinwurzeln (Ø <0,25 cm)) gemessen. Das vaskularisierte Gewebe in der Mitte der Wurzel enthält mehr lösliche Trockensubstanz (Tab. 12). Wenn der Brix-Gehalt als relevantes Qualitätskriterium gilt, so muss die Bildung von grossen Wurzeln über die Saatdichte gefördert werden.

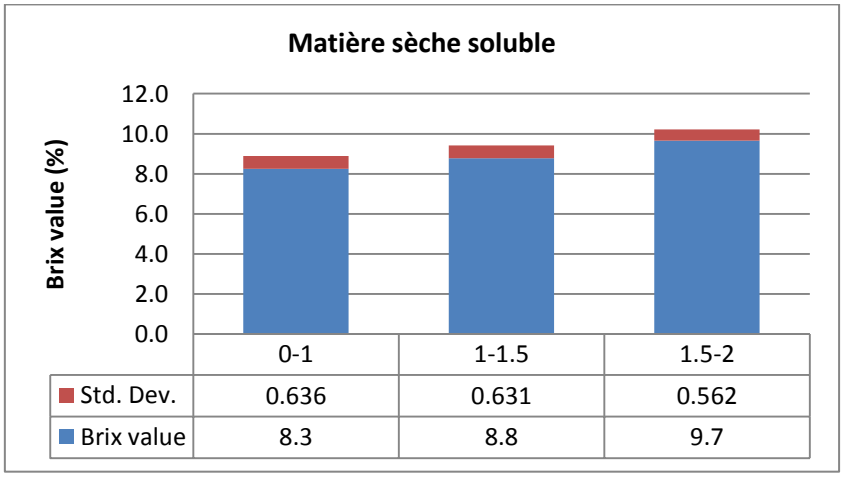


Figure 2. Teneur en matière sèche soluble [Brix %], en fonction de 3 classes de diamètre de racines. Moyennes des répétitions.
Abb. 2. Gehalt an löslicher Trockensubstanz [Brix %], in Abhängigkeit der 3 Kategorien von Wurzeldurchmesser. Mittelwert der Wiederholungen.

Tableau 11. Teneur en huile essentielle [%] et en matière sèche soluble [Brix %], en fonction de 3 classes de diamètre de racines. Moyennes des répétitions.
Tabelle 11. Gehalt an ätherischen Ölen [%] und an löslicher Trockensubstanz [Brix %], in Abhängigkeit der 3 Kategorien von Wurzeldurchmessern. Mittelwert der Wiederholungen.

Racines Wurzeln	Huile essentielle Äth. Öle [%]	Racines Wurzeln	Brix [%]
Ø < 0.5 cm	0.119^{a}	Ø < 1 cm	8.3^{b}
Ø 0.5 - 1.0 cm	0.110^{ab}	Ø 1.0 - 1.5 cm	8.8^{ab}
Ø > 1.5 cm	0.096^{b}	Ø 1.5-2.0 cm	9.7^{a}

Tableau 12. Teneur en huile essentielle [%] et en matière sèche soluble [Brix %], dans différentes parties de la racine (a. tissus vascularisés centraux ; b. périderme et cortex ; c. racines latérales et fines (Ø < 0.25 cm) Moyennes des répétitions.
Tabelle 12. Gehalt an ätherischen Ölen [%] und an löslicher Trockensubstanz [Brix %], in verschiedenen Wurzelteilen (a. zentrales vaskularisiertes Gewebe; b. Periderm und Cortex; c. Seiten- und Feinwurzeln (Ø < 0.25 cm)) Mittelwerte der Wiederholungen.

Racines Wurzeln	Huile essentielle Äth. Öle [%]	Brix [%] analyse 1	Brix [%] analyse 2
Centre de la racine, (tissus vascularisés) Wurzelzentrum, (vaskularisiertes Gewebe)	0.114^{a}	9.4^{a}	10.9^{a}
Epiderme et cortex Periderm und Cortex	0.017^{b}	8.7^{b}	10.1^{b}
Racines fines (Ø < 0.25 cm) Feinwurzeln (Ø < 0.25 cm)	0.010^{b}	7.2^{c}	8.9^{c}

Conclusions / Schlussfolgerungen

- Pour la Suisse, l'époque optimale de récolte des racines de *Pimpinella* est la première moitié du mois d'octobre. Le rendement n'augmente plus à partir du début octobre.
- Sauf à la densité de semis la plus faible (6g /m^2), déconseillée, le rendement en racines sèches n'a pas été influencé significativement par les autres procédés.
- La perte en eau au cours du séchage reste relativement stable durant la saison (en moyenne 22.3% de matière sèche). Aucun effet de la densité de plantation, ni de la date de récolte n'a été observé sur ce paramètre.
- La teneur en huile essentielle tend à diminuer légèrement à partir la mi-octobre. Dans cette essai, elle est demeurée toujours en dessous de la norme Ph. Helv. (0.2% de la matière sèche).
- La matière sèche soluble (Brix%) est corrélée au diamètre de racines. Les plus grosses en contiennent plus, comme le centre de la racine (tissus vascularisés)
- En fonction de ces résultats, une densité de semis de 18g/m^2 est le meilleur compromis entre le potentiel de récolte, la qualité de la drogue sèche et le temps de travail.

Für die Schweiz liegt der optimale Erntezeitpunkt von Bibernelle-Wurzeln in der ersten Oktoberhälfte. Ab Anfang Oktober steigt der Ertrag nicht mehr an.

- Ausser bei der nicht empfehlenswerten, niedrigsten Saatdichte (6g / m^2), konnte kein signifikanter Einfluss der Saatdichte auf den Ertrag an trockenen Wurzeln beobachtet werden.
- Der Wasserverlust (von durchschnittlich 22.3% Trockenmasse) beim Trocknen bleibt während der Saison relativ stabil. Es konnte kein Einfluss der Pflanzdichte und des Erntedatums auf diesen Parameter beobachtet werden.
- Der Gehalt an ätherischem Öl geht ab Mitte Oktober tendenziell leicht zurück. Im beschriebenen Versuch Test lag er stets unter der Ph. Helv. Norm (0.2% der Trockenmasse).
- Die lösliche Trockensubstanz (% Brix) korreliert mit dem Wurzeldurchmesser. Grössere Wurzeln und das Wurzelzentrum (vaskularisiertes Gewebe) enthalten mehr lösliche Trockensubstanz.
- Basierend auf diesen Ergebnissen ist eine Saatdichte von 18 g/m^2 der beste Kompromiss zwischen Ertragspotential, der Qualität der trockenen Ernteprodukt und der Arbeitszeit.

1^e date de récolte le 2 septembre 2014.
1. Erntedatum am 2. September

Annexes

Posters

Publications

Phytochemical variability of common tansy, an interesting species for veterinary medicine

Xavier Simonnet[1], Melanie Quennoz[1], Christoph Carlen[1,2]
[1] Mediplant, CH-1964 Conthey, Switzerland, www.mediplant.ch
[2] Agroscope Changins-Wädenswil Research Station ACW, CH-1964 Conthey, Switzerland, www.agroscope.ch

Introduction

Common tansy (*Tanacetum vulgare* L.) is a perennial, herbaceous flowering plant of the aster family, native to temperate Europe and Asia. Common tansy is mentioned in the literature as a plant with de-worming properties for livestock (Waller *et al*., 2011). Its use in commercial products is also reported (Valchev *et al*., 2009). However, only few studies have been devoted to the domestication and cultivation of this perennial species.
The aim of this project was to analyse the phytochemical variability present within common tansy.

Material and method

Plantation: 2.8 plants /m^2 planted in July 2004, 30 plants per accession, Conthey, CH.
Treatments: 27 accessions, (10 plants per accession analysed), harvest 2005.
Analysis: hydrodistillation of leaves and flowers, composition of esssential oil by SPME/GC.

Results

Significant differences between the accessions were recorded, such as the essential oil content of the leaves and flowers varying from 0.30 to 1.39 % (v/w).
High variations of the contents of several molecules in the essential oil such as α-thujone (0-86%), β-thujone (0-96%), chrysanthenone (0-82 %), lyratol (0-55%) and umbellulone (0-36%) were also observed (Table 1). This high variability concerning the composition of the essential oil is a valuable basis for a breeding program.
Important knowledge was also gained for breeding and cultivation of common tansy (floral biology, harvesting stage, pests, yields, location, cultivar rich in β-thujone).

Table 1. Mean composition of the essential oil of 27 accessions of common tansy and in brackets variability within an accession (min.-max.). Harvest stage: very beginning of flowering. (– indicates no detection of the molecule).

Acces-sions	Origin of the seeds	α-thujone (%)	β-thujone (%)	chrysan-thenon (%)	lyratol (%)	umbel-lulone (%)
TV-1	CH	-	**36** (0-70)	-	-	-
TV-2	CH	-	**49** (11-87)	-	-	-
TV-4	D	-	**88** (63-97)	-	-	-
TV-5	F	-	**13** (0-40)	**3** (0-16)	**5** (0-55)	**4** (0-12)
TV-6	DK	-	**20** (0-83)	-	-	**8** (0-32)
TV-7	HU	-	**11** (0-29)	-	-	-
TV-8	N	-	**19** (0-50)	-	-	**25** (12-36)
TV-9	B	-	**49** (11-92)	**25** (0-70)	-	**1** (0-6)
TV-10	D	-	**48** (0-80)	-	-	-
TV-11	CND	**2** (0-25)	**2** (0-23)	-	-	**7** (0-29)
TV-12	CND	-	**34** (0-93)	-	-	-
TV-13	D	-	**36** (0-67)	**5** (0-29)	-	-
TV-14	D	-	**20** (0-59)	-	-	-
TV-15	D	**19** (0-61)	**31** (0-71)	**9** (0-64)	-	-
TV-17	D	-	**11** (0-30)	**4** (0-26)	-	-
TV-19	RO	-	**5** (0-21)	**8** (0-82)	**5** (0-51)	-
TV-20	CH	-	**82** (27-94)	-	-	-
TV-23	D	-	**14** (0-93)	-	**22** (0-49)	-
TV-24	F	-	**45** (0-95)	-	-	-
TV-25	F	-	**24** (0-85)	-	**21** (0-42)	**4** (0-12)
TV-26	CH	-	**84** (11-96)	-	-	-
TV-28	CH	-	**31** (0-85)	-	-	-
TV-34	CND	-	**94** (89-96)	-	-	-
TV-35	CH	-	**92** (79-96)	-	-	-
TV-36	CH	**16** (0-55)	**1** (0-5)	-	-	-
TV-37	CH	**38** (0-86)	**17** (0-95)	-	-	-
TV-38	CH	-	**6** (0-18)	-	-	-

Conclusions

- A high variability between and within accessions, especially concerning the composition of the essential oil was recorded.
- This high variability is a valuable basis for a breeding program to develop a cultivar well adapted for veterinary medicine.

INTERREG IIIa N°4/FU/7.1/1

Melolontha sp. in Thymian

Catherine Baroffio, Claude-Alain Carron, Bertrand Gentizon[1]
Agroscope, CH-1964 Conthey. www.agroscope.ch. [1.] Andermatt Biocontrol. Grossdietwil. www.biocontrol.ch

Ziel

Eine Strategie zur Bekämpfung der Maikäfer, welche die Thymianparzelle in Le Prese befallen, entwickeln und definieren.

Material et Methoden

Varianten :

T	Standard	
G1	Galanem (Biocontrol)	1 Mio/m² / 2l Wasser /m² ⇨ 50 Mio verdünnt in 100 l Wasser für die 4 Wdhlg.
G2	Galanem (Biocontrol),	2 Mio/m² / 2l Wasser /m² ⇨ 100 Mio verdünnt in 100 l Wasser für die 4 Wdhlg.
N	Naturalis-L (Biocontrol)	0.5 % / 2l Wasser /m² ⇨ 0.5 l verdünnt in 100 l Wasser für die 4 Wdhlg.
R	Ravastop (Seydoux)	0.003l/m² / 2l Wasser /m² ⇨ 0.15 l verdünnt in 100 l Wasser für die 4 Wdhlg.
E	Ecofort Repulse (Seydoux)	0.002l/m² / 2l Wasser /m² ⇨ 0.1 l verdünnt in 100 l Wasser für die 4 Wdhlg.

Versuchseinrichtung:

- Teilflächen von 2.5m (= 5 Reihen) x 5m (12.5 m²)
- 4 Wiederholungen/Verfahren

T	R	E	G 2
G1	G2	N	T
N	E	G1	R
R	T	G2	E
E	G1	R	N
G 2	N	T	G1
Rép 1	Rép 2	Rép 3	Rép 4

Zwischenresultate

1.5 15-25.5	Besichtigung und Versuchseinrichtung Vorbereitung des Bodens
30.5 – 1.6	Anpflanzung
6.6	Anwendung der Produkte
14.7	Erste Auszählungen: • Die Pflanzen auf 5 Versuchsmetern ausgraben, den Zustand der Wurzeln auswerten. • Vorhandene Larven • Wiedereinpflanzung

Zusammenfassung
Nach einem Monat konnte kein signifikanter Unterschied zwischen den Varianten in Bezug auf den Zustand der Wurzeln festgestellt werden.
Erst nach der definitiven Auszählung bei der Ernte können die verschiedenen Varianten vollständig bewertet werden.

'Mattmark', a high yielding *Rhodiola rosea* cultivar launched in Switzerland

José F. Vouillamoz, Claude-Alain Carron, Catherine A. Baroffio, Christoph Carlen
Agroscope, CH-1964 Conthey, Switzerland; www.agroscope.ch

Introduction

Rhodiola rosea L., also called Golden Root or Roseroot, is an adaptogenic medicinal plant from alpine and arctic regions and has robust traditional and pharmacological evidence of use in fatigue, and emerging evidence supporting cognition and mood.

Breeding of a R. rosea cultivar is an important step to preserve natural populations of this species, to ensure supply of standardized raw material and to prevent frauds.

In this study, the phytochemical variability of salidroside and total rosavins in five natural populations in the Swiss Alps are tested in order to select the most interesting genotypes for a polycross to get a new cultivar.

Material and methods

Non-destructive rhizome cuttings were sampled in 2006 from 93 plants in five sites in the Swiss Alps (Fig. 1) and screened for their salidroside and rosavins contents by HPLC-DAD analysis.

Results

An important variability was observed among and within the populations, and no significant difference was observed between male and female plants (Fig. 2).

With an average content of 1,49% (± 1,15) for salidroside and 1,57% (± 0,74) for rosavins, the population in Mattmark (Saas Fee, Valais) turned out to have the most productive and vigorous genotypes. 4 male and 4 female genotypes of this population with high productivity and high contents of salidroside and rosavins were choosen (Tab.1) . A random polycross was performed with these 8 genotypes to produce seeds of 'Mattmark', the first cultivar of alpine *R. rosea* (Fig. 3).

Fig 3. Seed production of the new cultivar 'Mattmark' in Bruson (CH)

Fig 1. Swiss populations sampled: 1 Mattmark, 2 Binntal, 3 Nomnom, 4 Piano Canali, 5 Unteralp

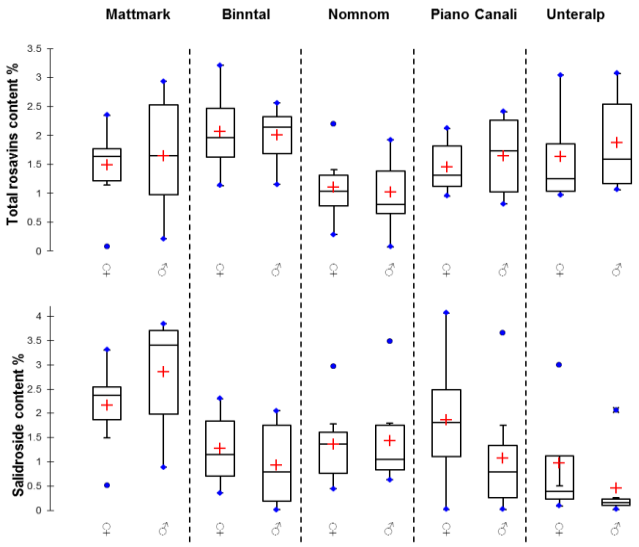


Fig 2. Variation of the contents in total rosavins and in salidroside [% of dry weight] in the rhizomes and roots of female (♀) and male (♂) *Rhodiola rosea* from five populations in the Swiss Alps.

Tab. 1. Selected genotypes (4 male and 4 female) from the population Mattmark for a polycross

		Salidroside %	Rosavins %	Nb
♀	**M9**	2.04	2.07	3
	M11	2.28	2.35	4
	M13	2.47	1.63	3
	M14	3.31	1.64	2
♂	**M2**	3.31	2.52	1
	M6	3.82	2.93	1
	M8	3.35	2.03	1
	M15	3.31	2.55	1

Conclusions

- A random polycross was performed with 8 selected genotypes to produce seeds of 'Mattmark', the first cultivar of alpine *R. rosea*
- This new cultivar 'Mattmark' showed high rhizome production and high salidroside and rosavins contents in the roots.
- The seeds of this cultivar are available through the company mediSeeds (www.mediseeds.ch).

Research Article

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Prediction of essential oil content of oregano by hand-held and Fourier transform NIR spectroscopy

Cédric Camps,[a*] Marianne Gérard,[a,b] Mélanie Quennoz,[b] Cécile Brabant,[c] Carine Oberson[c] and Xavier Simonnet[b]

Abstract

BACKGROUND: In the framework of a breeding programme, the analysis of hundreds of oregano samples to determine their essential oil content (EOC) is time-consuming and expensive in terms of labour. Therefore developing a new method that is rapid, accurate and less expensive to use would be an asset to breeders. The aim of the present study was to develop a method based on near-inrared (NIR) spectroscopy to determine the EOC of oregano dried powder. Two spectroscopic approaches were compared, the first using a hand-held NIR device and the second a Fourier transform (FT) NIR spectrometer.

RESULTS: Hand-held NIR (1000–1800 nm) measurements and partial least squares regression allowed the determination of EOC with R^2 and SEP values of 0.58 and 0.81 mL per 100 g dry matter (DM) respectively. Measurements with FT-NIR (1000–2500 nm) allowed the determination of EOC with R^2 and SEP values of 0.91 and 0.68 mL per 100 g DM respectively. RPD, RER and RPIQ values for the model implemented with FT-NIR data were satisfactory for screening application, while those obtained with hand-held NIR data were below the level required to consider the model as enough accurate for screening application.

CONCLUSION: The FT-NIR approach allowed the development of an accurate model for EOC prediction. Although the hand-held NIR approach is promising, it needs additional development before it can be used in practice.

Keywords: hand-held NIR; FT-NIR; PLS; essential oil content (EOC); oregano

INTRODUCTION

Owing to its high demand, especially in the food industry, as dry grass or in the form of essential oil,[1] oregano has been the subject of numerous studies, including breeding programmes.[2,3] Although the chemical composition differs depending on the species and variety,[4–6] the trade name 'oregano' includes species that are rich in monoterpenoid phenols, mainly carvacrol and occasionally thymol.[5,6] It has been shown that the essential oil, which is rich in these molecules, has antimicrobial[7] and antioxidant[4,7] properties that can be used not only for the benefit of human health but also in the farming and food industries.[7]

Currently, qualitative and quantitative analyses of the components of oregano or its essential oil by conventional methods (i.e. hydrodistillation, gas chromatography, high-performance liquid chromatography) are time-consuming and expensive. In terms of speed of analysis, such methods are difficult to use when a series of hundreds of samples has to be analyzed, as in the case of a breeding programme.[8] In the last 20 years a predictive, rapid and low-cost method based on near-infrared spectroscopy (NIRS) has been developed for determining the quality of various agricultural and food products. Several studies have already been carried out successfully in the field of aromatic and medicinal plants.[9,10] Studies have been reported on cumin,[11,12] fennel,[11,13] coriander,[11] green tea leaves,[14] sage,[15] thyme[9] and rosemary.[16]

The aim of the present study was to develop a method to quantify the contents of oregano essential oil by NIRS. The method developed must be fully usable in the context of a breeding programme. Two technologies have been tested, a hand-held NIR device and a Fourier transform (FT) NIR spectrometer, both adapted to the needs of a breeding programme.

MATERIALS AND METHODS

Plant material

The oregano samples used in this study comprised species and varieties grown in the experimental fields of Agroscope Research Station (Conthey, Switzerland). Samples were gathered from two harvest years (2009 and 2010) and stored at room temperature in

* *Correspondence To: Cedric Camps, Agroscope Research Station, Research Department of Production and Plant Protection of Crops in Alpine Areas/Greenhouse Crops, Route des Vergers 18, CH-1964 Conthey, Switzerland. E-mail: cedric.camps@acw.admin.ch*

a *Agroscope Research Station, Research Department of Production and Plant Protection of Crops in Alpine Areas/Greenhouse Crops, Route des Vergers 18, CH-1964, Conthey, Switzerland*

b *Mediplant, Route des Vergers 18, CH-1964, Conthey, Switzerland*

c *Agroscope Research Station, Research Department of Arable Crop Plant Breeding and Genetic Resources, Route de Duillier 50, CP 1012, CH-1260, Nyon, Switzerland*

the dark. Several species were studied in order to have the widest range of essential oil content, namely *O. vulgare*, *O. minutiflorum*, *O. syriacum* (ssp. *syriacum* and ssp. *bevanii*), *O. vulgare* ssp. *hirtum* (variety of seed supplier Bolier), *O. vulgare* (var. Carva: *O. vulgare* ssp. *viridulum* × *O. vulgare* ssp. *hirtum*), *O. vulgare* ssp. *hirtum* (var. Origalia). A total of 101 samples were used in the present study.

Determination of essential oil content

Essential oils were obtained by hydrodistillation of samples of dried leaves according to the standard method.[17] All samples were distilled 1 week before analysis by NIRS, providing reference data necessary for the calibration step of the model. The dry matter (DM) content of samples was measured by drying at 105 °C for 12 h. The essential oil content (EOC) was expressed in mL per 100 g DM.

Hand-held NIR approach

Fractions of oregano dry samples were pulverized in a laboratory mill (10 000 rpm, 0.5 mm grid; Variable-speed Rotor Mill PULVERISETTE 14, Fritsch GmbH, Idar-Oberstein, Germany). The powder was carefully placed in closed vials and stored at room temperature in the dark. A first set of 74 samples (calibration set) and a second set of 27 samples (test set) were used for the calibration and validation steps respectively.

Spectra were acquired in reflectance mode using a MEMS-based PHAZIR (NIR PHAZIR 1018, Anatec, Eke, Belgium). Samples of oregano dry powder were placed in adapted vials closed with a plastic cap (vials for PHAZIR PCX-ACC-4 solids adapter, 15 mm i.d.). Spectral acquisition was carried out in direct contact analysis mode by placing the vials in the specific PHAZIR adapter situated at the end of the NIR pistol. Absorbance spectra (average of 30 scans) were recorded at a resolution of 8 nm from 1000 to 1800 nm. Before analyzing the set of samples, a white reference scan was carried out using a piece of Spectralon®.

Within this framework, NIR measurements were performed three times by rotating the vial a few degrees between each measurement. A total of 222 (3 measurements × 74 samples) spectra were collected to calibrate the model for the prediction of EOC, and 81 (3 measurements × 27 samples) spectra were collected to constitute the validation set. In order to compensate the effects of uncontrolled baseline and intensity variations, spectra were pretreated using a second-derivative method.[10]

FT-NIR approach

Spectra were acquired in reflectance mode using an FT-NIR spectrometer (NIRFlex Solids, Büchi, Flawil, Switzerland). Powder of dried leaves was presented to the instrument in a rotating glass Petri dish, and NIR spectra were collected from 1000 to 2500 nm at a resolution of 12 cm^{-1}.

NIR measurements were performed six times by rotating the Petri dish between each measurement. A total of 444 (6 measurements × 74 samples) spectra were collected to calibrate the model for the prediction of EOC, and 27 (1 measurement × 27 samples) spectra were collected to constitute the validation set. Spectra were pretreated by the standard normal variate (SNV) method[18] and detrending.

Data analysis

Partial least squares regression (PLS) was carried out to produce linear models of prediction between spectral data and reference values (EOC). The models were built in three steps, i.e. (1) calibration, (2) cross-validation and (3) validation. Cross-validation was performed using a leave-*k*-out procedure, were *k* is the number of spectral acquisitions per sample.[19] The optimal number of latent variables (LV) introduced in the models corresponded to a compromise that allowed us to obtain a model presenting on the one hand the relatively lowest RMSECV value and on the other hand the relatively highest R^2 value.[20]

The accuracy of the predictions is discussed according to the coefficient of determination of calibration (R^2) and the standard errors of calibration (SEC), cross-validation (SECV) and validation (SEP), while other calculated parameters allowed us to attempt an evaluation of model quality according to the range of reference data and the eventual bias measured when the external validation was performed:

$$R^2\,(\mathrm{C/CV/P}) = 1 - (\mathrm{PRESS/TSS})$$

$$\mathrm{SE\,(C/CV/P)} = \left[\sum_{i=1}^{n} (y_i - \hat{y}_i)^2/n\right]^{1/2}$$

$$\mathrm{bias} = \sum_{i=1}^{n} (\hat{y}_i/n) - \sum_{i\ =1}^{n} (y_i/n) = \bar{\hat{y}} - \bar{y}$$

$$\mathrm{SE\,(C/CV/P)_c} = \left[\sum_{i=1}^{n} (\hat{y}_i - \mathrm{bias} - y_i)^2/n\right]^{1/2}$$

$$\mathrm{RSE\,(C/CV/P)_c\,(\%)} = (100/\bar{y}) \left[\sum_{i\ =1}^{n} (\hat{y}_i - \mathrm{bias} - y_i)^2/n\right]^{1/2}$$

where $\hat{y}_i$ is the predicted value, y_i the mean value and y_i the actual value of EOC in the PLS model, n is the number of samples in the PLS model, PRESS is the prediction residual error of the sum of squares, TSS is the total sum of squares and the subscript 'c' indicates that the parameters (SE(C/CV/P) and RSE(C/CV/P)) have been corrected for bias.

The accuracy and robustness of the PLS models are discussed according to the following parameters, all corrected for bias value:

$$\text{coefficient of variation, } \mathrm{CV_c\,(\%)} = \mathrm{SEP_c/mean}$$

$$\text{ratio of performance to deviation, } \mathrm{RPD_c} = \mathrm{SD/SEP_c}$$

where SD is the standard deviation;

$$\text{ratio of } \mathrm{SEP_c} \text{ to reference data range, } \mathrm{RER_c} = (y_{\max} - y_{\min})/\mathrm{SEP_c}$$

where $y_{\max}$ and $y_{\min}$ are the maximum and minimum reference values of EOC respectively;

$$\text{ratio of } \mathrm{SEP_c} \text{to interquartile,}^{21}\ \mathrm{RPIQ_c} = (Q_3 - Q_1)/\mathrm{SEP_c}$$

where Q_3 and Q_1 are the values of the third and first quartiles of reference data respectively.

RESULTS

Reference data values

The EOC reference values obtained from hydrodistillation of the essential oils ranged from 0.23 to 10.1 mL per 100 g DM. The histogram of EOC values with superimposed normal density curve in

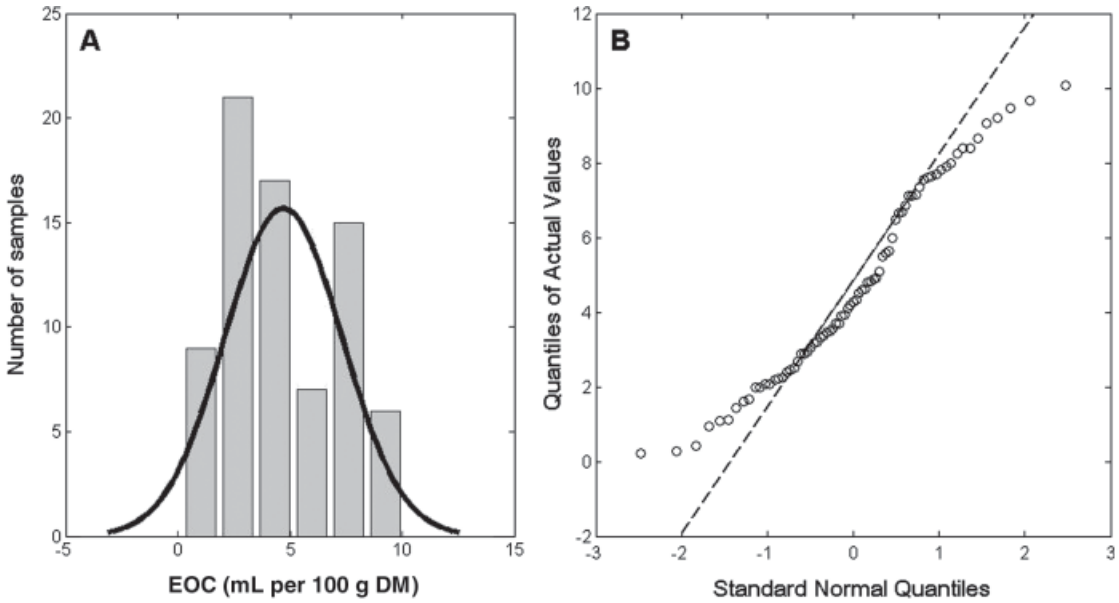

Figure 1. (A) Histogram of EOC values with superimposed normal density curve. (B) Quantile–quantile plot.

Table 1. PLS data of EOC determination (hand-held NIR and FT-NIR)

		PHAZIR 1018			FT-NIR		
PLS data	Unit	Calibration	Cross-validation	Validation	Calibration	Cross-validation	Validation
N	—	74	74	27	74	74	27
EOC range	mL per 100 g	0.23–10.1	0.23–10.1	0.4–8.1	0.23–10.1	0.23–10.1	0.4–8.1
EOC mean value	mL per 100 g	4.8	4.8	4.89	4.8	4.8	4.89
EOC SD	mL per 100 g	2.61	2.61	2.34	2.61	2.61	2.34
λ range	nm	939–1797	939–1797	939–1797	1000–2500	1000–2500	1000–2500
LV	—	3	3	3	6	6	6
R^2	—	0.92	0.92	0.58	0.93	0.94	0.91
SE(C/CV/P)	mL per 100 g	0.75	0.77	2.20	0.7	0.68	0.69
Bias	mL per 100 g	1.40×10^{-2}	1.55×10^{-2}	−2.04	4.5×10^{-7}	1.08×10^{-2}	8×10^{-2}
$SE(C/CV/P)_c$	mL per 100 g	0.75	0.77	0.81	0.7	0.68	0.68
$RSE(C/CV/P)_c$	Relative %	15	15	18	15	15	14
CV_c	Relative %	15	15	18	15	15	14
RPD_c	—	3.54	3.44	3.51 (1.30)[a]	3.7	3.82	3.24
$RPIQ_c$	—	6.19	6.01	5.03 (1.87)[a]	6.6	6.8	4.55
RER_c	—	13.17	12.78	9.45 (3.50)[a]	14.04	14.51	11.31
Spectral treatment		Golay second derivative (step 3)			SNV + detrending		

N, number of samples; EOC, essential oil content; SD, standard deviation; λ range, wavelength range of PLS model; LV, number of latent variables; R^2, determination coefficient; SE, standard error; RSE, relative standard error of prediction; CV, coefficient of variation; RPD, ratio of performance to deviation; RPIQ, ratio of performance to interquartile; RER, ratio of error to range; subscript 'c' (SE_c, RSE_c, CV_c, RPD_c, $RPIQ_c$ and RER_c), parameters calculated after bias correction; SNV, standard normal variate.
[a] Values in parentheses are RPD, RPIQ and RER before correction for bias.

Fig. 1A and the quantile–quantile plot in Fig. 1B illustrate the distribution of the EOC data set. The pattern of the quantile–quantile plot suggests a non-normal distribution of data, mainly at the extremities. Data analysis using Kolmogorov–Smirnov ($P = 1.03 \times 10^{-049}$) and Shapiro–Wilk ($P = 0.017$) tests at a threshold of 5% confirmed the non-normal distribution.

PLS model using hand-held NIRS

The PLS model data obtained using hand-held NIRS are reported in Table 1. Several parameters were calculated to evaluate the accuracy of the model and to measure the fit of the predicted values to the reference data. Figure 2 shows the actual *versus* predicted values for calibration (Fig. 2A) and validation (Fig. 2B). The calibration step was evaluated according to R^2 and SEC, for which values of 0.92 and 0.75 mL per 100 g DM respectively were obtained. The number of LV used was three, which is relatively small, thus avoiding potential overfitting of the model.[22] The cross-validation procedure presented a very small bias close to zero, while R^2 and SECV values were close to those obtained in calibration. Three parameters allowing us to evaluate the accuracy of the model according to the range of reference data were calculated, RPD_c, RER_c and $RPIQ_c$, whose values were 3.54, 13.17

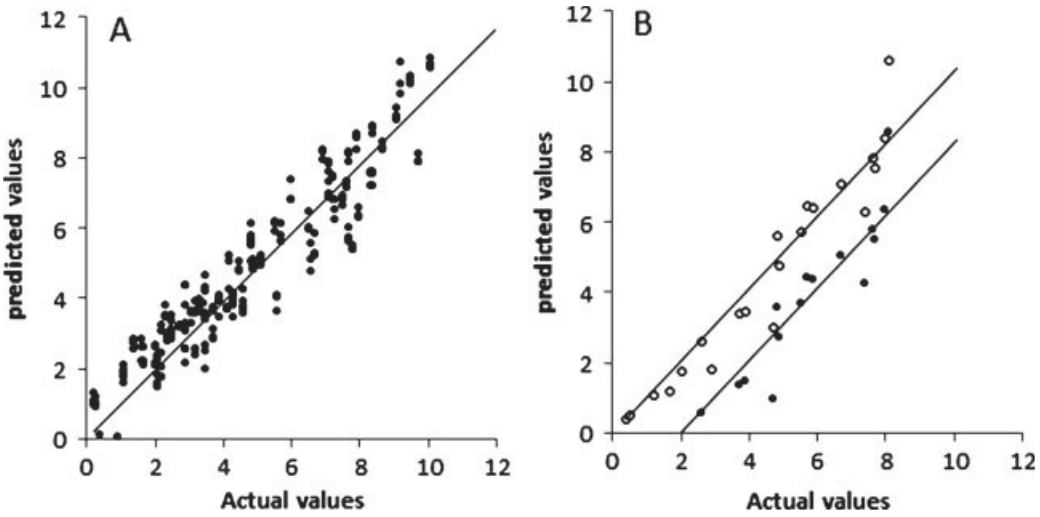

Figure 2. Actual *versus* predicted values of EOC using hand-held NIR device PHAZIR 1018: A, calibration; B, validation; ●, data without bias correction; ○, data corrected for bias value.

and 6.19 respectively for calibration. In cross-validation, RPD_c, RER_c and $RPIQ_c$ were 3.44, 12.78 and 6.01 respectively. For both calibration and cross-validation the RPD_c and RER_c values are greater than 3 and 10 respectively. This means that the model could be suitable for quantitative analysis.[23] RPIQ is used in the case of a non-normal distribution of the reference data set to standardize the SE value. RPIQ uses the inter-quartile ($IQ = Q_3 - Q_1$) parameter instead of SD to standardize the SE value. In the case of a non-normal distribution, IQ could be a better indicator of the data spread around the median. In the present study the RPIQ value indicates that the accuracy of the model is more than five times lower than the interquartile distance.

A validation step was performed with samples not used in the cross-validation model. The R^2 value obtained during validation ($R^2 = 0.58$) was lower than that obtained in cross-validation ($R^2 = 0.92$). An SEP value of 2.20 mL per 100 g DM was calculated, including a bias value of −2.04 mL per 100 g DM. Thus, after correcting for bias, the SEP_c value decreased to 0.81 mL per 100 g DM. Differently to R^2, the SEP_c value remained close to the SEC value obtained in the cross-validation step (0.77 mL per 100 g DM). RPD_c was greater than 3 (3.51), but RER_c decreased to 9.45. $RPIQ_c$ remained at a high level with a value of 5.03.

PLS model using FT-NIRS

The PLS model data obtained using FT-NIRS are reported in Table 1. Figure 3 shows the actual *versus* predicted values for calibration (Fig. 3A) and validation (Fig. 3B). The same parameters as for hand-held NIRS were calculated to evaluate the accuracy of the model and to measure the fit of the predicted values to the reference data. R^2 and SEC values of 0.93 and 0.7 mL per 100 g DM were obtained in the calibration step. Similar R^2 and SE values were obtained in cross-validation. The number of LV remained relatively small at six and the bias was negligible. RDP_c values of 3.7 and 3.82 and RER_c values of 14.04 and 14.51 were obtained in calibration and cross-validation respectively. In the validation step, R^2 and SEP values were at least equal to or better than those calculated in calibration. RPIQ values were 6.6 and 6.8 for calibration and cross-validation respectively and 4.55 in the validation step. No bias was measured during validation (0.08 mL per 100 g DM), in contrast to the model validation with the hand-held NIR device.

DISCUSSION

The aim of the present study was to evaluate the ability of a hand-held NIR device for determining the EOC of oregano dry powder and to compare its performance with that of an FT-NIR spectrometer commonly used in the laboratory. The advantages of a hand-held device are that it can be moved between several breeding sites and is cheaper than laboratory NIR equipment.

The results showed that the determination of EOC was possible with the FT-NIR spectrometer and promising with the hand-held NIR device. In terms of performance, chemometric analysis of the FT-NIR data allowed us to determine the EOC with an accuracy of about 0.70 mL per 100 g DM (cross-validation and validation). The R^2 value higher than 0.9 (calibration and validation) showed the good fit between reference and predicted data. Furthermore, no bias was measured in the model using FT-NIR data.

The model implemented using hand-held NIR data showed promising results but would not be usable in practice in its present state of development. Indeed, in terms of performance the model allowed a measurement accuracy of 0.77 mL per 100 g DM (calibration) and 0.81 mL per 100 g DM (validation), slightly lower than the FT-NIR results. The R^2 values, particularly in validation ($R^2 = 0.58$), showed that the reference and predicted values did not fit as well as with FT-NIR data. Moreover, a bias was measured in the validation step between reference and predicted values. This bias showed that the predicted values underestimated the reference values by about 2 mL per 100 g DM. The presence of such a bias is difficult to understand.

The bias is a systematic error that can have different origins in the chain of steps leading to the construction of a prediction model using NIRS data.

- The first two potential sources of error are a lack of reproducibility of the reference measurement and a lack of reproducibility of the sample preparation procedure between calibration and validation. In the present work the procedures were exactly the same when analysing samples of the calibration and validation steps.
- Another potential source of systematic error is a significant change in environmental conditions during spectral acquisition. In particular, variations in temperature and relative humidity could affect the quality of NIR spectra collected. In this study,

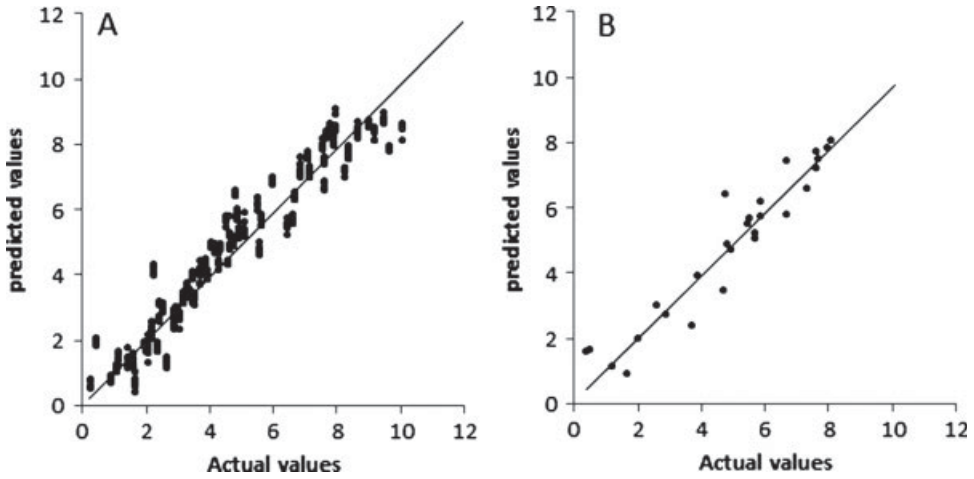

Figure 3. Actual *versus* predicted values of EOC using FT-NIR spectrometer: A, calibration; B, validation.

measures of calibration and validation were performed in the same laboratory under controlled environmental conditions.

- Also, a high genetic variability of plant samples could induce a systematic error between calibration and validation steps. In the present experiment, calibration was performed with samples of various genetic backgrounds (hybrids) to avoid or limit this effect. Storage of plant material for too long in unsuitable conditions could lead to chemical or physical deterioration, which may also introduce a bias. In the present study the spectral acquisitions of calibration and validation samples were carried out at different times, so it is possible that the samples for validation were altered. However, to confirm this hypothesis, a bias should be found in the model using the FT-NIR spectra, but this was not the case.
- A last potential source of bias is related to the manipulator itself. Indeed, a portable spectrometer requires working with the utmost rigour and perfect reproducibility. This parameter was not considered in this study and will be given special attention in future steps for the development of methods using a portable spectrometer.

Concerning the other calculated parameters, RPD, CV and RER are commonly used by NIR spectroscopists to evaluate their models and, more precisely, the error of the models as a function of the range values of the reference measure. To consider a model as 'correct' for 'plant screening', RPD and RER values have to be equal to or greater than 3 and 10 respectively.[23–25] RPD values of the model obtained with FT-NIR spectral data were higher than those of the model obtained with hand-held NIR spectral data. The RER value of the FT-NIR model reached 11.31 in validation, confirming the possible usability of such a device for screening samples. Concerning the model using hand-held NIR data, RPD and RER values were only satisfactory after correction for bias, which increased RPD from 1.30 to 3.51 and RER from 3.50 to 9.45. The relatively low RER value obtained after correction for bias (<10) confirms the relatively low R^2 value (0.58) and thus the non-usability of this model at its present stage of development.

RPIQ is a parameter allowing one to evaluate the spread of predicted *versus* reference data around the median in cases where the reference data distribution is non-normal (skewed distribution). Measurements of EOC in the present study followed a non-normal distribution as confirmed by Kolmogorov–Smirnov and Shapiro–Wilk tests. Thus RPIQ could be a more useful parameter than RPD to describe the obtained model of predictions. As stated above, RPIQ was higher for the model implemented with FT-NIR data (4.55) than for that implemented with hand-held NIR data before correction for bias (1.87). In the model using data from the hand-held NIR device, the RPIQ value of 1.87 means that the model error is less than two times smaller than the interquartile range of reference data, which is far from sufficient for good model performance. In contrast, the RPIQ value of 4.55 obtained with FT-NIR data means that the error of the model is less than four times smaller than the interquartile range of reference data. In this last case the performance of the model can be considered as good. Since the RPIQ parameter is a relatively recent index, no scale value has been published yet (contrary to the RPD parameter) allowing one to evaluate the prediction models.

In the present state of the model, with a 2 mL per 100 g DM bias measured during validation, the hand-held NIR device is not usable in practice. Additional samples of oregano allowing one to increase the variability of samples (various EOCs, geographical origins, cultivation practices, etc.) have to be collected to enrich the model and thus try to diminish the bias value.

CONCLUSION

The aim of this study was to investigate the potential of NIRS to facilitate the screening of hundreds or thousands samples of oregano, with particular emphasis on their EOC, in the context of a breeding programme.

Two approaches in terms of technology/device were attempted: hand-held NIRS and FT-NIRS. The approach using FT-NIR allowed the correct prediction of oregano EOC with an accuracy of 0.68 mL per 100 g DM. All parameters used to evaluate the performance of the model reached expected levels, indicating that the FT-NIR approach is suitable for good screening. Although the hand-held NIR approach is promising, the obtained results are not suitable for use in practice. The performance of the model ($SEP_c = 0.81$ mL per 100 g DM) is inferior to that obtained with the FT-NIR approach. Moreover, a bias correction of about 2 mL per 100 g DM had to be made to achieve an accuracy of 0.81 mL per 100 g DM. However, the RPIQ value calculated after bias correction is promising for future development of hand-held NIRS. In a next step the calibration data set has to be enriched with additional samples from different origins and different levels of EOC in order to minimize the bias value. The development of measurement

methods using portable tools must take into account the effect of the manipulator in order to minimize the systematic error between calibration and validation measurements.

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Genetic survey of *Rhodiola rosea* L. populations from the Swiss Alps based on SSR markers

Z. György [a,*], J.F. Vouillamoz [b], M. Ladányi [c], A. Pedryc [a]

[a] Corvinus University of Budapest, Department of Genetics and Plant Breeding, 1118 Budapest, Hungary
[b] Agroscope, Institute for Plant Production Sciences IPS, 1964 Conthey, Switzerland
[c] Corvinus University of Budapest, Department of Biometrics and Agrarinformatics, Hungary

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ABSTRACT

Rhodiola rosea is a perennial adaptogenic medicinal plant found in the cool climates of the northern hemisphere. This species is highly variable both in morphological and phytochemical traits. The genetic diversity of five populations located in the Swiss Alps was studied with twelve SSR markers. However, only eight markers turned out to be informative in this study. The primer pairs for these eight SSR markers produced 37 fragments. The number of alleles per locus ranged from two to eight. The observed heterozygosity was between 0.09 and 1.0, whereas the expected heterozygosity was between 0.13 and 0.72. The genetic diversity was in the same range for all five populations. Principal coordinate analysis revealed that individuals from different populations did not cluster together, which confirmed that diversity within and among the populations were almost equivalent. The genetic fragmentation of this alpine species despite of its fragmented and isolated habitats, did not happened yet. The results of the present study on the genetic diversity were consistent with an earlier study on the chemical diversity with the same individuals.

1. Introduction

Rhodiola rosea L. (*Crassulaceae*), commonly known as golden root or roseroot, is a traditional adaptogen plant that has been used for centuries in folk medicine in Scandinavia, Eastern Europe and Asia as a general immune-stimulant (Panossian et al., 2010). This herbaceous plant has a circumpolar distribution at high latitudes and elevations of the Northern hemisphere, mainly in Asia and Europe. According to Hegi (1963), its distribution in Europe stretches over Iceland and the British Isles across Scandinavia and as far south as the Pyrenees, the Alps, the Carpathian Mountains and other mountainous Balkan regions.

The thick rhizome and the roots of *R. rosea* contain pharmacologically important secondary metabolites, mainly salidroside and rosavins, that are responsible for increasing human resistance to fatigue, attention, memory and work productivity (Brown et al., 2002). However, this dioecious species is highly variable in both phytochemical (Kurkin et al., 1988; Wiedenfeld et al., 2007) and morphological traits (Ohba, 1981, 1989; Asdal et al., 2006), and plants with almost no salidroside or no rosavins have been observed in Switzerland (Malnoe et al., 2009).

To date, only a small number of studies have dealt with the genetic diversity in *R. rosea* populations from diverse locations. In Trentino (northern Italy), Zini et al. (2009) have developed eight microsatellite (SSR) primers that are specific to *R. rosea* and

* Corresponding author. Tel.: +36 1 482 6530; fax: +36 1 482 6343.
E-mail address: zsuzsanna.gyorgy@uni-corvinus.hu (Z. György).

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Table 1
Population sites of *Rhodiola rosea* analysed in the present study (Swiss cantons are abbreviated as VS = Valais, UR = Uri, TI = Ticino and GR = Graubünden).

Site	Altitude	Exposition
Mattmark (VS)	2100–2300 m	E–W
Binntal (VS)	1935–1980 m	N–W
Unteralp (UR)	1970–2140 m	S–W
Piano dei Canali (TI)	2000–2200 m	S
Nomnom (GR)	2020–2300 m	W

they have observed a significant deviation from Hardy Weinberg equilibrium in both analysed populations. In Scandinavia, no population-specific primer could be found in Sweden, Greenland and the Faroe Islands using microsatellites (SSR) and inter simple sequence repeats (ISSR) (Kylin, 2010). In Norway, Amplified Fragment Length Polymorphism (AFLP) analysis of natural populations has shown that intra-population variability was much higher than inter-populations variability, thus indicating a high level of gene flow that might be a result of seed dispersal rather than cross-pollination (Elameen et al., 2008). In Russia, on the contrary, a low level of diversity was detected at the population level with ISSR and SSR markers (György et al., 2012), but the number of individuals and markers was probably too low. Recently, You et al. (2013) developed 17 SSR markers and tested them on several *Rhodiola* species, though not *R. rosea*.

The aim of the present work was to characterize the inter- and intra-population genetic diversity among roseroot individuals from the populations of the Swiss Alps using SSR markers and compare the results with previous studies in order to set the basis for a new breeding program.

2. Materials and methods

2.1. Plant material

Cuttings of 93 plants of *R. rosea* plants were collected by Malnoe et al. (2009) in 2006 and 2007 from five sites in the Swiss Alps and were transplanted to the experimental field of Agroscope ACW in Bruson (Valais, Switzerland) at 1050 m a.s.l, where they are still cultivated. Out of these, 74 were used in the present study for genetic analysis: Mattmark $n = 16$, Binntal $n = 16$, Unteralp $n = 10$, Piano dei Canali $n = 16$ and Nomnom $n = 16$ (Table 1 and Fig. 1). Voucher samples are deposited at Agroscope ACW in Conthey and at CUB Dept. Genetics and Plant Breeding. Leaves were frozen in liquid nitrogen and stored at −80 °C. DNA was extracted with SP Plant Mini Kit (Omega, VWR International Kft, Budapest). DNA concentration and quality was assessed using NanoDrop (BioScience, Hungary) as well as on 1% agarose gel.

2.2. PCR amplification of SSR fragments

PCR was performed in a 25 μl reaction volume containing 20–80 ng DNA, 10× PCR reaction buffer, 2.5 mM $MgCl_2$, 0.02 mM dNTP mix, 2.5 μmol of each 5′ and 3′ end primers, 1 unit of *Taq* DNA polymerase (Fermentas, Szeged, Hungary) and sterile distilled water. The primers for the eight SSR loci described for roseroot by Zini et al. (2009) and the four most polymorphic SSR loci published by You et al. (2013) were used for the DNA amplification (Rs3, Rs4, Rs8, Rs11). The forward primers were fluorescently labelled with 6-FAM. PCR was carried out in a PTC 200 thermocycler (MJ Research, Budapest, Hungary) as described by Zini et al. (2009) and You et al. (2013). The PCR products were loaded on a 1% (w/v) ethidium bromide-stained agarose gel in 1xTBE buffer with xylencyanol loading buffer to verify the amplification. The amplified SSR fragments were run

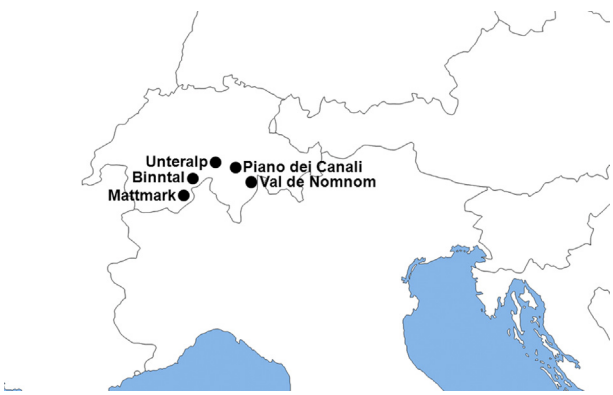

Fig. 1. Location of the studied roseroot populations in Switzerland.

in an automated sequencer ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Budapest, Hungary). Band scoring was analysed using Peak Scanner software 1.0 (Applied Biosystems 2006).

2.3. Data analysis

The results of chemical analysis were published earlier (Malnoe et al., 2009). Based on these results, clusters were created in the present study with Ward-linkage according to squared Euclidean distance.

Genetic relatedness was calculated by UPGMA (Unweighted Pair Group Method with Arithmetic averages) cluster analysis using Popgene version 1.32 (1997). Popgene was also used to estimate the expected (H_e) and observed (H_o) heterozygosity, as well as Shannon's Index (I) for co-dominant data markers. Principal Coordinates Analysis (PCoA) was performed with the software PAST (Hammer et al., 2001). The allelic SSR matrix was used for the analysis of molecular variance (AMOVA) implemented in Genalex 6.5 (Peakall and Smouse, 2012). AMOVA was used to estimate the partition of the genetic variation within and among the populations. The significance of the variance components was determined with a permutation test (999 replicates).

3. Results and discussion

Amplification was successful with eleven out of the twelve SSR markers. The primer pair RRF4 did not amplify any of the samples. Primers for marker RRE3 and RRE4 failed to amplify genomic DNA in many samples: with RRE3, amplification was successful in only 43% of the samples, of which 69% were monomorphic; with RRE4, amplification was successful in only 31% of the samples, of which 61% were monomorphic.

The number of alleles per locus ranged from two (RRE4, RRE9 and Rs4) to eight (RRE2), which is more than what was found by Zini et al. (2009) and Kylin (2010), and suggests a higher genetic diversity in the Swiss populations. The four markers developed by You et al. (2013) gave relatively low allele numbers. Marker Rs8 was the most polymorphic, with seven allele sizes detected.

The locus RRE9 was monomorphic, showing the alleles 146 and 155 for all the tested plants. As a consequence, only eight (RRC10, RRD6, RRE2, RRF3, Rs3, Rs4, Rs8, Rs11) out of the twelve markers turned out to be informative in this study, producing 37 alleles within the expected range based on published data (Zini et al., 2009; You et al., 2013), from 121 bp (RRF3) to 297 bp (Rs11). At the Rs8 locus, two new alleles were detected (209 and 212).

Genetic diversity parameters are given in Table 2. The observed heterozygosity (H_o) ranged from 0.0 at Rs4 and Rs3 for Unteralp and at Rs4 for Nomnom to 1.0 at RRD6, RRE2 and RRE3 for all five populations. The expected heterozygosity (H_e) (genetic diversity) ranged from 0.0 at Rs4 and Rs3 for Unteralp and at Rs4 for Nomnom to 0.81 at RRE2 for Nomnom. In comparison at the same loci, the observed heterozygosity ranged from 0.09 at RRE3 to 0.76 at RRF3 in Zini et al. (2009), and from 0.0 at RRE9 to 1.0 at RRC10 in Kylin (2010), while the expected heterozygosity ranged from 0.16 at RRE3 to 0.66 at RRC10 in Zini et al. (2009), and from 0.2 at RRE3 to 0.73 at RRC10 in Kylin (2010). With the markers developed by You et al. (2013), the observed heterozygosity ranged from 0.0 at Rs3 and Rs8 for *Rhodiola sacra* to 0.917 at Rs4 for *Rhodiola fastigiata* as well as at Rs11 for *R. sacra*, while the expected heterozygosity ranged from 0.156 at Rs3 for *R. sacra* to 0.867 at Rs3 for *Rhodiola crenulata*.

Genetic diversity was in the same range for all five populations. The smallest value of the means of Nei's index was 0.36 in the population from Unteralp, while the highest value was 0.54 in the population from Piano dei Canali. The values of the Shannon's informative index were the lowest in Unteralp with 0.55 and the highest in Piano dei Canali and in Binntal with 0.91.

The genetic parameters averaged over all five populations for the eight markers are listed in Table 3. The mean value of expected heterozygosity was 0.52, while the observed heterozygosity was 0.61. This indicates medium genetic variation among the Swiss roseroot populations, which is backed up by the mean value of Nei's genetic diversity at 0.52 as well as by the Shannon index at 0.94. Both Nei's genetic diversity and Shannon index (Tables 2and 3) indicate that the diversity within and among populations is equivalent.

Genetic relationships among the Swiss populations are illustrated in Fig. 2. The populations from Piano del Canali and from Binntal appeared to be the most closely related, Nomnom and Unteralp were also closely related, while the population from Mattmark was the most isolated. The clusters showed no correlation with the geographic landmark of the populations. This lack of correlation was also observed by Alrababah et al. (2011) between genetic and geographic distances in populations of *Pinus halepensis* in Jordan. The clusters based on the SSR data also showed no correlation with the dendrogram (Fig. 3) based on the glycoside content (salidroside and rosavins) of the same roseroot populations that was published in Malnoe et al. (2009). This was also the case when chemical composition and genetic diversity were compared in Norwegian roseroot populations (György et al., 2013).

Principal Coordinates Analysis (PCoA) of all 74 studied individuals (Fig. 4) showed that individuals from the same population do not necessarily cluster together and were scattered on both coordinates. Only the individuals from Mattmark and from Unteralp more or less grouped together alongside coordinate 2. PCoA confirmed that the Unteralp population was the least diverse, and that the diversity within and among the populations was almost equivalent, as shown in Tables 2 and 3. This is consistent with the chemical diversity in glycoside content that was observed in Malnoe et al. (2009) within and among the same five populations of the Swiss Alps: rosavins ranged from 1.02% to 2.07%, while salidroside ranged from 0.46% to 2.85%. Since an outstanding salidroside content was observed in the population in Mattmark, the most productive individuals were

Table 2
Genetic parameters for five Swiss populations of *Rhodiola rosea* based on 8 SSR markers. H_o = observed heterozygosity, H_e = expected heterozygosity, Nei = Nei's index of genetic diversity, Ave Het = Average heterozygosity, I=Shannon's Information Index.

Locus	H_o	H_e	Nei	Ave Het	I
Mattmark					
RRC10	0.25	0.23	0.22	0.22	0.38
RRD6	1.00	0.52	0.50	0.50	0.69
RRE2	1.00	0.73	0.71	0.71	1.38
RRE3	1.00	0.62	0.60	0.60	1.04
Rs4	0.25	0.23	0.22	0.22	0.38
Rs11	0.88	0.76	0.74	0.74	1.44
Rs3	0.25	0.23	0.22	0.22	0.38
Rs8	0.44	0.38	0.37	0.37	0.73
Mean	0.63	0.46	0.45	0.45	0.80
St.dev.	0.37	0.23	0.22	0.22	0.44
Nomnom					
RRC10	0.44	0.40	0.39	0.39	0.66
RRD6	1.00	0.64	0.62	0.62	1.04
RRE2	1.00	0.81	0.78	0.78	1.61
RRE3	1.00	0.62	0.60	0.60	1.04
Rs4	0.00	0.00	0.00	0.00	0.00
Rs11	0.31	0.28	0.28	0.28	0.54
Rs3	0.00	0.31	0.30	0.30	0.48
Rs8	1.00	0.66	0.64	0.64	1.06
Mean	0.59	0.47	0.45	0.45	0.80
St.dev.	0.46	0.26	0.26	0.26	0.49
Piano del Canali					
RRC10	0.50	0.57	0.55	0.55	0.88
RRD6	1.00	0.64	0.62	0.62	1.04
RRE2	1.00	0.68	0.66	0.66	1.28
RRE3	1.00	0.60	0.58	0.58	0.99
Rs4	0.25	0.23	0.21	0.21	0.38
Rs11	0.75	0.57	0.55	0.55	0.88
Rs3	0.06	0.47	0.45	0.45	0.64
Rs8	0.88	0.69	0.67	0.67	1.18
Mean	0.68	0.56	0.54	0.54	0.91
St.dev.	0.37	0.15	0.15	0.15	0.29
Binntal					
RRC10	0.31	0.51	0.50	0.50	0.69
RRD6	1.00	0.64	0.62	0.62	1.08
RRE2	1.00	0.75	0.73	0.73	1.55
RRE3	1.00	0.57	0.56	0.56	0.92
Rs4	0.13	0.12	0.12	0.12	0.23
Rs11	0.44	0.56	0.54	0.54	0.86
Rs3	0.13	0.48	0.47	0.47	0.66
Rs8	0.75	0.64	0.62	0.62	1.24
Mean	0.59	0.63	0.52	0.52	0.91
St.dev.	0.39	0.19	0.18	0.18	0.40
Unteralp					
RRC10	0.30	0.61	0.58	0.58	0.94
RRD6	1.00	0.66	0.63	0.63	1.04
RRE2	1.00	0.53	0.50	0.50	0.69
RRE3	1.00	0.53	0.50	0.50	0.69
Rs4	0.00	0.00	0.00	0.00	0.00
Rs11	0.20	0.19	0.18	0.18	0.33
Rs3	0.00	0.00	0.00	0.00	0.00
Rs8	0.90	0.52	0.50	0.50	0.69
Mean	0.55	0.38	0.36	0.36	0.55
St.dev.	0.47	0.27	0.26	0.26	0.40

isolated in an experimental field to generate by a polycross the first synthetic cultivar of roseroot that was named 'Mattmark' (Vouillamoz et al., 2012).

The AMOVA of the five populations showed that the vast majority of molecular variation was found within the populations (98% of total variance), while only 2% was observed among the populations, which is consistent with the highly scattered PCoA plot in Fig. 4. The AMOVA model is a linear additive model in which we express the total variance as the sum of variances between populations and among population. Whenever the diversity of the populations is very high, the proportion of the variances within population and total variance is high (in our case it is 98%). Therefore the remaining term of total variance (which is an extra variance added to the within population variance) that can be explained solely by the diversity between populations excluding the diversity within population (called as fixation index) is low (in our case: 100%–98% = 2%). It

Table 3
Genetic parameters averaged over all five Swiss roseroot populations based on eight SSR markers.

Locus	H_o	H_e	Nei	Ave Het	I
RRC10	0.36	0.54	0.53	0.45	0.82
RRD6	1.00	0.62	0.62	0.60	1.05
RRE2	1.00	0.72	0.72	0.68	1.50
RRE3	1.00	0.58	0.58	0.58	1.01
Rs4	0.14	0.13	0.13	0.11	0.25
Rs11	0.54	0.55	0.55	0.46	1.01
Rs3	0.09	0.42	0.41	0.29	0.68
Rs8	0.78	0.62	0.62	0.56	1.20
Mean	0.61	0.52	0.52	0.46	0.94
St.dev.	0.39	0.18	0.18	0.18	0.37

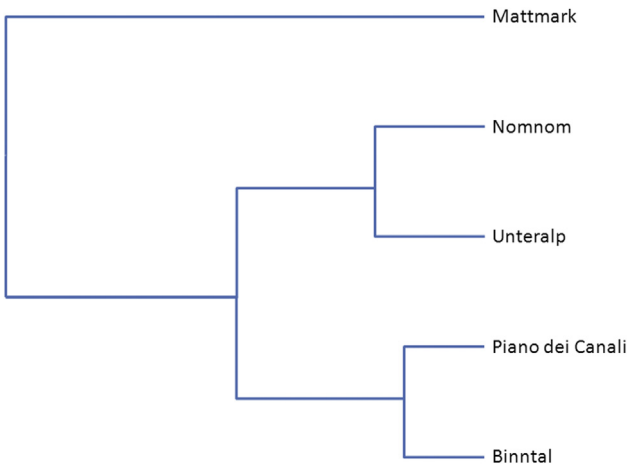

Fig. 2. Dendrogram (UPGMA) of the five Swiss roseroot populations based on Nei's (1978) genetic distance with eight SSR markers.

confirms that the diversity between populations is not significantly higher than the one within population which we stated as equivalent diversity rates of between and within populations.

Similar results were found by Lei et al. (2006) when studying genetic diversity of four populations of *R. crenulata* in the Hengduan Mountain region in China. The Shannon index showed that the genetic diversity within and between the populations of *R. crenulata* are approximately equivalent, while AMOVA showed that the variance within populations was higher (52.6%) than among populations (25.36% within locations and 22.02% and between locations). Similarly, in the study of Kozyrenko et al. (2011) studying the genetic diversity of *R. rosea* in Russia, a low level of diversity was detected at population level, while high genetic variation was observed at the species level. AMOVA revealed that the majority of the genetic variation was within populations (65.4%) and the variance among populations was only 34.6%. Our sampling area was far much smaller, which explains the 98% compared to their 65.4%.

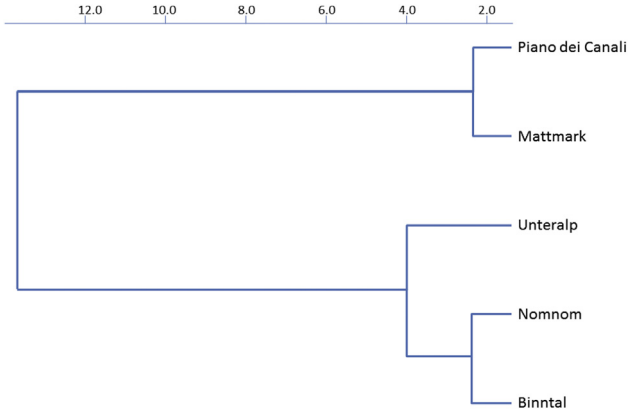

Fig. 3. Dendrogram (UPGMA) of the five Swiss roseroot populations based on the data of their glycoside content that were published in Malnoe et al. (2009).

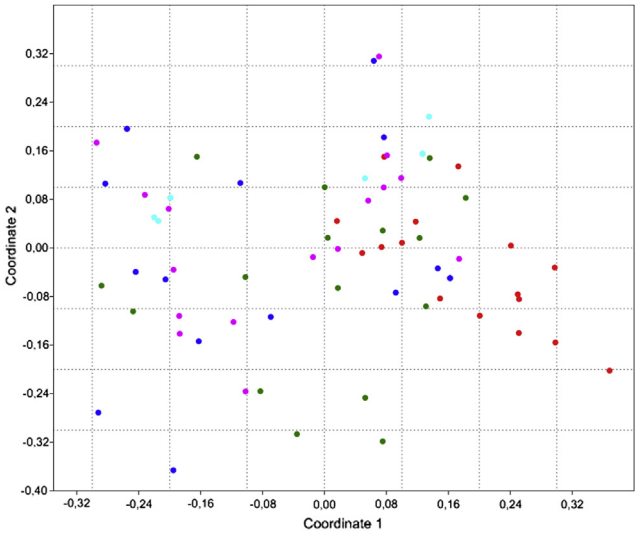

Fig. 4. Principal Coordinates Analysis of all 74 roseroot individuals assayed in this study. The different colours indicate members of the different populations (red – Mattmark, dark blue – Nomnom, pink – Piano dei C., green – Binntal, light blue - UnterAlp). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Out of the twelve *Rhodiola* specific SSR markers tested, eight turned out to be informative to assess the genetic diversity of Swiss roseroot populations. The studied markers showed correlation neither with the geographic distribution nor with the glycoside content of roseroot. Inter- and intrapopulation variability turned out to be in the same range in the Swiss Alps, which is probably the result of obligatory cross-pollination in this dioecious species. The genetic fragmentation of this alpine species despite of its fragmented and isolated habitats, did not happened yet.

Acknowledgement

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A Rapid Greenhouse Screening Method to Identify St. John's Wort (*Hypericum perforatum*) Accessions Resistant to *Colletotrichum gloeosporioides*

Vincent V. Michel[1]
Agroscope, CH-1964 Conthey, Switzerland

Nicole Debrunner and Xavier Simonnet
Médiplant, CH-1964 Conthey, Switzerland

Additional index words. anthracnose, disease, *Hypericum perforatum*, resistance breeding

Abstract. **Anthracnose is a major production constraint for st. john's wort (*Hypericum perforatum* L.) caused by the fungus *Colletotrichum gloeosporioides* (Penz.). A greenhouse screening method based on mortality was developed to eliminate accessions susceptible to anthracnose in the early stage of breeding for resistant cultivars. The mortality of 22 accessions of st. john's wort artificially inoculated with a strain of *C. gloeosporioides* was highly correlated between three greenhouse experiments (r = 0.799 to 0.923), even when done at two different places. The response of the greenhouse screening was equally highly correlated to the mortality in the field tested at two sites naturally infested with *C. gloeosporioides* (r = 0.700 to 0.865) but less well correlated with the mortality at a third field site (r = 0.495 to 0.672). Yield of st. john's wort was highly correlated with mortality (r = –0.747 to –0.846) at all three field sites, but a significant interaction between accession and site was observed. Therefore, an improvement of anthracnose resistance of st. john's wort should be based on a greenhouse screening of seedlings followed by multiple-site field testing of adult plants.**

The intensification of the st. john's wort (*Hypericum perforatum*) production in Switzerland at the end of the 1990s was accompanied by the appearance of anthracnose caused by *Colletotrichum gloeosporioides* (teleomorph *Glomerella cingulata*) (Debrunner et al., 2000), an important fungal disease of st. john's wort (Crompton et al., 1988). Reports on the occurrence of the disease in several European countries (Bomme, 1997; Debrunner et al., 2000; Schwarczinger and Vajna, 1998) can be related to the rapidly increasing surfaces planted to *H. perforatum*, largely attributable to the growing popularity of st. john's wort-based antidepressive drugs (Müller, 2005). For mild to moderate depression, *Hypericum* extracts show a similar efficacy and a better tolerability compared with standard antidepressant drugs (Kasper et al., 2010). The intensification of st. john's wort production led to a shift in the production mode to supply sufficient quantities of inflorescences needed for the transformation in herbal medicine. Wild collection was replaced by field cultivation to produce large quantities in a rational way and to protect natural populations (Lange, 2004). As a consequence of this intensive production, *C. gloeosporioides* became a major problem, especially in organic farming with a restricted use of fungicides, as is the case in the major part of the st. john's wort fields grown in Switzerland (Debrunner et al., 2000). Under such conditions, the pathogen can destroy this perennial crop in the first year of cultivation, especially when planted in more humid production areas and in heavy soils.

Because we are still at the beginning of the domestication process, breeding for anthracnose resistance offers the most promising way to reduce the impact of this disease, even in conventional farming systems. A breeding program was set up by Médiplant, a Swiss research and development institution focusing on the promotion of herbal and aromatic plants, with special emphasis on mountainous environments (Simonnet and Gaudin, 2000). From 1997 to 1999, a first step in st. john's wort improvement was achieved by screening 24 accessions in three different environments, resulting in the release of a resistant cultivar (Gaudin et al., 2002). However, field selection under natural infection with *C. gloeosporioides* is time- and space-consuming. Furthermore, conditions that are favorable for a natural infection vary from 1 year to the other, which can result in insufficient disease pressure in some years. A solution to this problem is the screening in the greenhouse for disease resistance before testing the more advanced breeding material under field conditions (Gardner, 1990).

The objective of this study was the development of a rapid greenhouse screening method for the identification of st. john's wort accessions that are resistant to anthracnose caused by *C. gloeosporioides*.

Materials and Methods

Accessions. Of the 26 st. john's wort accessions tested, 19 were used for both the greenhouse and field experiments. Additional two and five accessions were included in the greenhouse and field experiments, respectively. Next to the commercial cultivars, Topaz (Poland), Hyperimed (Germany), and Elixir (Canada), accessions from Switzerland (16 accessions), Germany (two accessions), Spain (two accessions), Australia (one accession), Austria (one accession), and Italy (one accession) were tested.

Seeds of the accessions were sown in multipot trays containing a commercial peat substrate (Brill 1 + Tonerde; Gebr. Brill Substrate GmbH & Co, Georgsdorf, Germany) and were placed in a greenhouse at 20 °C with a relative air humidity of 80%. Additional light was supplemented during the day by fluorescent tubes for 14 h. After emergence of the cotyledons, temperature was maintained at 20 °C during the day but was lowered to 15 °C during night. After 3 weeks, single seedlings at the two-true-leaf stage were transplanted in plastic pots (6 × 6 cm, 5 cm depth) containing a commercial peat substrate.

Inoculum. The *C. gloeosporioides* strain AN-16 was used to prepare the spore suspension for artificial inoculation in the greenhouse. This strain was isolated from a typical anthracnose stem lesion on st. john's wort at Conthey (Switzerland) and was identified based on morphological characteristics (Mordue, 1971) after growth on potato dextrose agar (PDA) and under light microscope. The identification was confirmed by the CABI Microbial Identification Service (Egham, U.K.). The strain was stored on PDA at 4 °C and periodically subcultured.

Spores were produced in a modified Richard's solution (Daniel et al., 1973). Two to three PDA cubes with mycelium were added to 100-mL aliquots in 300-mL Erlenmeyer flasks. They were placed on a rotary shaker (100 rpm) and incubated at room temperature for 5 d. Spores were harvested by filtration through cheesecloth followed by two consecutive centrifugations at 3913 g_n with resuspension of the pellets in 10 mL sterile deionized water (Daniel et al., 1973). Final concentration of the suspension was adjusted to 1×10^7 spores/mL by counting spores under light microscope using a counting chamber.

Greenhouse experiments. Artificial inoculation of 22 accessions was done twice in a greenhouse of the Agroscope crop improvement program at Nyon (Expt. ACW #1 and ACW #2) and once at Conthey (Expt. Médiplant) (Table 1). The experimental layout was a randomized complete block design (RCBD) with three replicates. At Nyon, each replicate was placed in a separate greenhouse compartment and consisted of 10 individual plants per accession. At Conthey, three replicates of five individual plants were all placed in the same greenhouse compartment. Plants

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[1]To whom reprint requests should be addressed; e-mail vincent.michel@agroscope.admin.ch.

Table 1. Mortality (%) of 26 st. john's wort accessions in the greenhouse after artificial inoculation with *Colletotrichum gloeosporioides* in the greenhouse and natural infection in the field at three sites.

	Greenhouse experiments[z]			Field experiments[y]		
St. john's wort accession	ACW #1	ACW #2	Médiplant	Bruson	Epines	Fougères
Topaz	41	30	60	0	3	3
Hyperimed	69	*0*[x]	100	10	27	23
Elixir	23	7	33	7	23	33
Hp #4	nt[w]	nt	nt	17	87	nt
Hp #5	nt	nt	nt	100	100	100
Hp #6	100	100	100	100	83	nt
Hp #7	3	0	0	0	0	10
Hp #8	86	41	93	53	87	nt
Hp #9	100	100	100	100	100	100
Hp #10	100	100	100	100	100	nt
Hp #11	100	100	100	100	100	100
Hp #12	90	23	27	40	80	40
Hp #13	100	100	100	100	100	100
Hp #14	100	100	100	100	100	100
Hp #15	100	*100*	100	100	100	100
Hp #16	96	*60*	100	27	100	27
Hp #17	43	3	27	0	0	nt
Hp #18	93	90	80	0	17	20
Hp #19	nt	nt	nt	0	23	50
Hp #20	93	50	87	0	93	60
Hp #21	3	0	7	0	27	30
Hp #22	nt	nt	nt	27	100	83
Hp #23	nt	nt	nt	3	100	nt
Hp #24	100	73	100	0	100	20
Hp #201	87	90	100	nt	nt	nt
Hp #208	57	40	73	nt	nt	nt

[z]Two experiments were conducted in the greenhouse of Agroscope Changins-Wädenswil at Nyon (ACW) and one in the greenhouse of Médiplant at Conthey. Mortality was rated 3 weeks after inoculation.
[y]Experiments were planted in Spring 1997 at the three sites natural infection of *Colletotrichum gloeosporioides*. Mortality was rated at harvest in Summer 1998.
[x]Number in *italic* = only one replicate of 10 plants (instead of three replicates).
[w]nt = not tested.

were inoculated 8 to 10 weeks after transplanting; plant height varied from 15 to 30 cm depending on the accession. The spore suspension, supplemented with 1 mL/L Tween 20 as a surfactant, was sprayed on the plants until runoff by the means of a handheld sprayer. A volume of 150 mL of spore suspension was needed to inoculate 220 plants, the equivalent of one replicate in the experiments at Nyon. After inoculation, plants were left in the greenhouse compartments and highly conducive conditions were created for 48 h. Therefore, the relative air humidity was set to 100% using a cold mist humidifier. During this period, the fluorescent tubes were removed. Air temperature was set to 24/20 °C (day/night, 14-h photoperiod) until the rating of mortality 3 weeks after inoculation. Plants were considered dead when the aboveground part of the plant was completely dry, with the exception of new sprouts growing from the basal part of the plant. Expts. ACW #1 and ACW #2 were conducted with 1 week interval at Nyon with inoculation on 19 and 26 Jan. 1999 for Expt. ACW #1 and ACW #2, respectively. Expt. Médiplant conducted at Conthey was inoculated on 15 Feb. 2000.

Field experiments. The sites for field experiments were located at Bruson (1060 m a.s.l.), Fougères, and Epines (both at 480 m a.s.l.). All three sites are situated in the mountainous canton (= state) of Valais in the southern part of Switzerland, the latter two sites in the main valley and Bruson in a side valley. At Bruson and Epines, 10- to 11-week-old seedlings of 24 accessions were planted on 21 and 15 May 1997, respectively. At Fougères, the number of accessions was restricted to 18 accessions and 10-week-old seedlings were planted on 14 May 1997. At all three sites, the experimental layout was a RCBD with three replicates. Ten plants per replicate were planted in double rows (five plants/row) at a density of 4.2, 3.1, and 3.1 plants per m^2 at Bruson, Epines, and Fougères, respectively. The experiments ended after the harvest in the second year in Fall 1998.

All plots were hand-weeded and irrigated regularly during the experimental period. No chemical plant protection measures were applied in the experiments. Before planting, basal N–P–K fertilizer was applied at a rate of 56 and 35 kg nitrogen (N), 24 and 15 kg of P_20_5, and 64 and 40 kg of K_20 per ha at Epines and Fougères, respectively. At Bruson, livestock manure at a rate of 50 m^3 per ha was used to enrich the soil before planting. In Spring 1998, N–P–K fertilizer was spread at Bruson, Epines, and Fougères at a rate of 56, 70, and 49 kg N; 24, 30, and 21 kg of P_20_5; and 64, 80 and 56 kg of K_20 per ha, respectively. Plants were harvested in 1997 and 1998 at full flowering and harvest date varied depending on the year, the accession, and the site. After harvest in 1997, plants were cut back to a length of 10 cm aboveground. The 1998 harvest data were used for yield analysis representing the first year with full yield potential of st. john's wort, which is normally cultivated for 2 to 3 years (Bomme, 1997). Harvest in 1998 occurred between 6 July and 4 August, 17 June and 13 July, and 9 June and 9 July at Bruson, Epines, and Fougères, respectively. At harvest, the top 15 cm of the inflorescences was collected manually and dry matter yield per experimental plot was determined. The resistance to the natural *C. gloeosporioides* infection in the field was assessed before the harvest in 1998 by recording the number of dead plants. At all three sites, no other disease than anthracnose and no infestation by insect pests were observed during the 2 years of cultivation.

Statistical analysis. The mortality rate of the st. john's wort accessions in the greenhouse and field experiments were compared by Spearman rank order correlation analysis imposed by the non-normal distribution even after arcsine transformation of the data (Little and Hills, 1978). Yield of the experimental plots was used as a basis for an analysis of variance to measure the influence of the accession and site on the dry matter production. The effect of the mortality in the field on yield was analyzed using Pearson product moment correlation analysis.

Results

The resistance of the st. john's wort accessions to anthracnose varied greatly in both the greenhouse and field experiments (Table 1). Highly susceptible accessions in the greenhouse (i.e., with 100% mortality in all three experiments) were also highly susceptible in the field (Hp #6, #9 to 11, #13 to 15) with at least 83% mortality. Two accessions (Hp #7 and #21) had less than 10% mortality in the greenhouse. In the field, however, the reaction of these presumably highly resistant accessions was less clear cut. Hp #7 was highly resistant, whereas the mortality of Hp #21 reached 30% at one site. In contrast, 'Topaz' and Hp #17, with an average mortality in the greenhouse of 44% and 24%, respectively, were highly resistant in the field.

The method to screen anthracnose resistance in the greenhouse using artificial inoculation proved to be consistent (Table 2). When the same material was tested twice at the same place (Nyon), the rank correlation coefficient was very high with 92% ($P < 0.001$), and screening at two different sites (Nyon and Conthey) by different persons was still highly correlated with at least 79% ($P < 0.001$). At two sites, Bruson and Epines, mortality in the field was highly correlated with a rank correlation coefficient of at least 70% ($P < 0.001$) with the greenhouse screenings. Mortality at the site Fougères had a lower rank correlation coefficient and was not significant for the greenhouse screening experiment at Médiplant.

Dry matter production was significantly affected by the field site and the accession (Table 3). Mortality in the field had a direct impact on the dry matter yield in the second year after planting (Fig. 1). Dry matter yield was significant negatively correlated ($P < 0.01$) at all three sites, but ranking of the accessions was site-specific (Table 4). St. john's wort

Table 2. Rank correlation coefficients of mortality ratings of st. john's wort accessions in three greenhouse and three field experiments.

	Greenhouse experiments[z]		Field experiments[y]		
	ACW #2	Médiplant	Bruson	Epines	Fougères
ACW #1	0.923***[x]	0.855***	0.726***	0.865***	0.657**
ACW #2	—	0.799***	0.752***	0.775***	0.672**
Médiplant		—	0.700***	0.828***	0.495 NS
Bruson			—	0.698***	0.829***
Epines				—	0.727***

[z]Two experiments were conducted in the greenhouse of Agroscope Changins-Wädenswil at Nyon (ACW) and one in the greenhouse of Médiplant at Conthey. Mortality was rated 3 weeks after artificial inoculation.
[y]Experiments were planted in Spring 1997 at the three sites with natural infection of *Colletotrichum gloeosporioides*. Mortality was rated at harvest in Summer 1998.
[x]Correlation coefficients followed by ***, **, or NS are significant at $P < 0.001$, $P < 0.01$, or nonsignificant ($P > 0.05$), respectively.

Table 3. Effect of st. john's wort accession and site on dry matter yield in the field.

	df	MS	F-value	Prob. > F
Block	2	15081.4	0.4751	0.6236
Accession	16	321454.3	10.1259	0.0001
Site	2	530509.3	16.7111	0.0001
Accession × site	22	60410.3	1.9029	0.0201
Error	80	31745.9		

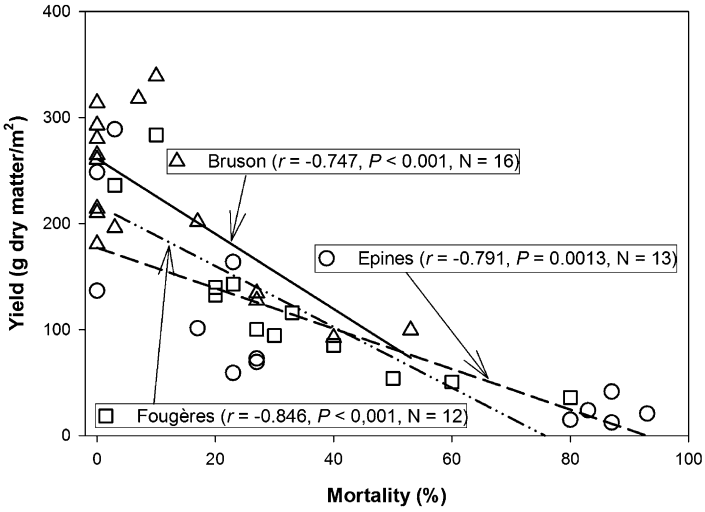

Fig. 1. Correlation between dry matter yield and mortality for the 1998 harvest of st. john's wort accessions in field experiments at three sites. Mortality was measured as percentage of dead plants (i.e., completely dry) before harvest. Accessions with 100% mortality were excluded from correlation analysis. For better visualization, non-transformed mortality data were used for graphical display, because correlation analysis with arcsine-transformed mortality data only differed slightly from the analysis using non-transformed data.

cultivar Hyperimed ranked at the highest position at Bruson and at the seventh position at Epines, whereas 'Topaz' was best at Epines and sixth at Bruson. Despite similar groups of accessions with high ('Topaz', 'Hyperimed', 'Elixir', Hp #7, #17, and #18) and low (Hp #6, #8, #12, #16, #20, and #22) dry matter yield at the three sites, significant interactions ($P = 0.02$) between accession and site were observed (Table 3).

Discussion

Considerable differences in resistance of st. john's wort to anthracnose could be detected in the greenhouse after artificial inoculation with a spore suspension. The wide range of resistance stretching from complete susceptibility to nearly immunity is not surprising because nearly all st. john's wort accessions tested were non-selected ecotypes, representing the large range of resistance of this species. The complete mortality in all greenhouse and field experiments of seven accessions confirmed the high virulence of *C. gloeosporioides,* which was tested as a potential biological control agent of *H. perforatum* in Canada (Hildebrand and Jensen, 1991). At the beginning of the improvement of a crop, the variation in resistance level allows to rapidly find resistant material. For this purpose, greenhouse inoculation methods can be most useful because they allow to screen the resistance of large numbers of accessions within a short period of time and with little need of space (Lu and Raid, 2013).

For the mass selection of st. john's wort, inoculation with *C. gloeosporioides* may take place before transplanting seedlings in pots, which would result in an important reduction of space and labor input. Highly susceptible accessions would thereby be eliminated before the labor-consuming transplanting. Before using such a mass screening scheme, however, the susceptibility of *H. perforatum* to *C. gloeosporioides* at different growing stages must be elucidated. Differences of susceptibility at the seedling stage were observed in cucumber (*Cucumis sativus*), where seedlings at the cotyledon stage were less susceptible to gummy stem blight than seedlings with one true leaf, which in their turn were less susceptible than seedlings with three true leaves (Amand and Wehner, 1995).

The major part of the st. john's wort accessions was collected in a relatively small area of Switzerland. The range of resistance levels represented by this sample indicates wide variability of this trait. Such a large variability in mortality of st. john's wort accessions was reported from Australia where several Australian and Canadian ecotypes were inoculated with two strains of *C. gloeosporioides* (Shepherd, 1995). For crop improvement purpose, such variability is of great interest, which gains additionally in value for the facultative apomictic st. john's wort (Mártonfi et al., 1996). Intra- and interspecific hybridizations of *H. perforatum* are difficult and ploidy level of the offspring can vary considerably (Schulte et al., 1999). The broad genetic diversity of st. johns' wort, as expressed in large variability in resistance to anthracnose, encourages therefore an improvement by selection of ecotypes rather than pedigree breeding.

If the first screening is done in the greenhouse, the results have to be transposable to field conditions. The high rank correlation coefficients between the greenhouse and the field experiments (Table 2) indicate that the level of resistance detected in the greenhouse after artificial inoculation is also valid in the field under natural infection. The only nonsignificant rank correlation coefficient between the Fougères field experiment and the Médiplant greenhouse experiment might be the result of the lower number of accessions tested at Fougères and the smaller number of plants per accession used in the Médiplant greenhouse experiment, which increased the variation of the results. All the remaining eight rank correlation coefficients varied between 0.865 and 0.657 and were highly significant. A slightly higher correlation coefficient of 0.89 was reported by Pande et al. (2011) between the screening of chickpea seedlings in the greenhouse and adult plants in the field for their resistance to *Ascochyta* blight caused by *Ascochyta rabiei* (Pass.) Labr. This higher correlation coefficient can be explained by the repeated artificial inoculation of the chickpea with a spore suspension of *A. rabiei* in the field. In contrast, our field screening of *H. perforatum* was conducted in plots naturally infested with *C. gloeosporioides* without additional inoculation.

Table 4. Dry matter yield (g/m^2) of 17 st. john's wort accessions at three sites with natural infection of *Colletotrichum gloeosporioides* in Summer 1998.[z]

St. john's wort accession[y]	Bruson		Epines		Fougères	
	Yield	Rank	Yield	Rank	Yield	Rank
Topaz	265 abcd[x]	6	289 a	1	236 ab	2
Hyperimed	339 a	1	69 bc	7	143 abc	3
Elixir	318 ab	2	164 abc	3	116 bc	6
Hp #4	202 abcde	10	41 c	9	nt[w]	
Hp #6	0		24 c	10	nt	
Hp #7	280 abc	5	248 ab	2	284 a	1
Hp #8	99 e	15	12 c	13	nt	
Hp #12	92 e	16	15 c	12	85 bc	9
Hp #16	135 cde	13	0		100 bc	7
Hp #17	314 ab	3	137 abc	4	nt	
Hp #18	293 ab	4	101 bc	5	133 abc	5
Hp #19	214 abcde	8	59 c	8	54 c	10
Hp #20	181 bcde	12	21 c	11	51 c	11
Hp #21	261 abcd	7	73 bc	6	94 bc	8
Hp #22	128 de	14	0		36 c	12
Hp #23	196 abcde	11	0		nt	
Hp #24	210 abcde	9	0		140 abc	4

[z]Experiments were planted in Spring 1997 and harvested in Summer 1998.
[y]Accessions Hp #5, Hp #9, Hp #10, Hp #11, Hp #13, Hp #14, and Hp #15 had no yield at all the three sites and were therefore excluded from analysis.
[x]Numbers in columns followed by the same letter are not significantly different (Tukey test, $P = 0.05$). Dry matter values of 0 were excluded from data analysis.
[w]nt = not tested.

The greenhouse tests were especially efficient in detecting highly susceptible accessions in the field. In contrast, the determination of resistant accessions in the field was not clearly linked to the response to artificial inoculation in the greenhouse (Table 1). Therefore, the greenhouse screening is suitable to discard the highly susceptible material, which allows a considerable decrease of the size of field tests. This second step of improvement is necessary not only to improve the anthracnose resistance, but also to measure agronomic traits such as yield, flowering date, growth type, cultivar stability, and phytochemical traits. Testing at multiple sites furthermore increases the quality of the field tests as the major factor that influences the SE between accessions is the environment (Campbell and Lipps, 1998).

The detection of susceptible accessions in the field is of major importance because the mortality is significantly correlated to the dry matter yield (Fig. 1). If important yield reduction caused by fungal pathogens is well known in crop production (Russell, 1978), loss of 100% yield resulting from a fungal disease is rather exceptional. This might be explained by the high susceptibility of some of the accessions tested. Another reason might be the augmentation of the pathogen population over a period of more than 1 year between planting and harvesting in the second year. A similar increase of the anthracnose pressure between the first and the second year of st. john's wort cultivation was also observed during cultivar tests in Germany (Schenk and Gärber, 2002). A rapid spread of the spores within the field experiments most probably occurred, because spores of *C. gloeosporioides* are reported to be easily dispersed by rainfall (Yang and TeBeest, 1992).

Field testing of selected accessions after greenhouse screening is stressed by the significant interactions between accessions and sites for dry matter yield (Table 3). Among the accessions tested at all three sites, the highest ranking Hp #7 and 'Topaz' had the highest yield across all sites. In contrast, 'Hyperimed' and 'Elixir', belonging to the group with the highest yield at Bruson, had a significantly lower yield than the highest ranking accessions at Epines ('Hyperimed') and at Fougères ('Elixir'). For the use of a cultivar on a larger area, yield stability, as shown by 'Topaz' and Hp #7, is of major importance (Plaisted and Peterson, 1959).

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