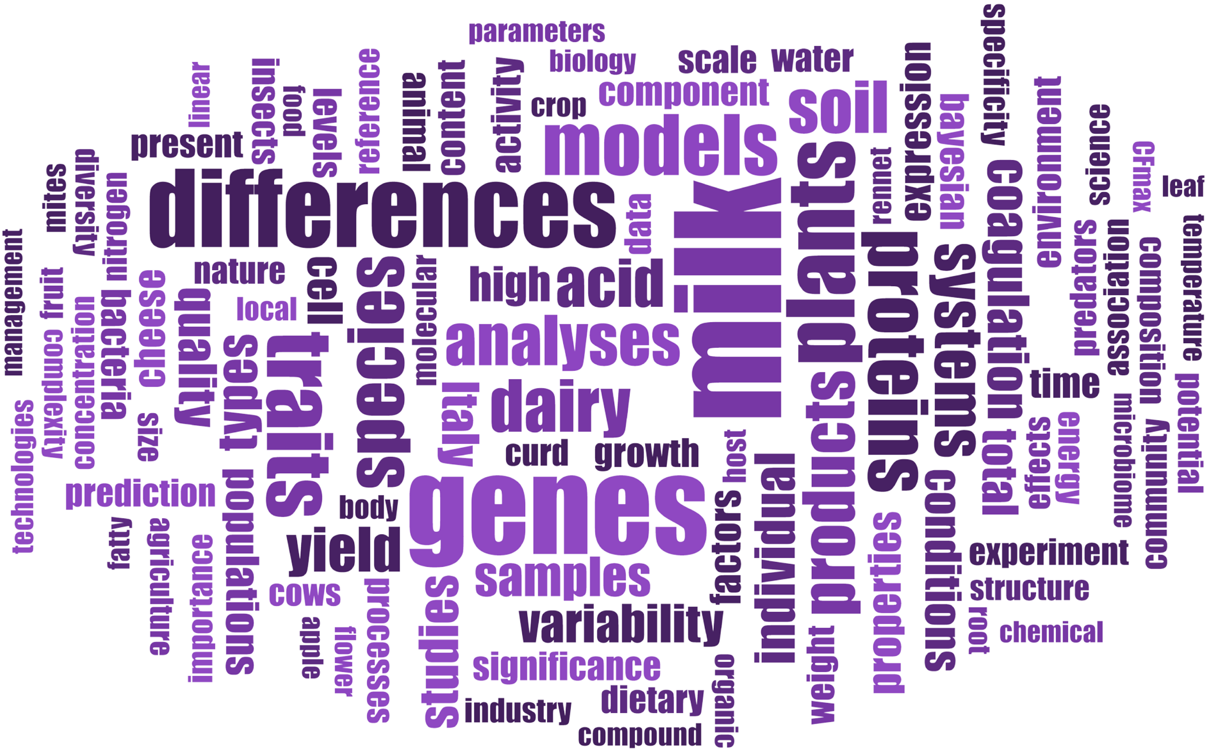
DAFNAE

RESEARCH YEARBOOK
2015



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PADOVA UNIVERSITY PRESS

RESEARCH YEARBOOK 2015

Edited by Alessandro Botton, Paolo Carletti, Martino Cassandro, Roberta Masin,
Mauro Penasa, Silvia Quaggiotti, Chiara Rizzi

Università degli Studi di Padova
via 8 Febbraio 2, Padova

www.padovauniversitypress.it

ISBN 978-88-6938-074-7

UNIVERSITY of PADOVA

DEPARTMENT OF AGRONOMY FOOD NATURAL RESOURCES
ANIMALS AND ENVIRONMENT

RESEARCH YEARBOOK

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Contributions of the National Research Council (CNR) center in DAFNAE:

INTRODUCTION

It is a great pleasure for me to introduce the 2015 edition of the Research Yearbook of the Department of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE) of the University of Padova. Following the two previous editions, it focuses on the top level scientific products of the Department and presents the abstracts of the peer-reviewed papers as retrieved from the ISI and Scopus databases.

These publications represent only a part of the relevant deliverables of the wide research activity carried out within the Department. Indeed, an incredible number of other documents, such as book chapters, conference contributions, abstracts, reports, and fact sheets were also released.

This great effort involved all the wide expertise present in the Department and was facilitated by the contribution of the technical staff and the enthusiastic participation of PhD students, postdocs, and grant holders that represent the freshest energy of the Department.

The research activity developed by DAFNAE has been accompanied by an impressive offer of undergraduate, MS and PhD courses, in which the DAFNAE members can promptly transmit to the students the knowledges derived from their research activities, carried out both in field and lab.

I am very proud for the enthusiasm and the vitality of the Department and I am sure that this booklet will be a remarkable instrument to disseminate the research and innovation produced by DAFNAE.

I deeply thank all the colleagues who contributed with their papers, with a special acknowledgement to the group of editors who took care of this issue.

We are ready for the 2016 edition!

Maurizio Borin
Head
Department of Agronomy, Food,
Natural resources, Animals and Environment

THE RESEARCH PAPERS: STATISTICS AND COMMENTS

The release of the DAFNAE research yearbook represents a very important annual event. It is the moment when the production and quality of the research activity and the degree of collaboration and internationalization of the department are summarized.

Although in the first edition of the yearbook we predicted for the decade 2011-2020 an average number of documents per year published in peer review journal equal to 119, the actual number of scientific documents published in 2012, 2013, 2014, and 2015 was higher, being equal to 151, 154, 123 and 150, respectively.

The 150 scientific documents published in 2015 (+22% compared with 2014) produced a total of 1,110 pages (+8% respect to 2014), on 88 (-6% respect to 2014) different scientific journals. The Journal of Dairy Science (Q1 in the subject categories of Scopus/WOS), with 14 documents, was the scientific journal most highly used by DAFNAE researchers in 2015, as in the previous year.

The average number of papers per researcher was 2.42 and the average number of authors per document was 5.6, ranging from 1 to 16 co-authors.

The documents collected in the present Research yearbook can be divided into 143 scientific articles, 3 reviews, 3 conference papers and 1 letter.

A relevant result obtained in the year 2015 was the high proportion of papers published in journals in the Q1 (first quartile) level of impact factor of the subject category. Table 1 shows the distribution of frequency of scientific documents for quartile of impact factor and for each scientific category. More than 75% of the published papers were categorized Q1.

The categories where all documents were published, are reported in Table 2. The most productive scientific categories were Animal science (19.4%), Genetics and genomics (15.3%), and Food science and technology (14.7%).

Table 1. Distribution of frequency of scientific documents, for quartile of impact factor per each scientific category (Scopus database and Scimago Journal & Country Rank).

Scientific category	Q1	Q2	Q3	Q4	unknown Q
Animal science	15	5	-	9	-
Genetics and Genomics	19	3	-	-	1
Food science and technology	17	2	1	2	-
Plant science	20	-	1	-	-
Ecology and Environmental science	18	2	-	-	-

Entomology	9	4	-	1	-
Agronomy and Soil science	8	2	1	-	1
Microbiology	7	-	2	-	-
Total, n.	113	18	5	12	2
Total, %	75.4	12.0	3.3	8.0	1.3

The quota of permanent research staff per document can be used as an indicator of the degree of self-sufficiency of the Department in the long period. Indeed, the total number of authors per documents is based on the permanent and temporary research staff of the department plus the external collaborations. In 2015, the average self-sufficiency degree of DAFNAE was 37%, ranging from 20 % in the Microbiology category to 49% in the Animal science category.

These results indicate the importance of the temporary research staff and external collaborations to guarantee both a high research quality and a high scientific productivity in the future.

Extensive and widespread international and national collaborations are guaranteed by 25 prestigious scientific research institutions in Italy, and by international research centers of high scientific level, located in more than 18 countries in 5 continents.

The internationalization process of DAFNAE is growing up fast with many initiatives, together with many new partnerships and collaborations with excellent research centers around the world, with particular attention to those located in countries such as Australia, Brazil and China, which are considered for the governance of the University of Padova among the priorities for scientific collaboration.

Table 2. Distribution of frequency of scientific documents, number of authors and % of ownership per document, for scientific category (Scopus database).

Category	# of documents	% of total documents	% of documents with foreign co-authors	Average # of authors per document *	Average % of ownership per document **
Animal science	29	19.4	24.1	7.0 (min. 3 – max 16)	49 (min. 10–max 100)
Genetics and Genomics	23	15.3	56.5	5.6 (min. 3 – max 13)	37 (min. 8 – max 75)
Food science and technology	22	14.7	13.6	4.9 (min. 2 – max 9)	45 (min. 11 – max 80)

Plant science	21	14.0	52.4	7.2 (min. 4 – max 16)	28 (min. 9 – max 50)
Ecology and Envi-ronmental science	20	13.3	40.0	4.6 (min. 2 – max 8)	28 (min. 13 – max 50)
Entomology	14	9.3	50.0	4.4 (min. 3 – max 7)	45 (min. 20 – max 100)
Agronomy and Soil science	12	8.0	41.7	5.9 (min. 2 – max 11)	27 (min. 9 – max 67)
Microbiology	9	6.0	33.3	7.0 (min. 3 – max 10)	20 (min. 0 – max 40)
TOTAL	150	100	38.0	5.6 (min. 1 – max 16)	37 (min. 0 – max 100)

* Total number of authors is based on permanent and temporary research staff of the department plus the external collaborations.

** Ownership is considered by counting the permanent research staff of University of Padova (researchers, associated professors and full professors) over the total number of authors per document.

In conclusion, this edition of the DAFNAE research yearbook aims to give a snapshot of the main research activities carried out in 2015, with a special analysis on the trends realized in the last 4 years of activity of DAFNAE and with a special focus on the quality of the research documents, the role of the young collaborators and temporary staff on the total productivity, together with the external collaborations. This report would like to inspire the future DAFNAE research and its further improvement under a collaborative perspective. A special acknowledgment to all those who have worked at all levels to qualify the research activities of DAFNAE and a great thank to those who have allowed the realization of this volume.

Martino CASSANDRO

DAFNAE RESEARCH STAFF

Full Professors

Name	Lastname	Phone	Email
ANDREA	BATTISTI	0498272804	andrea.battisti@unipd.it
ANTONIO	BERTI	0498272828	antonio.berti@unipd.it
GIOVANNI	BITTANTE	3204347297	giovanni.bittante@unipd.it
MAURIZIO	BORIN	0498272838	maurizio.borin@unipd.it
SERGIO	CASELLA	0498272922	sergio.casella@unipd.it
MARTINO	CASSANDRO	0498272666	martino.cassandro@unipd.it
ANDREA	CURIONI	0498272624	andrea.curioni@unipd.it
CARLO	DUSO	0498272805	carlo.duso@unipd.it
LUIGI	GALLO	0498272657	luigi.gallo@unipd.it
MARGHERITA	LUCCHIN	0498272816	margherita.lucchin@unipd.it
ROBERTO	MANTOVANI	0498272661	roberto.mantovani@unipd.it
GIULIANO	MOSCA	0498272830	giuliano.mosca@unipd.it
SERENELLA	NARDI	0498272911	serenella.nardi@unipd.it
GIUSEPPINA	PELLIZZARI	0498272806	giuseppina.pellizzari@unipd.it
MAURIZIO	RAMANZIN	0498272654	maurizio.ramanzin@unipd.it
PAOLO	SAMBO	0498272860	paolo.sambo@unipd.it
STEFANO	SCHIAVON	0498272644	stefano.schiavon@unipd.it
GEROLAMO	XICCATO	0498272639	gerolamo.xiccato@unipd.it
GIUSEPPE	ZANIN	0498272819	giuseppe.zanin@unipd.it

Associate Professors

Name	Lastname	Phone	Email
GIANNI	BARCACCIA	0498272814	gianni.barcaccia@unipd.it
MARINA	BASAGLIA	0498272921	marina.basaglia@unipd.it
STEFANO	BONA	0498272831	stefano.bona@unipd.it
CLAUDIO	BONGHI	0498272844	claudio.bonghi@unipd.it

ALESSIO	CECCHINATO	0498272646	alessio.cecchinato@unipd.it
GIUSEPPE	CONCHERI	3351012466	giuseppe.concheri@unipd.it
VIVIANA	CORICH	0498272925	viviana.corich@unipd.it
MASSIMO	DE MARCHI	0498272627	massimo.demarchi@unipd.it
MASSIMO	FACCOLI	0498272891	massimo.faccoli@unipd.it
ROSSELLA	GHISI	0498272910	rossella.ghisi@unipd.it
ALESSIO	GIACOMINI	0498272924	alessio.giacomini@unipd.it
ANNA	LANTE	0498272920	anna.lante@unipd.it
MARIO	MALAGOLI	0498272908	mario.malagoli@unipd.it
ANTONIO	MASI	0498272932	antonio.masi@unipd.it
FRANCESCO	MORARI	0498272857	francesco.morari@unipd.it
GABRIELLA	PASINI	0498272919	gabriella.pasini@unipd.it
ANDREA	PITACCO	0498272848	andrea.pitacco@unipd.it
BENEDETTO	RUPERTI	0498272845	benedetto.ruperti@unipd.it
MICHELE	SCOTTON	0498272833	michele.scotton@unipd.it
ANDREA	SQUARTINI	0498272923	squart@unipd.it
ENRICO	STURARO	0498272641	enrico.sturaro@unipd.it
FRANCO	TAGLIAPIETRA	0498272622	franco.tagliapietra@unipd.it
TEOFILO	VAMERALI	0498272861	teofilo.vamerali@unipd.it
SERENA	VAROTTO	0498272858	serena.varotto@unipd.it

Researchers with permanent position

Name	**Lastname**	**Phone**	**Email**
ALESSANDRO	BOTTON	0498272849	alessandro.botton@unipd.it
PAOLO	CARLETTI	0498272934	paolo.carletti@unipd.it
ANTONELLA	CRAPISI	0498272918	antonella.crapisi@unipd.it
CHIARA	DE FASSI NEGRELLI RIZZI	0498272625	chiara.rizzi@unipd.it
GIOVANNA	LOMOLINO	0498272917	giovanna.lomolino@unipd.it
STEFANO	MACOLINO	0498272834	stefano.macolino@unipd.it
LORENZO	MARINI	0498272807	lorenzo.marini@unipd.it

ROBERTA	MASIN	0498272884	roberta.masin@unipd.it
LUCA	MAZZON	0498272813	lmazzon@unipd.it
NICOLA	MORI	0498272802	nicola.mori@unipd.it
MAURO	PENASA	0498272629	mauro.penasa@unipd.it
SILVIA	QUAGGIOTTI	0498272913	silvia.quaggiotti@unipd.it
RINA	VERDIGLIONE	0498272635	rina.verdiglione@unipd.it
SIMONE	VINCENZI	0438453052	simone.vincenzi@unipd.it
GIAMPAOLO	ZANIN	0498272902	paolo.zanin@unipd.it

Researchers with temporary position

Name	**Lastname**	**Phone**	**Email**
NICOLA	DAL FERRO	0498272818	nicola.dalferro@unipd.it
LORENZO	FAVARO	0498272800	lorenzo.favaro@unipd.it
GIULIO	GALLA		giulio.galla@unipd.it
MATTEO	MARANGON		matteo.marangon@unipd.it
FRANCO	MEGGIO		franco.meggio@unipd.it
CARLO	NICOLETTO	0498272826	carlo.nicoletto@unipd.it
ALBERTO	POZZEBON	0498272798	alberto.pozzebon@unipd.it
CRISTINA	SARTORI		cristina.sartori@unipd.it
PIERGIORGIO	STEVANATO	0498272938	stevanato@unipd.it
ALESSANDRO	VANNOZZI		alessandro.vannozzi@unipd.it

DAFNAE technical and administrative staff

Name	**Lastaname**	**Phone**	**Email**
SARA	BACELLE	0498272881	sara.bacelle@unipd.it
LOREDANA	BALLIN	0498272930	loredana.ballin@unipd.it
ELISA	BARZON	0498272631	elisa.barzon@unipd.it
ALICE	BELLUCO	0498272825	alice.belluco@unipd.it
MASSIMO	CAGNIN	0498272927	massimo.cagnin@unipd.it

CARLO	CAMAROTTO	0498272837	carlo.camarotto@unipd.it
VALERIO	CARDILLO	0498272843	valerio.cardillo@unipd.it
MILENA	CARLOT	0438453743	milena.carlot@unipd.it
LORENZO	CAROTTA	0498272577	lorenzo.carotta@unipd.it
ELISABETTA	CARRARO	0498272840	elisabetta.carraro@unipd.it
LUCA	CARRARO		luca.carraro@unipd.it
RAFFAELLA	CATTARINUSSI	0498272542	raffaella.cattarinussi@unipd.it
LAURA	CECCHINATO	0498272940	laura.cecchinato@unipd.it
ROBERTO	CHIMETTO	0498272571	roberto.chimetto@unipd.it
SANTA	DA MOLIN	0498272672	viva.damolin@unipd.it
FLAVIO	DA RONCH		flavio.daronch@unipd.it
CLAUDIA	DAL BUONO	0498272897	claudia.dalbuono@unipd.it
PATRIZIA	DALL’ARA	0498272801	patrizia.dallara@unipd.it
ROBERTO	DEGAN	0498272578	roberto.degan@unipd.it
FLAVIO	FACCHINELLI	0498272842	flavio.facchinelli@unipd.it
DONATELLA	FAGOTTO	0498272836	donatella.fagotto@unipd.it
VITTORIO	FERRARI	0498272852	vittorio.ferrari@unipd.it
DEBORAH	FRANCESCHI	0438453052	deborah.franceschi@unipd.it
LORENZO	GIORATO	0498272568	lorenzo.giorato@unipd.it
VALTER	GIRALDO	0498272568	valter.giraldo@unipd.it
LUCA	GRIGOLETTO	0498272678	luca.grigoletto@unipd.it
SIMONE	GUZZON	0498272815	simone.guzzon@unipd.it
ROSARIO	LA BIUNDA	0498272866	rosario.labiunda@unipd.it
LUCIANO	MAGRO	0498272672	luciano.magro@unipd.it
ADRIANO	MASSIGNAN	0498272578	adriano.massignan@unipd.it
LAMARIKA	MOLINARI	0498272939	lamarika.molinari@unipd.it
STEFANO	MONNI	0498272586	stefano.monni@unipd.it
ROSALBA	MORO	0498272617	rosalba.moro@unipd.it
LAURA	NICOLETTI	0498272851	laura.nicoletti@unipd.it
PAOLO	PAOLUCCI	0498272809	paolo.paolucci@unipd.it
ALBERTO	PASIMENI	0498272649	alberto.pasimeni@unipd.it

DIEGO	PIZZEGHELLO	0498272915	diego.pizzeghello@unipd.it
VALENTINO	PIZZOCCHERO	0498272926	valentino.pizzocchero@unipd.it
RICCARDO	POLESE		riccardo.polese@unipd.it
ANGELA	RASORI	0498272843	angela.rasori@unipd.it
NICOLETTA	REDIGOLO	0498272539	nicoletta.redigolo@unipd.it
MARTINA	SALMASO	0498272664	martina.salmaso@unipd.it
ALBERTO	SIMONETTO	0498272568	alberto.simonetto@unipd.it
STEFANO	‘NETTI’ TIOZZO	0498272663	stefano.tiozzo@unipd.it
ARCANGELA	TONO	0498272835	arcangela.tono@unipd.it
ANNARITA	TRENTIN	0498272937	annarita.trentin@unipd.it
TIZIANA	TURRA	0498272671	tiziana.turra@unipd.it
MARA	VEGRO	0498272933	mara.vegro@unipd.it
STEFANIA	ZANNONI	0498272928	stefania.zannoni@unipd.it

Agronomy and soil science

Application of smoothed particle hydrodynamics (SPH) and pore morphologic model to predict saturated water conductivity from X-ray CT imaging in a silty loam Cambisol

Dal Ferro, N.[a], *Strozzi, A.G.*[b], *Duwig, C.*[c], *Delmas, P.*[d], *Charrier, P.*[e], *Morari, F.*[a]

[a] Department of Agronomy, Food Natural resources, Animals and Environment,
Agripolis University of Padova, Viale Dell'Università 16, Legnaro, Padova, Italy
[b] UNAM, CCADET, Ciudad Universitaria, Mexico Cuidad, Mexico
[c] IRD/UJF-Grenoble 1/CNRS/G-INP, LTHE, UMR 5564, BP 53, Grenoble Cedex 9, France
[d] Department of Computer Science, The University of Auckland, Private Bag 92019,
New Zealand
[e] Laboratoire 3S-R, CNRS/UMR/UJF/INP BP 53, Grenoble Cedex 9, France

Abstract

This study aims to estimate saturated hydraulic conductivity in a silty loam soil and compare modelled data with experimental ones. The flow characteristics of twelve undisturbed soil cores (5 cm in diameter x 6 cm high) were measured in the laboratory after performing X-ray computed microtomography (microCT) analysis. MicroCT 3D imaging was integrated with an existing pore morphologic model and a numerical simulation based on mesh-free smoothed particle hydrodynamics (SPH) to calculate the water flow through the macropore network (pores > 40μm). Results showed that the proposed SPH method was able to predict hydraulic conductivity of large-sized samples as falling in the range of the experimental ones. By contrast the morphologic model generally underestimated the water flow and was slightly affected by the pore shape. Increasing microCT imaging resolution and expanding the variability with other soil types will improve the understanding of the role of micropore size and morphology on water conductivity.

Keywords: Pore size distribution; Saturated hydraulic conductivity; Soil structure; X-ray computed microtomography

Geoderma

Volume 255-256, Pages 27-34

Assessing microclimate conditions of surface soil layers to improve weed emergence modelling

Loddo, D. [a], *Masin, R.* [b], *Gasparini, V.* [b], *Meggio, F.* [b], *Pitacco, A.* [b], *Zanin, G.* [b]

[a] Istituto di Biologia Agro-ambientale e Forestale (IBAF), CNR, Legnaro, PD, Italy
[b] Dipartimento di Agronomia Animali Alimenti Risorse Naturali e Ambiente, Università di Padova, Agripolis, Italy

Abstract

Thermal or hydrothermal models for weed emergence prediction are useful tools for Integrated Weed Management (IWM) and require an accumulation of Growing Degree Days (GDD) to be calculated through the comparison of base temperature for germination with daily average soil temperature. Consequently, the accuracy of measurements or estimations of soil temperature, which has a strong depth-dependant variability, affects the predictive quality. Emergence models for arable conditions adopt soil temperature measured at a depth of 3-5 cm, but this may not be adequate for no-till conditions. Daily and hourly soil temperatures were measured at depths of 2, 5, 10, 20 and 50 cm during the period of weed emergence and the respective means (T_d and T_h) were calculated. Accumulations of GDD were computed for *Abutilon theophrasti*, *Chenopodium album* and *Sorghum halepense* with values of T_d and T_h measured at depths of 2, 5 and 10 cm. The emergence curves calculated for each species with the AlertInf model were compared to identify estimation variability due to the adoption of values of soil temperature measured at different depths. Despite the great depth-dependent differences observed for T_d and T_h, differences among the emergence curves estimated for each species were not significant from the point of view of weed control. The adoption of T_h measured at a depth of 5 cm could be the best compromise to guarantee model accuracy without complicating measurements. However, further studies are required to adjust and calibrate models developed for arable fields to no-till conditions.

Keywords: Integrated weed management; Soil temperature profile; Thermal time; Weed emergence modelling

Italian Journal of Agrometeorology

Volume 20, Issue 2, Pages 19-26

Characterization of chemical–physical, structural and morphological properties of biochars from biowastes produced at different temperatures

Pituello, C.[a], Francioso, O.[b], Simonetti, G.[a], Pisi, A.[b], Torreggiani, A.[c], Berti, A.[a], Morari, F.[a]

[a] Dipartimento di Agronomia Animali Alimenti Risorse Naturali e Ambiente, Università di Padova, Viale dell'Università 16, Padova, Italy
[b] Dipartimento di Scienze Agrarie, Università di Bologna, V.le Fanin 40, Bologna, Italy
[c] ISOF, Consiglio Nazionale delle Ricerche, Via Gobetti 101, Bologna, Italy

Abstract

Purpose: Biochar production from biowastes (e.g. digestate) is currently one of the more innovative and unexplored fields of research. A complete characterization of these materials, also according to the production temperature, would be a key tool to assess their potential use as soil amendments. Material and methods: For this purpose, five feedstocks (sewage sludge, municipal organic waste, cattle manure and silage digestates, poultry litter and vineyard pruning residues) were pyrolyzed at different temperatures. Structural and morphological transformations of biomasses during heating were followed by using FT-IR, scanning electron microscopy (SEM) and hyperspectral enhanced dark-field microscopy, a novel technique that provides both spectral and spatial information in one measurement. In addition, biochar microstructure (i.e. surface area and pore size distribution) using CO_2 and N_2 adsorption isotherms was investigated. Specific density was also analysed by a helium pycnometer. Results and discussion: Biochars exhibited considerable chemical, structural and morphological differences depending on temperature and feedstock type. Moreover, specific density and surface area increased with the temperature. In particular, heating was able to produce a sharp increase of mesopore and micropore volume especially at 450 and 550 °C, but with different intensities for each feedstock. Thanks to the hyperspectral analysis, distinctive spectral patterns depending on the biochar chemical composition as well as the spatial distribution of the components were found. Conclusions: The results demonstrated that, from a physical–chemical point of view, it is not possible to identify an "ideal" biochar able to improve both soil nutrient content and structure. On the contrary, depending on feedstocks and temperature, each biochar exhibits specific features that would make it suitable for a specific purpose.

Keywords: Biochar; Chemical–physical properties; Feedstocks; Temperature pyrolysis

Journal of Soils and Sediments

Volume 15, Issue 4, Pages 792-804

Combining crop sensing and simulation modeling to assess withinfield corn nitrogen stress

Zanella, V.[a], *Ortiz, B.V.*[b], *Thorp, K.*[c], *Morari, F.*[a], *Mosca, G.*[a], *Hoogenboom, G.*[d]

[a] Dipartimento di Agronomia Animali Alimenti Risorse Naturali e Ambiente, Università di Padova, Padova, Italy
[b] Crop, Soil and Environmental Sciences, Auburn University, Auburn, AL, United States
[c] Arid Land Agricultural Research Center, USDA ARS, Maricopa, AZ, United States
[d] Biological Systems Engineering, Washington State University, Prosser, DC, United States

Abstract

Increasing nitrogen (N) use efficiency could be possible by better in-season N fertilization management. The goal of this study was to test and develop a methodology for combining normalized difference vegetation index data and simulation modeling to assess spatial variability of corn N stress and in-season N rate. Using 2008-2009 data from five corn fields located in north Italy, spatial modeling calibration and simulation were conducted in the CERES-Maize model in DSSAT using the interface with the Geospatial Simulation (GeoSim) tool in the Quantum GIS software. Spatial simulation of yield variability and N stress were possible.

Keywords: Canopy sensing; Corn; Nitrogen management; Spatial simulation modeling

Precision Agriculture 2015 - Papers Presented at the 10th European Conference on Precision Agriculture, ECPA 2015

Pages 391-398

Effect of incident rainfall redistribution by maize canopy on soil moisture at the crop row scale

Martello, M.[a], Dal Ferro, N.[a], Bortolini, L.[b], Morari, F.[a]

[a] Department of Agronomy, Food, Natural resources, Animals and Environment, Agripolis - University of Padova, Viale Dell'Università 16, Legnaro (Padova), Italy
[b] Department of Land, Environment, Agriculture and Forestry, Agripolis - University of Padova, Viale Dell'Università 16, Legnaro (Padova), Italy

Abstract

The optimization of irrigation use in agriculture is a key challenge to increase farm profitability and reduce its ecological footprint. To this context, an understanding of more efficient irrigation systems includes the assessment of water redistribution at the microscale. This study aimed to investigate rainfall interception by maize canopy and to model the soil water dynamics at row scale as a result of rain and sprinkler irrigation with HYDRUS 2D/3D. On average, 78% of rainfall below the maize canopy was intercepted by the leaves and transferred along the stem (stemflow), while only 22% reached the ground directly (throughfall). In addition, redistribution of the water with respect to the amount (both rain and irrigation) showed that the stemflow/throughfall ratio decreased logarithmically at increasing values of incident rainfall, suggesting the plant capacity to confine the water close to the roots and diminish water stress conditions. This was also underlined by higher soil moisture values observed in the row than in the inter-row at decreasing rainfall events. Modelled data highlighted different behavior in terms of soil water dynamics between simulated irrigation water distributions, although they did not show significant changes in terms of crop water use efficiency. These results were most likely affected by the soil type (silty-loam) where the experiment was conducted, as it had unfavorable physical conditions for the rapid vertical water movement that would have increased infiltration and drainage.

Keywords: Hydrus 2D/3D; Irrigation; Maize; Soil water content; Stemflow

Water (Switzerland)

Volume 7, Issue 5, Pages 2254-2271

Fatty acid methyl ester (FAME) succession in different substrates as affected by the co-application of three pesticides

Cardinali, A., Pizzeghello, D., Zanin, G.

Department of Agronomy, Food, Natural Resources, Animals and Environment,
DAFNAE, University of Padua, Viale dell'Università, Legnaro, PD, Italy

Abstract

Introduction: In intensive agriculture areas the use of pesticides can alter soil properties and microbial community structure with the risk of reducing soil quality. Materials and Methods: In this study the fatty acid methyl esters (FAMEs) evolution has been studied in a factorial lab experiment combining five substrates (a soil, two aged composts and their mixtures) treated with a co-application of three pesticides (azoxystrobin, chlorotoluron and epoxiconazole), with two extraction methods, and two incubation times (0 and 58 days). FAMEs extraction followed the microbial identification system (MIDI) and ester-linked method (EL). Results and Discussion: The pesticides showed high persistence, as revealed by half-life ($t_{1/2}$) values ranging from 168 to 298 days, which confirms their recalcitrance to degradation. However, $t_{1/2}$ values were affected by substrate and compost age down to 8 days for chlorotoluron in S and up to 453 days for epoxiconazole in 12M. Fifty-six FAMEs were detected. A nalysis of variance (ANOVA) showed that the EL method detected a higher number of FAMEs and unique FAMEs than the MIDI one, whereas principal component analysis (PCA) highlighted that the monosaturated 18:1ω9c and cyclopropane 19:0ω10c/19ω6 were the most significant FAMEs grouping by extraction method. The cyclopropyl to monoenoic acids ratio evidenced higher stress conditions when pesticides were applied to compost and compost+soil than solely soil, as well as with final time. Conclusion: Overall, FAMEs profiles showed the importance of the extraction method for both substrate and incubation time, the $t_{1/2}$ values highlighted the effectiveness of solely soil and the less mature compost in reducing the persistence of pesticides.

PLoS ONE

Volume 10, Issue 12, e0145501

From real soils to 3D-printed soils: reproduction of complex pore network at the real size in a silty-loam soil

Dal Ferro, N., Morari, F.

Dep. of Agronomy, Food, Natural Resources, Animals and Environment, Agripolis, Univ. of Padova, Viale Dell'Università 16, Legnaro (Padova), Italy

Abstract

Pore complexity and micro-heterogeneity are pivotal in characterizing biogeochemical processes in soils. Recent advances in X-ray computed microtomography (microCT) allow the 3D soil morphology characterization of undisturbed samples, although its geometrical reproduction at very small spatial scales is still challenging. Here, by combining X-ray microCT with 3D multijet printing technology, we aimed to evaluate the reproducibility of 3D-printing soil structures at the original scale with a resolution of 80 mm and compare the hydraulic properties of original soil samples with those obtained from the soil-like prototypes. Results showed that soil-like prototypes were similar to the original samples in terms of total porosity and pore shape. By contrast the pore connectivity was reduced by incomplete wax removal from pore cavities after the 3D printing procedure. Encouraging results were also obtained in terms of hydraulic conductivity since measurements were successfully conducted on five out of six samples, showing positive correlation with experimental data. We are confident that future developments of 3D-printing technologies and of their combination with microCT will help to further the understanding of soil micro-heterogeneity and its effects on soil-water dynamics.

Keywords: 3D printers; Complex networks; Computerized tomography; Printing; Soil moisture

Soil Science Society of America Journal

Volume 79, Issue 4, Pages 1008-1017

Low stabilization of aboveground crop residue carbon in sandy soils of Swedish long-term experiments

Poeplau, C.[ab], Kätterer, T.[a], Bolinder, M.A.[a], Börjesson, G.[c], Berti, A.[d], Lugato, E.[e]

[a] Swedish University of Agricultural Sciences (SLU), Department of Ecology, Box 7044, Uppsala, Sweden
[b] Thuenen Institute of Climate-Smart Agriculture, Bundesallee 50, Braunschweig, Germany
[c] Swedish University of Agricultural Sciences (SLU), Department of Soil and Environment, Box 7014, Uppsala, Sweden
[d] Dipartimento di Agronomia Animali Alimenti Risorse Naturali e Ambiente, Università di Padova, viale dell'Università 16, Legnaro, Padova, Italy
[e] European Commission, Joint Research Centre, Institute for Environment and Sustainability, via E. Fermi 2749, Ispra, VA, Italy

Abstract

Soil organic carbon (SOC) storage can be increased by incorporating crop residues such as straw. However, the use of straw as a bioenergy source is an alternative option. There is currently great uncertainty concerning the effects of residue incorporation/removal, but estimates can be improved by using well-documented, frequently sampled long-term experiments (LTEs). This study examined the effect of straw incorporation on SOC stocks in six Swedish LTEs (duration of 27-56 years), using data from 5 to 28 sampling occasions. A total of 16 pairs of straw incorporation (SI) and straw removal (SR) treatments were compared and modelled with the ICBM/2 model (two young pools with distinct humification coefficients, h), which enabled us to clearly isolate the effect of straw carbon input. The model results were compared to the Ultuna frame trial, as the first and only parameterization site of ICBM. At five out of six sites, the humification coefficient for straw (h_{litter}) was much smaller (0-0.09) than the ICBM default h value for plant material (0.125). The derived h_{litter} values and thus the stabilization of straw derived carbon increased significantly with clay content. An Italian site with five pairs of SI and SR treatments was used to test the performance of ICBM/2 under contrasting pedoclimatic conditions. Similar to the Swedish sites, the best model fits were found with hlitter values ranging from 0 to 0.05 increasing with nitrogen fertilization (range of 0-240 kg N ha^{-1} yr^{-1}), which was attributed to changes in substrate use efficiency of microbes. However, this trend was not consistent for all sites. For future applications of ICBM/2, we suggest using the validated clay function to derive h_{litter} for common levels of nitrogen fertilization. The efficiency of incorporating straw to increase SOC stocks depends on soil texture and using it for bioenergy production could be a more sustainable and climate-smart option.

Keywords: Arable soil; Carbon sequestration; Crop residue management; Humification coefficient; ICBM; Soil carbon modelling

Geoderma

Volume 237, Pages 246-255

Magnetic purification of curcumin from *Curcuma longa* rhizome by novel naked maghemite nanoparticles

Magro, M.[ab], Campos, R.[c], Baratella, D.[a], Ferreira, M.I.[c], Bonaiuto, E.[a], Corraducci, V.[a], Uliana, M.R.[c], Lima, G.P.P.[c], Santagata, S.[d], Sambo, P.[d], Vianello, F.[ab]

[a] Department of Comparative Biomedicine and Food Safety, University of Padua, Agripolis, Viale dell'Università 16, Legnaro, (PD), Italy
[b] Regional Centre of Advanced Technologies and Materials, Department of Physical Chemistry, Palacky University in Olomouc, Olomouc, Czech Republic
[c] Department of Chemistry and Biochemistry, Universidade Estadual Paulista (UNESP), Botucatu, São Paulo, Brazil
[d] Department of Agronomy, Food, Natural Resources, Animals and Environment, University of Padua, Legnaro, PD, Italy

Abstract

Naked maghemite nanoparticles, namely, surface active maghemite nanoparticles (SAMNs), characterized by a diameter of about 10 nm, possessing peculiar colloidal stability, surface chemistry, and superparamagnetism, present fundamental requisites for the development of effective magnetic purification processes for biomolecules in complex matrices. Polyphenolic molecules presenting functionalities with different proclivities toward iron chelation were studied as probes for testing SAMN suitability for magnetic purification. Thus, the binding efficiency and reversibility on SAMNs of phenolic compounds of interest in the pharmaceutical and food industries, namely, catechin, tyrosine, hydroxytyrosine, ferulic acid, coumaric acid, rosmarinic acid, naringenin, curcumin, and cyanidin-3-glucoside, were evaluated. Curcumin emerged as an elective compound, suitable for magnetic purification by SAMNs from complex matrices. A combination of curcumin, demethoxycurcumin, and bis-demethoxycurcumin was recovered by a single magnetic purification step from extracts of *Curcuma longa* rhizomes, with a purity >98% and a purification yield of 45%, curcumin being >80% of the total purified curcuminoids.

Keywords: *Curcuma longa*; Curcumin; Curcuminoids; Magnetic nanoparticles; Magnetic purification; Polyphenols

Journal of Agricultural and Food Chemistry

Volume 63, Issue 3, Pages 912-920

Modeling soil–plant dynamics: Assessing simulation accuracy by comparison with spatially distributed crop yield measurements

Manoli, G.[a], *Bonetti, S.*[b], *Scudiero, E.*[c], *Morari, F.*[d], *Putti, M.*[e], *Teatini, P.*[f]

[a] Nicholas School of the Environment, Duke Univ, Durham, NC, United States
[b] Pratt School of Engineering, Duke Univ, Durham, NC, United States
[c] USDA–ARS, US Salinity Lab, Riverside, CA, United States
[d] Dep. of Agronomy, Food, Natural Resources, Animals and Environment, Univ. of Padova, Padova, Italy
[e] Dep. of Mathematics, Univ. of Padova, Padova, Italy
[f] Dep. of Civil, Architectural and Environmental Engineering, Univ. of Padova, Padova, Italy

Abstract

Coupling hydrological models with plant physiology is crucial to capture the feedback mechanisms occurring within the soil–plant–atmosphere continuum. However, the ability of such models to describe the spatial variability of plant responses to different environmental factors remains to be proven, especially at large scales (field or watershed). We used an innovative threedimensional soil–plant model to quantify temporal and spatial variability of crop productivity at the field scale, and we assessed simulation accuracy by comparison with spatially distributed crop yield measurements. A 25-ha field located in the Venice coastland, Italy, cultivated with a maize (*Zea mays* L.) crop and characterized by a highly heterogeneous soil subject to salt contamination, has been extensively studied by soil sampling, geophysical surveys, and hydrological monitoring. Based on these observations, field-scale simulations of soil moisture dynamics coupled with plant transpiration, photosynthesis, and growth were run and compared with crop yield maps of different growing seasons. The model captured the observed crop productivity (grain yield varying between 2 and 15 Mg ha^{-1}), but the accuracy of the predicted spatial patterns was limited by the available information on soil heterogeneities. Further model uncertainties are related to the characterization of the rooting systems and their responses to environmental factors (soil characteristics, precipitation) that were shown to be crucial to describe the effect of drought conditions on growth processes. These results demonstrate that large-scale mechanistic simulations of soil–plant systems require a trade-off between site characterization, model processes, and computational efficiency, offering an open challenge for future ecohydrological research.

Vadose Zone Journal

Volume 14, Issue 12, 13p

Protein hydrolysates as biostimulants in horticulture

Colla, G.[a], Nardi, S.[b], Cardarelli, M.[c], Ertani, A.[b], Lucini, L.[d], Canaguier, R.[e], Rouphael, Y.[f]

[a] Department of Agriculture, Forestry, Nature and Energy, University of Tuscia, via San Camillo De Lellis snc, Viterbo, Italy
[b] Department of Agronomy, Food, Natural Resources, Animals and Environment. University of Padova, Agripolis, Legnaro, Italy
[c] Consiglio per la Ricerca in Agricoltura e l'analisi dell'economia agraria, Centro di ricerca per lo studio delle Relazioni tra Pianta e Suolo, Via della Navicella 2-4, Roma, Italy
[d] Institute of Environmental and Agricultural Chemistry, Università Cattolica del Sacro Cuore, Piacenza, Italy
[e] Nixe Laboratory, Sophia Antipolis Cedex, France
[f] Department of Agricultural Sciences, University of Naples Federico II, V

Abstract

In recent years, new strategies have been proposed in order to improve the sustainability of production systems for horticultural crops. A promising tool would be the use of substances and/or microorganisms defined also as 'biostimulants' able to enhance crop quality parameters, nutrient efficiency and abiotic stress tolerance. Protein hydrolysates (PHs) are an important group of plant biostimulants based on a mixture of peptides and amino acids that have received increasing attention in the recent years due to their positive effects on crop performances. PHs are mainly produced by enzymatic and/or chemical hydrolysis of proteins from animal- or plant-derived raw materials. The current review gives an overview of the biostimulant properties of PHs on productivity and product quality of horticultural crops, in particular fruit trees, vegetables, flower crops and ornamentals. After a brief introduction on PHs as plant biostimulants, this review focuses on the classification and chemical composition of PHs according to the source of proteins and method of protein hydrolysis. The plant uptake and transport of amino acids and peptides and the effects of PHs on primary and secondary metabolism as well as the biochemical and physiological processes conferring tolerance to abiotic stress are also covered. The review concludes by proposing several perspectives for future research aiming to understand the mode of action of PHs based on their composition and also to define the suitable time and dose of application.

Keywords: Amino acids; Biostimulants; Enzymatic hydrolysis; Horticultural crops; Peptides; Product quality

Scientia Horticulturae

Volume 196, Pages 28-38

Spontaneous aggregation of humic acid observed with AFM at different pH

Colombo, C.[a], Palumbo, G.[a], Angelico, R.[a], Cho, H.G.[b], Francioso, O.[c], Ertani, A.[d], Nardi, S.[d]

[a] Dipartimento Agricoltura Ambiente Alimenti (DAAA), Università del Molise, Via De Sanctis 86100, Campobasso (CB), Italy
[b] Department of Geology and Research Institute of Natural Science, Gyeongsang National University, Jinju, South Korea
[c] Dipartimento di Scienze Agrarie (DipSA), Università di Bologna, V.le Fanin 40, Bologna, Italy
[d] Dipartimento di Agronomia Animali Alimenti Risorse Naturali e Ambiente - DAFNAE, Viale dell'Università 16, Agripolis, Legnaro, Italy

Abstract

Atomic force microscopy in contact (AFM-C) mode was used to investigate the molecular dynamics of leonardite humic acid (HA) aggregate formed at different pH values. HA nanoparticles dispersed at pH values ranging from 2 to 12 were observed on a mica surface under dry conditions. The most clearly resolved and well-resulted AFM images of single particle were obtained at pH 5, where HA appeared as supramolecular particles with a conic shape and a hole in the centre. Those observations suggested that HA formed under these conditions exhibited a pseudo-amphiphilic nature, with secluded hydrophobic domains and polar subunits in direct contact with hydrophilic mica surface. Based on molecular simulation methods, a lignin-carbohydrate complex (LCC) model was proposed to explain the HA ring-like morphology. The LCC model optimized the parameters of -O-4 linkages between 14 units of 1-4 phenyl propanoid, and resulted in an optimized structure comprising 45-50 linear helical molecules looped spirally around a central cavity. Those results added new insights on the adsorption mechanism of HA on polar surfaces as a function of pH, which was relevant from the point of view of natural aggregation in soil environment.

Keywords: Atomic force microscopy (AFM); Humic acid; Leonardite; Lignin-carbohydrate complex (LCC) model; Molecular conformation; Particle size

Chemosphere

Volume 138, Pages 821-828

Animal science

Associative effects of poor-quality forages combined with food industry byproducts determined *in vitro* with an automated gas-production system

Tagliapietra, F.[a], *Cattani, M.*[b], *Guadagnin, M.*[b], *Haddi, M.L.*[c], *Sulas, L.*[d], *Muresu, R.*[d], *Squartini, A.*[a], *Schiavon, S.*[a], *Bailoni, L.*[b]

[a] Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, 35020, Legnaro, PD, Italy
[b] Department of Comparative Biomedicine and Food Science (BCA), University of Padova, Viale dell'Università 16, 35020, Legnaro, PD, Italy
[c] Département de Biochimie et Microbiologie, Université de Constantine, Route Ain El Bey, 25000, Constantine, Algeria
[d] CNR, Istituto per Il Sistema Produzione Animale in Ambiente Mediterraneo (ISPAAM), Traversa la Crucca 3, Località Baldinca 07100 Li Punti, Sassari, Italy

Abstract

This experiment aimed to investigate the associative effects among two low-quality forages (crown daisy, milk thistle) and three agro-industrial byproducts (apple pomace, citrus pulp, tomato peel), by means of an automated gas production (GP) system. All feeds were incubated alone or as 50:50 mixtures of each forage with each byproduct. Samples (0.500 ± 0.0010 g) of single feeds or mixtures were incubated for 96 h, in three replicates in individual bottles (310 mL), with 75 mL of buffered rumen fluid. Bottles were vented by an open-close valve when the internal pressure reached 3.4 kPa. The metabolisable energy content of single feeds and mixtures was computed from GP at 24 h and feed chemical composition. Feed substrates were ranked for GP in the following way: byproducts, mixtures, and forages. The two forages did not differ for GP and metabolisable energy content, although differences were observed among byproducts and among mixtures. Both forages interacted positively with apple pomace from 6 h ($P < 0.001$) to 24 h ($P = 0.029$) of incubation and with citrus pulp at 12 h ($P = 0.005$) and 24 h ($P = 0.012$), whereas no associative effects were detected when forages were incubated with tomato peels. Results suggest that *in vitro* fermentability of low-quality forages could be efficiently improved by combining these two forages with apple pomace or citrus pulp. These findings are relevant, because the use of low-quality forages and byproducts in ruminant feeding is considered important for improving the environmental and economic sustainability of forage systems in arid and semi-arid areas.

Keywords: Feed combination; Food byproducts; *In vitro* gas production; Low-quality forages.

Animal Production Science

Volume 55, Issue 9, Pages 1117-1122

A comparison of milk clotting characteristics and quality traits of Rendena and Holstein-Friesian cows

Varotto, A., De Marchi, M., Penasa, M., Cassandro, M.

Dipartimento di Agronomia, Animali, Alimenti, Risorse naturali e Ambiente, Università di Padova, Italy

Abstract

Milk coagulation properties (MCP) and composition, as predicted by mid-infrared spectroscopy (MIRS), were compared between Rendena (RE) local breed and Holstein- Friesian (HF) cows using 4614 individual milk samples from 28 single-breed herds. Records of rennet coagulation time (RCT, min), curd firmness (a_{30}, mm), daily milk yield and quality traits were analysed using a linear mixed model which included fixed effects of breed, herd-test-date nested within breed, lactation stage, parity, and two-way interactions between the main effects. Random effects were cow nested within breed and residual. Milk from RE coagulated 2.1 min earlier and showed a firmer curd by 4.8 mm than that of HF cows ($P<0.05$). Milk yield (+9.7 kg/d) and fat content (+0.22 g/100 g) were greater for HF than RE ($P<0.05$), while protein (+0.05 g/100 g) and casein (+0.06 g/100 g) contents were greater in milk from RE cows ($P<0.05$). Rennet coagulation time was shortest at the beginning of lactation, and a_{30} was firmest at the beginning and end of lactation. Results from this study suggest that milk of RE is more suitable for cheese processing than that of HF cows. Milk clotting characteristics of the local breed should be taken into account when developing strategies useful for its valorisation.

Keywords: Local cattle breed; Mid-infrared spectroscopy; Milk coagulation

Italian Journal of Animal Science

Volume 14, Issue 2, Pages 202-206

Bayesian regression models outperform partial least squares methods for predicting milk components and technological properties using infrared spectral data

Ferragina, A.[a]*, de los Campos, G.*[bc]*, Vazquez, A.I.*[b]*, Cecchinato, A.*[a]*, Bittante, G.*[a]

[a] Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, 35020 Legnaro, Italy
[b] Epidemiology and Biostatistics Department, Michigan State University, East Lansing 48824
[c] Department of Statistics and Probability, Michigan State University, East Lansing 48824

Abstract

The aim of this study was to assess the performance of Bayesian models commonly used for genomic selection to predict "difficult-to-predict" dairy traits, such as milk fatty acid (FA) expressed as percentage of total fatty acids, and technological properties, such as fresh cheese yield and protein recovery, using Fourier-transform infrared (FTIR) spectral data. Our main hypothesis was that Bayesian models that can estimate shrinkage and perform variable selection may improve our ability to predict FA traits and technological traits above and beyond what can be achieved using the current calibration models (e.g., partial least squares, PLS). To this end, we assessed a series of Bayesian methods and compared their prediction performance with that of PLS. The comparison between models was done using the same sets of data (i.e., same samples, same variability, same spectral treatment) for each trait. Data consisted of 1,264 individual milk samples collected from Brown Swiss cows for which gas chromatographic FA composition, milk coagulation properties, and cheese-yield traits were available. For each sample, 2 spectra in the infrared region from 5,011 to 925 cm^{-1} were available and averaged before data analysis. Three Bayesian models: Bayesian ridge regression (Bayes RR), Bayes A, and Bayes B, and 2 reference models: PLS and modified PLS (MPLS) procedures, were used to calibrate equations for each of the traits. The Bayesian models used were implemented in the R package BGLR (http://cran.r-project.org/web/packages/BGLR/index.html), whereas the PLS and MPLS were those implemented in the WinISI II software (Infrasoft International LLC, State College, PA). Prediction accuracy was estimated for each trait and model using 25 replicates of a training-testing validation procedure. Compared with PLS, which is currently the most widely used calibration method, MPLS and the 3 Bayesian methods showed significantly greater prediction accuracy. Accuracy increased in moving from calibration to external validation methods, and in moving from PLS and MPLS to Bayesian methods, particularly Bayes A and Bayes B. The maximum R^2 value of validation was obtained with Bayes B and Bayes A. For the FA, C10:0 (% of each FA on total FA basis) had the highest R^2 (0.75, achieved with Bayes A and Bayes B), and among the technological traits, fresh cheese yield R^2 of 0.82 (achieved with Bayes B). These 2 methods have proven to be useful instruments in shrinking and selecting very informative wavelengths and inferring the structure and functions of the analyzed traits. We conclude that Bayesian models are powerful tools for deriving calibration equations, and, importantly, these equations can be easily developed using existing open-source software. As part of our study, we provide scripts based on the open source R software BGLR, which can be used to train customized prediction equations for other traits or populations.

Keywords: Bayesian method; Cheese yield; Fatty acid; Infrared spectroscopy; Milk trait

Journal of Dairy Science

Volume 98, Issue 11, Pages 8133-8151

Canine indolent and aggressive lymphoma: clinical spectrum with histologic correlation

Aresu, L.[a], Martini, V.[b], Rossi, F.[c], Vignoli, M.[d], Sampaolo, M.[c], Aricò, A.[a], Laganga, P.[c], Pierini, A.[c], Frayssinet, P.[e], Mantovani, R.[f], Marconato, L.[c]

[a] Department of Comparative Biomedicine and Food Science, University of Padova, Legnaro, Italy
[b] Department of Veterinary Sciences and Public Health, University of Milan, Milan, Italy
[c] Centro Oncologico Veterinario, Sasso Marconi, Italy
[d] Clinica Veterinaria dell'Orologio, Sasso Marconi, Italy
[e] Urodelia, St Lys, France
[f] Department of Agronomy Food Natural Resources Animals & Environment, University of Padova, Legnaro, Italy

Abstract

Sixty-three dogs with newly diagnosed lymphoma underwent complete staging and received the same chemotherapy. Diffuse large B-cell lymphoma was the leading histotype (44.4%), followed by peripheral T-cell lymphoma (20.6%). Indolent lymphomas accounted for 30.2% of cases. Most dogs with aggressive B-cell lymphoma had stage IV disease. Dogs with indolent and aggressive T-cell lymphoma had more often stage V disease and were symptomatic. Liver and bone marrow were predominantly involved in B-cell and T-cell lymphoma, respectively. The clinical stage was significantly related to substage, sex and total lactic dehydrogenase (LDH) levels. Aggressive B-cell lymphomas were more likely to achieve remission. Median survival was 55 days for aggressive and indolent T-cell lymphoma, 200 and 256 days for indolent and aggressive B-cell lymphoma, respectively. The prognosis of advanced indolent lymphoma does not appear to be appreciably different from that of aggressive disease. Familiarity with the various histotypes is critical to make the correct diagnosis and drive therapy.

Keywords: Clinical stage; Dog; Histological subtype; Lymphoma

Veterinary and Comparative Oncology

Volume 13, Issue 4, Pages 348-362

Carcass characteristics and meat quality traits of the Padovana chicken breed, a commercial line, and their cross

Cassandro, M., De Marchi, M., Penasa, M., Rizzi, C.

Dipartimento di Agronomia, Animali, Alimenti, Risorse naturali e Ambiente, University of Padua, Italy

Abstract

The objective of this study was to compare the Padovana Camosciata local chicken breed (PC; n=59), the slow-growing line Berlanda-Gaina (BG; n=62), and their cross (BGxPC; n=57) for carcass and meat quality features. Animals were reared under the same experimental conditions and slaughtered at 3 different ages. An analysis of variance was performed on carcass and meat traits using a linear model that included fixed effects of genotype, sex, age at slaughter, and interactions between them. The PC local breed was approximately 1 kg lighter (P<0.001) at slaughter and exhibited greater dressing percentage (+1.50%; P<0.05) than BG. Breast skin of PC was bluer (-2.74; P<0.001), and breast muscle was darker (-2.65; P<0.001) and redder (+0.48; P<0.001) than that of BG. The pH (+0.16; P<0.001), thawing (+0.90%; P<0.01) and cooking (+2.28%; P<0.001) losses determined on breast muscle were higher for PC than BG. Crossbred animals performed better than the average of BG and PC chickens for breast weight (+22.81 g; P<0.01) and dressing percentage (+1.38%; P<0.05). Breast skin of BGxPC was darker (-1.74; P<0.05), less red (-0.23; P<0.05), and bluer (-1.54; P<0.01) than the average of BG and PC, and breast muscle was more yellow (+0.64; P<0.05) for BGxPC. Cooking losses were lower (-0.99%; P<0.05) for crossbred than the average of BG and PC chickens. Results confirmed the specificity of meat characteristics of PC local breed and demonstrated the potential benefit of crossbreeding to improve production traits of PC breed without compromising the peculiar quality of its meat.

Keywords: Chicken; Crossbreeding; Local breed; Meat quality

Italian Journal of Animal Science

Volume 14, Issue 3, Pages 304-309

Carcass quality and uniformity of heavy pigs fed restrictive diets with progressive reductions in crude protein and indispensable amino acids

Gallo, L.[a], *Dalla Montà, G.*[a], *Carraro, L.*[a], *Cecchinato, A.*[a], *Carnier, P.*[b], *Schiavon, S.*[a]

[a] Department of Agronomy Food Natural Resources Animals and Environment, University of Padova, Viale Università 16, 35020 Legnaro, PD, Italy
[b] Department of Comparative Biomedicine and Food Science, University of Padova, Viale Università 16, 35020 Legnaro, PD, Italy

Abstract

This study investigated the effects of four dietary treatments characterized by 0 to 20% progressive reduction of the dietary crude protein (CP) and indispensable amino acid (AA) contents on carcass quality and uniformity of pigs fed restrictively and slaughtered around 165 kg body weight (BW). Carcass data from 233 pigs from a feeding study that involved 3 batches of 80 crossbred pigs each were used. Pigs, offspring of 12 boars, were assigned to 1 of 4 dietary treatments (10 pigs per pen, gilts and barrows, and 2 pens per treatment in each batch) with diets formulated to contain 146 to 117 and 133 to 108 g/kg of CP and 7.3 to 5.8 and 5.7 to 4.7 g/kg of total Lys in early (90 to 130 kg BW) and late (130 to 165 kg BW) finishing, respectively. After slaughter, the carcasses were processed and the weight of the commercial lean (neck, loin, shoulder, and ham) and fat cuts (backfat, belly, and jowl) was recorded. The coefficient of variation was used to describe uniformity of the most important carcass traits, and the corresponding confidence intervals were computed to make comparisons across dietary treatments. Carcass weight and midline backfat thickness averaged 137 kg and 31 mm, respectively, and were not affected by diets. Diets did not influence the weight of commercial cuts and their proportion on carcass weight, with the sole exception of loins. Pigs fed diets containing the 2 lowest CP content had a slight lower proportion of loins in the carcass compared with pigs fed the conventional CP diet ($P < 0.05$). Most carcass traits were affected by sex and by sire effects, but interactions between diet and sex or sire was only episodic. Uniformity of carcass weight, backfat thickness, and weight of loins and dressed hams was not influenced by the dietary treatments. In conclusion, the content of CP, Lys, and other indispensable AA of conventional diets for finishing heavy pigs may be reduced by 20% without impairing the weight of carcass and primal cuts and the yields of dressed hams. This feeding strategy will contribute to the heavy pig industry by reducing feed costs (i.e., decreasing the dietary provision of soybean meal and crystalline AA) and minimizing the nitrogen excretion, without affecting carcass quality.

Keywords: Amino acid content; Carcass quality; Crude protein content; Heavy pigs; Nutrition

Livestock Science

Volume 172, Pages 50-58

Comparison of rumen fluid inoculum vs. faecal inoculum on predicted methane production using a fully automated *in vitro* gas production system

Ramin, M.[a], *Lerose, D.*[b], *Tagliapietra, F.*[b], *Huhtanen, P.*[a]

[a] Department of Agricultural Research for Northern Sweden, Swedish University of Agricultural Sciences, SE-901 83 Umeå, Sweden
[b] Università degli studi di Padova, DAFNAE, viale dell'università 16, 35020 Padova, Italy

Abstract

The aim of the present study was to compare *in vitro* methane (CH_4) production and other fermentation parameters from different substrates incubated either in rumen or faecal inoculum. Five different substrates were incubated in two different inocula and gas recordings were made using an automated *in vitro* gas production system. The substrates were: particulate matter obtained from reticulo-rumen (RR) digesta and faeces (FC) by wet sieving, timothy hay (H), first cut grass silage (S) and a mixture of grass silage and barley (50:50; SB). One gram of each substrate was incubated either in 60 ml buffered rumen or faecal inoculum taken from Swedish lactating dairy cows for 48 h. The results indicated that *in vitro* total gas production, predicted *in vivo* CH_4 production and the ratio of CH_4 production to total gas production were greater ($P < 0.01$) for substrates incubated in rumen inoculum as compared to faecal inoculum. Mean of predicted *in vivo* CH_4 production was greater for substrates incubated in rumen inoculum (23.5 ml/g DM, 29.0 ml/g OM) as compared to faecal inoculum (11.2 ml/g DM, 14.3 ml/g OM). Predicted CH_4 production based on volatile fatty acids (VFA) stoichiometry equations (CH_4VFA) showed no difference in CH_4 per mol VFA ($P = 0.44$) between the two sources of inoculum used for all substrates. Molar proportions of propionate were higher and that of butyrate were lower ($P < 0.01$) for all substrates incubated in faecal inoculum compared to rumen inoculum. No difference ($P = 0.13$) in molar proportions of acetate was observed. Digestibility of neutral detergent fibre (aNDFomD) and true organic matter digestibility (TOMD) were lower ($P < 0.01$) for all substrates incubated in faecal inoculum compared to rumen inoculum. It can be concluded that using faecal inoculum tended to give lower values of predicted *in vivo* CH_4 production as compared to rumen inoculum. The discrepancy between observed and stoichiometric CH_4 production suggests an existence of acetogenesis in the hindgut.

Keywords: Faeces; Hindgut fermentation; Methane; Ruminants; VFA

Livestock Science

Volume 181, Pages 65-71

Determination by GCxGC of fatty acid and conjugated linoleic acid (CLA) isomer profiles in six selected tissues of lambs fed on pasture or on indoor diets with and without rumen-protected CLA

Pellattiero, E., Cecchinato, A., Tagliapietra, F., Schiavon, S., Bittante, G.

Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy

Abstract

In this study GCxGC was used to study the effects of pasture, hay, concentrate (indoor), and indoor plus 8 g/day of a rumen-protected conjugated linoleic acid (indoor-CLA) diets on the detailed fatty acid (FA) profiles of six tissues (muscles, fatty tissues, and liver) collected from 36 lambs. This powerful technique allowed the quantification of 128 FAs, of which 21 SFAs, 16 MUFAs, 19 PUFAs were identified by reference standards. The diets had similar, but not identical, effects on FA profiles (g/100 g FA) in the various tissues, as both indoor diets reduced total PUFAs (from 8.91 ± 6.27 to 8.06 ± 5.97; $p < 0.05$) and n-3 PUFAs (from 2.70 ± 2.37 to 1.50 ± 1.69; $p < 0.01$) and increased n-6 PUFA (from 3.76 ± 2.46 to 4.58 ± 3.42; $p < 0.01$), branched (from 2.37 ± 2.05 to 3.23 ± 0.54; $p < 0.01$), odd-chain FAs (from 5.88 ± 5.33 to 7.07 ± 1.51; $p < 0.01$) compared to pasture. Indoor-CLA increased CLA*c*9,*t*11 (from 0.42 ± 0.13 to 0.53 ± 0.19; $p < 0.01$), CLA*t*10,*c*12 (from 0.07 ± 0.06 to 0.12 ± 0.22; $p < 0.05$), and CLAc11,*t*13 (from 0.02 ± 0.04 to 0.05 ± 0.04; $p < 0.05$) compared to indoor.

Keywords: Fatty acids; Lambs; Rumen-protected conjugated linoleic acid; Tissues; Two-dimensional gas chromatography

Journal of Agricultural and Food Chemistry

Volume 63, Issue 3, Pages 963-974

Effect of dairy farming system, herd, season, parity, and days in milk on modeling of the coagulation, curd firming, and syneresis of bovine milk

Bittante, G., Cipolat-Gotet, C., Malchiodi, F., Sturaro, E., Tagliapietra, F., Schiavon, S., Cecchinato, A.

Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, 35020 Legnaro, Italy

Abstract

The objectives of this study were to characterize the variation in curd firmness model parameters obtained from coagulating bovine milk samples, and to investigate the effects of the dairy system, season, individual farm, and factors related to individual cows (days in milk and parity). Individual milk samples (n = 1,264) were collected during the evening milking of 85 farms representing different environments and farming systems in the northeastern Italian Alps. The dairy herds were classified into 4 farming system categories: traditional system with tied animals (29 herds), modern dairy systems with traditional feeding based on hay and compound feed (30 herds), modern dairy system with total mixed ration (TMR) that included silage as a large proportion of the diet (9 herds), and modern dairy system with silage-free TMR (17 herds). Milk samples were analyzed for milk composition and coagulation properties, and parameters were modeled using curd firmness measures (CF_t) collected every 15 s from a lacto-dynamographic analysis of 90 min. When compared with traditional milk coagulation properties (MCP), the curd firming measures showed greater variability and yielded a more accurate description of the milk coagulation process: the model converged for 93.1% of the milk samples, allowing estimation of 4 CF_t parameters and 2 derived traits [maximum CF (CF_{max}) and time from rennet addition to CF_{max} (t_{max})] for each sample. The milk samples whose CF_t equations did not converge showed longer rennet coagulation times obtained from the model (RCT_{eq}) and higher somatic cell score, and came from less-productive cows. Among the sources of variation tested for the CF_t parameters, dairy herd system yielded the greatest differences for the contrast between the traditional farm and the 3 modern farms, with the latter showing earlier coagulation and greater instant syneresis rate constant (k_{SR}). The use of TMR yielded a greater t_{max} because of a higher instant curd-firming rate constant (k_{CF}). Season of sampling was found to be very important, yielding higher values during winter for all traits except k_{CF} and k_{SR}. All CF_t traits were affected by individual cow factors. For parity, milk produced by first-lactation cows showed higher k_{CF} and k_{SR}, but delays in achieving CF_{max}. With respect to stage of lactation, RCT_{eq} and potential asymptotic CF increased during the middle of lactation and stabilized thereafter, whereas the 2 instant rate constants presented the opposite pattern, with the lowest (k_{CF}) and highest (k_{SR}) values occurring in mid lactation. The new challenge offered by prolonging the test interval and individual modeling of milk technological properties allowed us to study the effects of parameters related to the environment and to individual cows. This novel strategy may be useful for investigating the genetic variability of these new coagulation traits.

Keywords: Curd firming; Dairy system; Milk coagulation; Modeling; Syneresis

Journal of Dairy Science

Volume 98, Issue 4, Pages 2759-2774

Animal science

Effect of genotype, gender and feed restriction on growth, meat quality and the occurrence of white striping and wooden breast in broiler chickens

Trocino, A.[a], Piccirillo, A.[a], Birolo, M.[b], Radaelli, G.[a], Bertotto, D.[a], Filiou, E.[b], Petracci, M.[c], Xiccato, G.[b]

[a] Department of Comparative Biomedicine and Food Science (BCA), University of Padova, Viale dell'Università 16, I-35020 Legnaro, Padova, Italy
[b] Department of Agronomy Food Natural Resources Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, I-35020 Legnaro, Padova, Italy
[c] Department of Agricultural and Food Sciences, Alma Mater Studiorum, University of Bologna, I-47521 Cesena, Forlì Cesena, Italy

Abstract

Due to their importance for the control of meat quality in broiler chickens, the present study aimed at identifying the factors associated with the occurrence of myopathies and characterizing the meat properties when affected by myopathies. To this aim, a total of 768 broiler chickens were reared until slaughter (46 d) to evaluate the effect of genotype, gender, and feeding regime (ad libitum vs. restricted rate, 80% from 13 to 21 d of age) on performance and meat quality. Standard broilers were heavier (3,270 vs. 3,139 g; $P < 0.001$) and showed lower feed conversion (1.56 vs. 1.61; $P < 0.001$) than the high-yield broilers. Males showed higher final live weight (3,492 vs. 2,845 g) and lower feed conversion (1.54 vs. 1.63) than females ($P < 0.001$). Feed restriction decreased final live weight (3,194 vs. 3,142 g; $P < 0.01$) and feed conversion (1.60 vs. 1.57; $P < 0.01$) compared to ad libitum feeding. At gross examination, feed restriction tended to increase white-striped breasts (69.5 vs. 79.5%; $P < 0.10$), whereas females showed less wooden breasts than males (8.0 vs. 16.3%; $P < 0.05$). White-striped fillets had higher pHu (5.87 vs. 5.83), and lower a* (-0.81 vs. -0.59) and b* color indexes (13.7 vs. 14.5) ($P < 0.05$), whereas wooden breast fillets exhibited higher cooking losses (25.6 vs. 22.1%) and AK-shear force (4.23 vs. 2.84 kg/g) compared with normal fillets ($P < 0.001$). At histological examination, 3.1% of pectoralis major were normal, 26.6% mildly degenerated, 45.3% moderately degenerated, and 25.0% severely degenerated. In conclusion, genotype had a moderate effect on growth without modifying myopathy occurrence. In contrast, gender and feed restriction affected performance, meat quality, and breast abnormalities.

Keywords: Growth; Histology; Meat quality; Myopathies

Poultry Science

Volume 94, Issue 12, Pages 2996-3004

Effect of protein shortage and conjugated linoleic acid supplementation on quality traits and modelling of coagulation, curd firming and syneresis of Holstein-Friesian milk

Cesaro, G., Schiavon, S.

University of Padova, Department of Agronomy, Food, Natural resources, Animals and
Environment (DAFNAE), Viale dell'Università 16, Legnaro (PD), Italy

Abstract

Aim of the present study was to evaluate the effect of diets with optimal (CP 15% DM) or suboptimal (CP 12.3% DM) protein content, supplemented (CLA+) or not (CLA-) with rumen-protected conjugated linoleic acid (rpCLA) on some cheesemaking properties. Twenty Holstein-Fresian mid lactating dairy cows have been reared following a 4x4 Latin square experimental design of 4 periods, 3 weeks each. Individual milk samples, collected during the third week of each period, were analysed for chemical composition, traditional milk coagulation properties (MCP: RCT, k_{20} and a_{30}) and for recording curd firmness (CF) every 15 s over a 90 min period. Data acquired from each sample were used to model CF over time calculating the following parameters: rennet coagulation time (RCT_{eq}), asymptotic potential CF (CF_P), CF rate constant (k_{CF}), syneresis rate constant (k_{CF}), maximum CF achieved within 90 min (CF_{max}) and time to CF_{max} (t_{max}). Data were analysed using period, diet and group (random) as sources of variation. Cows evidenced a strong individual variability within groups and were classified as early (RCT<20 min) or late (RCT>20 min) coagulating cows. Dietary protein shortage reduced milk protein and lactose content, while rpCLA supplementation depressed milk fat synthesis. Results showed that traditional MCP parameters were worsened by reduction of dietary protein in the case of milk produced by early coagulating cows, while rpCLA supplementation affected negatively all three traits on all cows. The study of CF model parameters evidenced that CP12 diets have improved CF (CF_P and CF_{max}) respect to CP15 when fed to late coagulating cows while worsened CF (CF_P and CF_{max}) and reduced k_{CF} when fed to early coagulating cows. The results of the present study underline the complex relationship between dietary fat and protein and their consequences on milk technological properties highlighting the need for further investigations.

Keywords: Bovine milk; Curd firming modelling; Dietary protein; Milk coagulation properties; Rumen-protected conjugated linoleic acid

Poljoprivreda

Volume 21, Issue 1 (Supplement), Pages 78-85

Effects of dairy system, herd within dairy system, and individual cow characteristics on the volatile organic compound profile of ripened model cheeses

Bergamaschi, M.[ab], Aprea, E.[a], Betta, E.[a], Biasioli, F.[a], Cipolat-Gotet, C.[b], Cecchinato, A.[b], Bittante, G.[b], Gasperi, F.[a]

[a] Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach (FEM), Via E. Mach 1, 38010 San Michele all'Adige (TN), Italy
[b] Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy

Abstract

The objective of this work was to study the effect of dairy system, herd within dairy system, and characteristics of individual cows (parity, days in milk, and daily milk yield) on the volatile organic compound profile of model cheeses produced under controlled conditions from the milk of individual cows of the Brown Swiss breed. One hundred fifty model cheeses were selected from 1,272 produced for a wider study of the phenotypic and genetic variability of Brown Swiss cows. In our study, we selected 30 herds representing 5 different dairy systems. The cows sampled presented different milk yields (12.3-43.2 kg/d), stages of lactation (10-412 d in milk), and parity (1-7). In total, 55 volatile compounds were detected by solid-phase microextraction and gas chromatography-mass spectrometry, including 14 alcohols, 13 esters, 11 free fatty acids, 8 ketones, 4 aldehydes, 3 lactones, 1 terpene, and 1 pyrazine. The most important sources of variation in the volatile organic profiles of model cheeses were dairy system (18 compounds) and days in milk (10 compounds), followed by parity (3 compounds) and milk yield (5 compounds). The model cheeses produced from the milk of tied cows reared on traditional farms had lower quantities of 3-methyl-butan-1-ol, 6-pentyloxan-2-one, 2-phenylethanol, and dihydrofuran-2(3H)-one compared with those reared in freestalls on modern farms. Of these, milk from farms using total mixed rations had higher contents of alcohols (hexan-1-ol, octan-1-ol) and esters (ethyl butanoate, ethyl pentanoate, ethyl hexanoate, and ethyl octanoate) and lower contents of acetic acid compared with those using separate feeds. Moreover, dairy systems that added silage to the total mixed ration produced cheeses with lower levels of volatile organic compounds, in particular alcohols (butan-1-ol, pentan-1-ol, heptan-1-ol), compared with those that did not. The amounts of butan-2-ol, butanoic acid, ethyl-2-methylpropanoate, ethyl-3-methylbutanoate, and 6-propyloxan-2-one increased linearly during lactation, whereas octan-1-ol, 3-methyl-3-buten-1-ol, 2-butoxyethanol, 6-pentyloxan-2-one, and 2,6-dimethylpyrazine showed a more complex pattern during lactation. The effect of the number of lactations (parity) was significant for octan-1-ol, butanoic acid, and heptanoic acid. Finally, concentrations of octan-1-ol, 2-phenylethanol, pentanoic acid, and heptanoic acid increased with increasing daily milk yield, whereas dihydrofuran-2(3H)-one decreased. In conclusion, the volatile organic compound profile of model cheeses from the milk of individual cows was affected by dairy farming system and stage of lactation and, to lesser extent, by parity and daily milk yield.

Keywords: Aroma; Cheese quality; Lactation stage; Milk yield; Solid-phase microextraction/gas chromatography-mass spectrometry (SPME/GC-MS)

Journal of Dairy Science

Volume 98, Issue 4, Pages 2183-2196

Effects of floor type, stocking density, slaughter age and gender on productive and qualitative traits of rabbits reared in collective pens

Trocino, A.[a], *Filiou, E.*[b], *Tazzoli, M.*[b], *Birolo, M.*[b], *Zuffellato, A.*[c], *Xiccato, G.*[b]

[a] Department of Comparative Biomedicine and Food Science (BCA), University of Padova, Viale dell'Università 16, I-35020 Legnaro, Padova, Italy
[b] Department of Agronomy Food Natural Resources Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, I-35020 Legnaro, Padova, Italy
[c] Veronesi Verona S.p.A., Via Valpantena 18/G, I-37142 Quinto di Valpantena, Verona, Italy

Abstract

At 34 days of age, 376 crossbred rabbits of both sexes were housed in 16 open-top collective pens (1.68 m^2) according to a 2x2x2 factorial arrangement with two types of pen floor (plastic v. wooden slatted), two stocking densities (12 v. 16 animals/m^2) and two slaughter ages (76 v. 83 days). The rabbits were examined for growth performance, slaughter results and meat quality. The effect of gender was also examined. The percentage of rabbits with wounds due to aggression varied with stocking density (8.2% v. 26.2% for 12 v. 16 animals/m^2; $P \leq 0.001$), slaughter age (15.0% v. 22.0% at 76 v. 83 days; $P \leq 0.10$) and gender (11.3% v. 25.8% for females v. males; $P \leq 0.001$). Rearing rabbits on a plastic rather than a wooden slatted floor promoted slaughter weight (2795 v. 2567 g; $P \leq 0.001$), dressing percentage (61.4% v. 60.9%; $P \leq 0.01$), dissectible fat (2.4% v. 2.0%; $P \leq 0.01$) and hind leg muscle-to-bone ratio (5.81 v. 5.35; $P \leq 0.001$). Increased stocking density impaired daily growth (38.5 v. 35.9 g/day; $P \leq 0.05$) and feed intake (140 v. 134 g/day; $P \leq 0.01$) during the second period (55 days to slaughter) and decreased slaughter weight (2725 v. 2637 g; $P \leq 0.01$). At the older slaughter age, the feed conversion ratio was impaired (2.98 v. 3.18; $P \leq 0.001$); the slaughter weight (2574 g v. 2788 g; $P \leq 0.001$), dissectible fat (2.0% v. 2.4%; $P \leq 0.01$) and hind leg muscle-to-bone ratio (5.41 v. 5.75; $P \leq 0.01$) increased; meat thawing losses, cooking losses and shear force decreased ($P \leq 0.05$). The main differences between the females and males were found in the slaughter for transport losses (2.6% v. 2.2%; $P \leq 0.01$) and longissimus lumborum proportions (13.0% v. 12.4%; $P \leq 0.01$). In conclusion, the growth performance of pen-housed rabbits was largely determined by the type of floor and less affected by stocking density. The meat quality depended on ontogenetic factors, such as slaughter age and gender, and not on housing conditions. The differences in the percentages of wounded animals owing to experimental factors deserve further investigation from the perspective of animal welfare issues.

Keywords: Gender; Growth performance; Meat quality; Pen housing; Stocking density

Animal

Volume 9, Issue 5, Pages 855-861

Growth performance, and carcass and raw ham quality of crossbred heavy pigs from four genetic groups fed low protein diets for dry-cured ham production

Schiavon, S.[a], Carraro, L.[a], Dalla Bona, M.[a], Cesaro, G.[a], Carnier, P.[b], Tagliapietra, F.[a], Sturaro, E.[a], Galassi, G.[c], Malagutti, L.[c], Trevisi, E.[d], Crovetto, G.M.[c], Cecchinato, A.[a], Gallo, L.[a]

[a] Department of Agronomy, Food, Natural Resources, Animals and Environment, University of Padua, 35020 Legnaro, PD, Italy
[b] Department of Comparative Biomedicine and Food Science (BCA), University of Padua, 35020 Legnaro, PD, Italy
[c] Department of Agricultural and Environmental Sciences, University of Milan, 20133 Milan, Italy
[d] Institute of Zootechnics, Faculty of Agriculture, Food and Environmental Science, Università Cattolica del Sacro Cuore, via Emilia Parmense 84, 29122 Piacenza, Italy

Abstract

This study investigated growth performance, and carcass and raw ham quality of pigs from 4 genetic groups (GG), ANAS (A), DanBred (D), Goland (G) and Topig (T), fed conventional (CONV) or low-protein (LP) diets. In each of 3 trials, 96 pigs were housed in 8 pens in groups of 12 on the basis of their GG, sex (gilts and barrows), and BW. Each pen was assigned a CONV or LP diet. The CONV diets in early (89-120 kg BW) and late (121-165 kg BW) finishing contained 13.1 and 13.2. MJ/kg of ME, 147 and 132 g CP/kg, and 6.0 and 4.4 g/kg of standardized ileal digestible (SID) lysine, respectively. The LP diets in early and late finishing contained only 119 and 103 g/kg of CP, and 4.8 and 3.5 g/kg of SID lysine, respectively. Restricted feed allowance was increased on a weekly basis from 2.3 to 3.2 kg/d during the experiment. Automated feeding stations measured individual feed intake, and pigs were weighed at 3-weeks intervals until slaughter. Hot carcass was weighed and dissected into lean and fat primal cuts. After 24 h of chilling, hams were dressed, weighed, and scored for roundness (0 = low to 4 = high), fat cover thickness (-4 = thin to 4 = thick), marbling (0 = absent to 4 = evident), lean color (-4 = pale to 4 = dark), bicolor and veining (0 = absent to 4 = evident). In addition, ham backfat thickness was measured with a ruler. Dietary CP reduction did not influence final BW (166 kg), daily gain (0.67 kg/d), carcass weight (136 kg) or yield (0.82), but reduced feed efficiency by 5.2% ($P <$ 0.001). Irrespective of GG, the reduction in protein supply increased fat cover depth ($P < 0.001$) and marbling ($P = 0.009$) of hams, but did not influence other traits. Compared to A, hams from D were heavier (+6.4%; $P < 0.001$), and had a thinner cover fat (19.4 vs 24.8 mm, $P = 0.001$), a greater marbling score (2.05 vs 1.44 points; $P < 0.001$) and a paler lean color (-0.61 vs 0.34 points; $P < 0.001$). Ham quality of A, G and T was comparable, but the marbling score of T was higher and similar to that of D. The use of low-protein diets would reduce the environmental impact of heavy pig production with some improvement in ham quality (> subcutaneous fat depth), although increased marbling is expected. They should preferably be used with GGs that have low marbling scores under conventional conditions.

Keywords: Carcass; Dry-cured ham; Growth; Low-protein diets; Pig

Animal Feed Science and Technology

Volume 208, Pages 170-181

Influence of N shortage and conjugated linoleic acid supplementation on some productive, digestive, and metabolic parameters of lactating cows

Schiavon, S., Cesaro, G., Tagliapietra, F., Gallo, L., Bittante, G.

Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, Legnaro (PD), Italy

Abstract

The effects of a shortage of dietary N and of a rumen protected CLA (rpCLA) supplement on DMI, rumination, rumen fluid characteristics, milk yield (MY), and milk N/N intake ratio (ENU) were studied in mid-late lactating cows. Twenty cows housed in 4 pens in groups of 5, homogeneous for parity, days in milk (DIM) and MY, were fed on 4 different diets: with 150 g (CP15) or 123 g (CP12) of CP/kg DM, with or without an rpCLA supplement (containing 6.34 g/d of C18:2*c*9,*t*11 and 6.14 g/d of C18:2*t*10,*c*12). A 4 x 4 Latin Square experimental design was used with periods of 3 wks, although the sequence of the 4 treatments (CP15 or CP12, with or without rpCLA) was such that each group received CP15 or CP12 for 6 consecutive wks. The CP12 diet was formulated from CP15 by replacing soybean meal with barley grain to maintain similar energy, fiber content and feed particle size. Rumination activity, DMI, and MY were recorded daily. Rumen fluid was analyzed for VFA and ammonia N content, and milk for quality traits. Nutrient digestibility was estimated using Lignin(sa) as a marker. Period, treatment and group (random) were included as sources of variation in the statistical analysis. Dietary CP restriction tended to reduce DMI (-7.7%; $P = 0.09$) and digestibility, but increased time spent in rumination (+10%; $P = 0.009$), decreased rumen fluid ammonia N (-36%; $P < 0.001$), and reduced MY (-4.8%; $P = 0.047$) and milk protein content (-4.7%, $P = 0.026$); it had no influence on the ratio between energy-corrected milk yield and DMI. CP restriction reduced N intake by 122 g/d and N in milk by 14 g/d, did not influence N in feces, but increased ENU from 0.31 to 0.36 ($P < 0.01$). A low marginal response of 115 g milk N/kg to the increased N intake from soybean meal was found. The addition of rpCLA tended to reduce DMI (-8.1%; $P = 0.07$) and decreased milk fat content (-15%; $P = 0.002$), but decreased N in milk only when added to CP12 (CP x rpCLA interaction, $P = 0.016$). A shortage of N supply increases ENU without apparent alteration of BW, BCS and blood metabolites. Long-term investigations to clarify the role of body N reserves and of a shortage of CP supplies on N partitioning are needed.

Keywords: Conjugated linoleic acid; Dairy cows; Low-protein diet; Milk yield; Nitrogen partition; Rumination

Animal Feed Science and Technology

Volume 208, Pages 86-97

Lungworms in Alpine ibex (*Capra ibex*) in the eastern Alps, Italy: An ecological approach

Cassini, R.[a], Párraga, M.A.[b], Signorini, M.[a], Frangipane di Regalbono, A.[a], Sturaro, E.[b], Rossi, L.[c], Ramanzin, M.[b]

[a] Department of Animal Medicine, Production and Health, University of Padova, Viale dell'Università, 16, 35020 Legnaro, PD, Italy
[b] Department of Agronomy, Food, Natural Resources, Animals and Environment, University of Padova, Viale dell'Università, 16, 35020 Legnaro, PD, Italy
[c] Department of Veterinary Sciences, University of Torino, L.go Braccini, 2, 10095 Grugliasco, TO, Italy

Abstract

Host-parasite relationships have been frequently investigated in mountain dwelling ungulates, though mostly focusing on gastrointestinal nematodes. On the contrary, very few studies were conducted on broncopulmonary nematodes, which may result in severe parenchymal lesions and act as predisposing factor for multifactorial pneumonia. The epidemiological and ecological features of lungworms infecting an Alpine ibex population in the Eastern Alps, Italy, were non-invasively investigated by means of a modified Baermann technique with an original quantitative methodology. Out of a total of 269 samples collected monthly from July to November 2013 and from July to October 2014, 212 (78.8%) were positive for *Muellerius* and 26 (9.7%) for *Protostrongylus*, whereas *Neostrongylus* and *Cystocaulus* were less prevalent (4.1% and 0.7%, respectively). None of the investigated samples tested positive for dictyocaulids. The genus *Muellerius* showed the highest larval output intensity (134.2 L1/g), followed by *Protostrongylus* with 33.8 L1/g. A contrasting age-related pattern of *Muellerius* and *Protostrongylus* was revealed, with the former significantly more prevalent and abundant in adult animals, while the latter in kids. Due to the limited accessibility of the study area during winter and spring, it was difficult to describe clear seasonal trends in larval output, although *Muellerius* showed a minimum in the late summer and a rise in the autumn. The newly developed diagnostic method showed a fair repeatability, thus representing an interesting tool to investigate the ecology of lungworms in protected species, such as the A. ibex. Based on results, ibex in the Marmolada massif seem to have an ecologically stable relationship with their lungworm community.

Keywords: Alpine ibex; Ecology; Italy; Lungworm; Protostrongylidae

Veterinary Parasitology

Volume 214, Issue 1-2, Pages 132-138

Milk coagulation properties of cattle breeds reared in Alpine area

Visentin, G., Penasa, M., Gottardo, P., Niero, G., Isaia, M., Cassandro, M., De Marchi, M.

University of Padova, Department of Agronomy, Food, Natural Resources, Animals and Environment, Viale dell'Universitá 16, 35020 Legnaro (PD), Italy

Abstract

The aim of the present study was to apply mid-infrared spectroscopy prediction models developed for milk coagulation properties (MCP) to a spectral dataset of 123,240 records collected over a 2-year period in the Alpine area, and to investigate sources of variation of the predicted MCP. Mixed linear models included fixed effects of breed, month and year of sampling, days in milk, parity, and the interactions between the main effects. Random effects were herd nested within breed, cow nested within breed, and the residual. All fixed effects were significant (P<0.01) in explaining the variation of MCP. In particular, milk clotting characteristics varied significantly among breeds, and local Alpine Grey breed exhibited the most favourable processing characteristics. Milk coagulation properties varied across lactation and were at their worst after the peak.

Keywords: Local breeds; Mid-infrared spectroscopy; Milk quality

Poljoprivreda

Volume 21, Issue 1 (Supplement), Pages 237-240

Modeling of coagulation, curd firming, and syneresis of milk from Sarda ewes

Vacca, G.M.[a], Pazzola, M.[a], Dettori, M.L.[a], Pira, E.[a], Malchiodi, F.[b], Cipolat-Gotet, C.[b], Cecchinato, A.[b], Bittante, G.[b]

[a] Department of Veterinary Medicine, University of Sassari, Via Vienna 2, 07100 Sassari, Italy
[b] Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, viale dell'Università 16, 35020 Legnaro (PD), Italy

Abstract

This study investigated the modeling of curd-firming (CF) over time (CF_t) of sheep milk. Milk samples from 1,121 Sarda ewes from 23 flocks were analyzed for coagulation properties. Lactodynamographic analyses were conducted for up to 60 min, and 240 CF individual observations from each sample were recorded. Individual sample CF_t equation parameters (RCT_{eq}, rennet coagulation time; CF_P, asymptotic potential value of curd firmness; k_{CF}, curd-firming instant rate constant; and k_{SR}, curd syneresis instant rate constant) were estimated, and the derived traits (CF_{max}, the point at which CF_t attained its maximum level, and t_{max}, the time at which CF_{max} was attained) were calculated. The incidence of noncoagulating milk samples was 0.4%. The iterative estimation procedure applied to the individual coagulation data showed a small number of not-converged samples (4.4%), which had late coagulation and an almost linear pattern of the ascending part of the CF_t curve that caused a high value of CF_P, a low value of k_{CF}, and a high value of k_{SR}. Converged samples were classified on the basis of their CF_t curves into no-k_{SR} (18.0%), low-k_{SR} (72.6%), and high-k_{SR} (4.5%). A CF_t that was growing continuously because of the lack of the syneresis process characterized the no-k_{SR} samples. The high-k_{SR} samples had a much larger CF_P, a smaller k_{CF}, and an anticipation of t_{max}, whereas the low-k_{SR} samples had a fast k_{CF} and a slower k_{SR}. The part of the average CF_t curves that showed an increase was similar among the 3 different syneretic groups, whereas the part that decreased was different because of the expulsion of whey from the curd. The traditional milk coagulation properties recorded within 30 min were not able to detect any appreciable differences among the 4 groups of coagulating samples, which could lead to a large underestimation of the maximum CF of all samples (if predicted by a_{30}), with the exception of the no-k_{SR} samples. Large individual variability was found and was likely caused by the effects of the dairy system, such as flock size (on CF_{max}, t_{max}, and % ewes with no-k_{SR} milk), flock within flock size (representing 11 to 43% of total variance for % ewes with no-k_{SR} milk and CF_{max}, respectively), days in milk (on all model parameters and CF_{max}), parity (on RCT_{eq}, k_{SR}, and CF_{max}), daily milk yield (on RCT_{eq} and CF_{max}), and position of the individual pendulum that significantly affected model parameters and derived traits. In conclusion, the results showed that the modeling of coagulation, curd-firming, and syneresis is a suitable tool to achieve a deeper interpretation of the coagulation and curd-firming processes of sheep milk and also to study curd syneresis.

Keywords: Cheese-making property; Curd-firming modeling; Milk coagulation property; Ovine milk; Syneresis

Journal of Dairy Science

Volume 98, Issue 4, Pages 2245-2259

Nitrogen and energy partitioning in two genetic groups of pigs fed low-protein diets at 130 kg body weight

Galassi, G.[a], *Malagutti, L.*[a], *Colombini, S.*[a], *Rapetti, L.*[a], *Gallo, L.*[b], *Schiavon, S.*[b], *Tagliapietra, F.*[b], *Crovetto G.M.*[a]

[a] Dipartimento di Scienze Agrarie e Ambientali, University of Milan, Italy
[b] Dipartimento di Agronomia Animali Alimenti Risorse Naturali e Ambiente, University of Padua, Italy

Abstract

The aim was to evaluate the effect of lowprotein (LP) or low-amino acid diets on digestibility, energy and nitrogen (N) utilization in 2 genetic groups (GG) of pigs (129±11 kg BW). Duroc Large White (A) pigs were chosen to represent a traditional GG for ham production, and Danbred Duroc (D) pigs to represent a GG with fast growing rate and high carcass lean yield. Dietary treatments: a conventional diet (CONV) containing 13.2% CP, and two LP diets, one with LP (10.4%) and low essential AA (LP1), the second with LP (9.7%) and high essential AA (LP2). Compared to CONV, LP2 had the same essential AA content per unit feed, while LP1 the same essential AA content per unit CP. Feed was restricted (DMI=6.8% $BW^{0.75}$). Four consecutive digestibility/balances periods were conducted with 24 barrows, 12 A and 12 D. Metabolic cages and respiration chambers were used. No significant difference between diets was registered for digestibility. Nitrogen excreted: 41.3, 33.4 and 29.0 g/d (P=0.009), for CONV, LP1 and LP2 diets, respectively. Nitrogen retention was similar between the diets. Heat production (HP) was the lowest for LP diets. There was a tendency (P=0.079) for a lower energy digestibility in D group. The D pigs also had a higher HP and hence a lower retained energy in comparison with the A pigs. In conclusion: it is possible to reduce N excretion using very LP diets and LP low AA diets; Danbred GG have a higher heat production and a lower energy retention than A pigs.

Keywords: Digestibility; Energy; Genotypes; Nitrogen; Pig

Italian Journal of Animal Science

Volume 14, Issue 3, Pages 293-298

Optimizing feed efficiency and nitrogen excretion in growing rabbits by increasing dietary energy with high-starch, high-soluble fibre, low-insoluble fibre supply at low protein levels

Tazzoli, M.[a], *Trocino, A.*[b], *Birolo, M.*[a], *Radaelli, G.*[b], *Xiccato, G.*[a]

[a] Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, I-35020 Legnaro, Padova, Italy
[b] Department of Comparative Biomedicine and Food Science (BCA), University of Padova, Viale dell'Università 16, I-35020 Legnaro, Padova, Italy

Abstract

A total of 282 crossbred rabbits of both genders were divided into six experimental groups and fed *ad libitum* six diets formulated in a 2 x 3 factorial arrangement based on three dietary energy levels (low, approximately 8.45 MJ/kg; medium, 9.9 MJ/kg: and high, 11.5 MJ/kg) and two crude protein levels (15% and 17%). The increase of dietary energy was obtained by a simultaneous increase of dietary starch (9.1%, 13.7%, and 17.9%) and soluble fibre (4.8%, 7.1%, and 9.4%) at the expense of acid detergent fibre (23.0%, 18.0%, and 13.5%). Growth performance and nitrogen excretion from weaning until slaughter, and digestive traits were monitored. The increase of dietary energy enhanced the apparent digestibility of dry matter (50.8%, 60.5%, and 71.5%; $P < 0.001$), gross energy (50.3%, 59.7%, and 70.7%; $P < 0.001$), soluble fibre (73.1%, 77.8%, and 82.9%; $P < 0.10$), and neutral detergent fibre (16.1%, 29.0%, and 48.1%; $P < 0.001$). In the caecum, the total volatile fatty acid (VFA) content increased (63.0, 79.2, and 78.5. mmol/L; $P < 0.05$), and the propionate rate decreased (4.0%, 4.2%, and 3.5% mol VFA; $P < 0.05$); in the jejunum, the villi-to-crypt ratio tended to decrease (4.18, 3.89, and 3.57; $P < 0.10$). The increase of dietary energy supply decreased feed intake (181 g/d, 157 g/d, and 135 g/d; $P < 0.001$), which improved feed conversion ratios (3.98, 3.40, and 2.87; $P < 0.001$), and decreased nitrogen excretion (3.15 g/d, 2.56 g/d, and 1.93 g/d; $P < 0.001$). The increase of dietary energy significantly decreased mortality (15.8%, 4.9%, and 4.9%; $P < 0.05$). When dietary crude protein increased from 15% to 17%, apparent digestibility increased for dry matter, crude protein, total dietary fibre, and gross energy ($P < 0.01$). Growth performance during the trial was not affected, but nitrogen excretion decreased significantly (2.73 g/d vs. 2.37 g/d; $P < 0.001$). In conclusion, the increase of dietary energy by the simultaneous increase of dietary starch and soluble fibre to replace insoluble fibre optimized feed efficiency and controlled nitrogen excretion in growing rabbits with positive effects on digestive traits and health. The reduction in dietary protein level was determined to be an useful strategy to reduce farm nitrogen excretion.

Keywords: Caecal fermentation; Digestive physiology; Growth performance; Health

Livestock Science

Volume 172, Pages 59-68

Prediction of gross feed efficiency in Italian Holstein Friesian bulls

Finocchiaro, R.[a], *Penasa, M.*[b], *Marusi, M.*[a], *De Marchi, M.*[b], *Cassandro, M.*[b]

[a] Italian Holstein Friesian Cattle Breeders Association (ANAFI), Via Bergamo 262, loc. Migliaro, Cremona, Italy
[b] University of Padova, Department of Agronomy, Food, Natural resources, Animals and Environment, Viale dell'Università 16, 35020 Legnaro (PD), Italy

Abstract

The aim of this study was to predict gross feed efficiency of Italian Holstein Friesian bulls selected for production, functional and type traits. A total of 12,238 bulls, from the April 2015 genetic evaluation, were used. Predicted daily gross feed efficiency (pFE) was obtained as ratio between milk yield (MY) and predicted dry matter intake (pDMI). Phenotypic trend for MY, predicted body weight (pBW) and pFE were calculated by the bull birth year. The results suggest that pFE can be successfully selected to increase profitability of dairy cattle using the current milk recording system. Direct measurements on DMI should be considered to confirm results of pFE obtained in the present study.

Keywords: Correlation; Genetic trend; Gross feed efficiency; Holstein Friesian bulls

Poljoprivreda

Volume 21, Issue 1 (Supplement), Pages 83-85

Study of milk protein composition and coagulation properties of Burlina local cattle breed

Niero, G., Penasa, M., De Marchi, M., Visentin, G., Cassandro, M.

University of Padova, Department of Agronomy, Food, Natural Resources, Animals and
Environment, Viale dell'Università 16, 35020 Legnaro (PD), Italy

Abstract

Burlina is a dual-purpose cattle breed, mainly reared in mountain areas of Veneto Region (Italy). The aim of this study was to investigate milk protein composition and milk coagulation properties (MCP) of Burlina breed. A total of 80 individual milk samples were collected and milk casein fractions were detected and quantified with reversed-phase high-performance liquid chromatographic analysis whereas MCP were determined with Formagraph. Sources of casein fractions variation and MCP were investigated using a linear model including herd, parity, and days in milk as fixed effects. Casein fractions showed increasing concentrations across days in milk, but not specific trends across parities. Milk coagulation properties exhibited better values in early than late lactation stages. Also, they deteriorated across parities.

Keywords: Casein fraction; Local breed; Milk coagulation property

Poljoprivreda

Volume 21, Issue 1 (Supplement), Pages 101-104

Sustainable milk production in different dairy cattle systems and valorisation of environmental chain on the basis of added value

Cassandro, M.

University of Padova, Department of Agronomy, Food, Natural Resources, Animals and Environment, Viale dell'Università 16, 35020 Legnaro (PD), Italy

Abstract

Aim of this review is to estimate milk yield and predicted methane emissions added values in local and cosmopolitan cow breeds reared in Italian circumstances. Nowadays it is well known that over the next 50 years, the world's farmers will be asked to produce more food than has been produced in the past thousand years, and in this concern it will be in environmentally sustainable way. The review will higlight the differences between intensive and extensive agricultural systems and this will be discussed and evaluated in dairy cattle production system context. In conclusion, animal genetic resources need to be evaluated not only per unit of output but for other direct and indirect output units related to social and human returns supporting different animal production systems, intensive or extensive ones. The intensive and extensive farming systems are not replaceable to each other, but they should be combined in order to respond to different social and environmental needs, so, to define the best sustainable production system. Moreover, both systems should also consider the modern demands that nowadays agriculture requires as, guarantee for food security. Therefore each system, intensive or extensive, should improve the animal products technological characteristics and at the same time reduce the carbon footprint.

Keywords: Added values; Animal production; Cattle breeds; Production and environmental chains; Sustainable systems

Poljoprivreda

Volume 21, Issue 1 (Supplement), Pages 22-27

Testicular growth and comb and wattles development in three Italian chicken genotypes reared under freerange conditions

Rizzi, C., Verdiglione, R.

Dipartimento di Agronomia, Animali, Alimenti, Risorse naturali e Ambiente, Università di Padova, Italy

Abstract

Male chickens belonging to three Italian purebreds – *Ermellinata di Rovigo* (ER), *Robusta lionata* (RL) and *Robusta maculata* (RM) – were studied. All the birds were reared under the same rearing conditions (from May until autumn). Chickens were reared under infra-red lamps from birth until 4 weeks of age with a 24L:0D photoperiod. Then they were kept outdoor: the photoperiod changed according to the season (from 16L:8D to 12L:12D). At 138 and 168 days of age 20 birds/breed were weighed and then slaughtered. Testicular samples were collected, after evisceration, processed and embedded in paraffine wax. Sections were stained for morphological observations, observed with light microscope, and then classified according to the testis maturation stage. *Ermellinata di Rovigo* chickens showed the lowest ($P<0.01$) body weight and the highest ($P<0.01$) testes weight; testes maturity was higher ($P<0.01$) in ER than in RL, whereas RM was intermediate. For each genotype testes weight and testes maturity did not significantly differ with aging. Correlations between testes weight and body, comb, and wattles weight, according to the breed, were calculated at 168 days of age. For ER no significant correlation was found, whereas RL showed a significant ($P<0.01$) positive relationship between testes weight and body weight, and sexual secondary characters. *Robusta maculata* showed a significant correlation between testes weight and comb ($P<0.01$) and wattles weight ($P<0.10$). Our results suggest that under the studied environmental conditions ER showed the highest testes development according to its more precocious achievement of adult body weight, whereas RL was the least precocious purebred.

Keywords: Chicken; Local breed; Seasonality; Sexual maturity; Testis

Italian Journal of Animal Science

Volume 14, Issue 2, Pages 266-271

The environmental impact of cow milk in the northeast of Italy

Dalla Riva, A.[a]*, Burek, J.*[b]*, Kim, D.*[b]*, Thoma, G.*[b]*, Cassandro, M.*[a]*, De Marchi, M.*[a]

[a] University of Padova, Department of Agronomy, Food, Natural resources, Animals and Environment, Viale dell'Università 16, 35020 Legnaro (PD), Italy
[b] University of Arkansas, Ralph E. Martin Department of Chemical Engineering, 3202 Bell Engineering Center, Fayetteville, AR 72701-1201, United States

Abstract

This study presents a "from cradle to farm gate" Life Cycle Assessment on cow milk produced in Northeast Italy. System boundaries consider milk and meat delivered at farm gate, including all upstream emissions. All farm activities were considered. Inputs and outputs required in one year are counted and information about 34 dairy farms are used to represent the production area. Different allocation approaches were used to share resources and emissions between milk and meat. Functional unit was one kg of raw milk. The Ecoinvent v3.1 and Agri-footprint v1.0 database were used for secondary data, and SimaPro© 8 was the main software in the analysis. The following impact categories were investigated: Climate Change (CC), Terrestrial Acidification (TA), Freshwater Eutrophication (FE), Land Occupation (LO), Water Depletion (WD) and Cumulative Fossil Energy Demand (CFED). Purchased feed production was the first emitter, followed by on-farm crop production, animals and manure management emissions. Considering the most debated impact categories, 1.80-2.19 kg CO_2eq and 8.84-10.78 MJ represent, respectively, CC and CFED per kg of raw milk. This research could be applied in regional studies on environmental impact of Italian dairy production.

Keywords: Dairy farm; Environmental impact; LCA; Milk

Poljoprivreda

Volume 21, Issue 1 (Supplement), Pages 105-108

The physical form of corn influences the rumen bacterial biodiversity – preliminary results

Rossi, G.[a], Tagliapietra, F.[a], Pinloche, E.[b], De La Fuente, G.[c], Schiavon, S.[a], Maccarana, L.[d], Bittante, G.[a], Newbold, C.J.[b]

[a] Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Legnaro (PD), Italy
[b] Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, SY23 3AL, United Kingdom
[c] Department of Animal Production, University of Lleida, Alcalde Rovira Roure 191, 25198 Lleida, Spain
[d] Department of Comparative Biomedicine and Food Science (BCA), University of Padova, Legnaro (PD), Italy

Abstract

The aim of this study was to investigate the rumen bacteria in terms of genetic biodiversity and variation due to different physical form of corn in cow diet. A total of twenty dry cows were fed for 3 months with the same diet, only differed for corn physical form, ten received corn grains, while the other ones received corn flour. To investigate the biodiversity of the bacterial 16S rRNA gene clone library analysis has been conducted and then the sequencing has been carried out using Ion Torrent PGM™ System. Bacterial population was tested using R statistical software. The Kruskal-Wallis one-way analysis of variance (Kruskal-Wallis, 1952) confirmed that the bacterial populations were different when the animals were fed grain compared with flour corn. Both the OTU's abundance (Operational Taxonomic Unit) and the biodiversity indexes presented a significant difference among the two sample groups, underlining the large changes that take place even with small diet modifications in ruminal environment. There is still the need to deepen how exactly the diet changes the rumen phylogenetic structure and the consequences on bacteria's activity.

Keywords: Diet composition; OTUs; Physical form; Rumen bacterial biodiversity

Poljoprivreda

Volume 21, Issue 1 (Supplement), Pages 93-96

The use of 2-dimensional gas chromatography to investigate the effect of rumen-protected conjugated linoleic acid, breed, and lactation stage on the fatty acid profile of sheep milk

Pellattiero, E., Cecchinato, A., Tagliapietra, F., Schiavon, S., Bittante, G.

Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy

Abstract

In this study, 2-dimensional gas chromatography (GC x GC) was used to obtain a detailed fatty acid (FA) profile of sheep milk and to evaluate the effects of a rumen-protected conjugated linoleic acid (rpCLA) supply, breed, days in milk (DIM), sampling period, and number of lambs suckling on the FA profile. Twenty-four ewes, from 3 autochthonous breeds of the Veneto Alps (Brogna, Foza, and Lamon), were housed in 6 pens (2 pens/breed), according to DIM (38 ± 23 d) and body weight (61 ± 13 kg). The ewes and their offspring of 3 pens (1 pen/breed) were fed ad libitum a total mixed ration (control), and the other animals received the same diet supplemented with 12 g/d per ewe, plus 4 g/d for each lamb older than 30 d, of an rpCLA mixture. The study lasted 63 d. Two composite milk samples for each ewe were prepared during the first and second months of the trial. The pooled milk samples were analyzed in duplicate for FA profile by 2-dimensional gas chromatography, which allowed us to obtain a detailed FA profile of sheep milk, with 170 different FA detected, including many that were present in small concentrations. The milk relative proportions of individual FA, groups of FA, or FA indices were analyzed by PROC MIXED of SAS (SAS Institute Inc., Cary, NC), considering diet, breed, DIM, and sampling period as sources of variation. The random effect of animal was used to test diet, breed, and DIM, whereas the effects of period were tested on the residual. Breed had a small influence on milk FA profile, mainly on branched- and odd-chain FA. Within breed, animal repeatability for the relative proportions of milk FA was notable for almost all monounsaturated FA and for saturated FA with 14 to 19 carbon atoms, except C16:0, and less so for polyunsaturated FA. The inclusion of rpCLA (CLA *cis*-9,*trans*-11 and CLA *trans*-10,*cis*-12) increased the presence of the same CLA isomers in the milk as well as that of CLA *trans*-9,*trans*-11, and decreased the proportions of de novo-synthesized short-chain FA. From a cluster analysis based on the matrix of correlation coefficients among all FA relative proportions, 3 main FA groups were observed: the first included mainly odd- or branched-chain saturated FA, C18:0, C16:0 and CLA *trans*-10,*cis*-12; the second included monounsaturated FA or polyunsaturated FA with 16 to 20 carbons, CLA *cis*-9,*trans*-11, and CLA *trans*-9,*trans*-11; and the third included short- to medium-chain saturated FA, polyunsaturated FA with 2 to 5 double bonds, and 3 CLA isomers not affected by rpCLA addition (CLA *trans*-11,*cis*-13, CLA *cis*-9,*cis*-11, and CLA *cis*-10,*cis*-12).

Keywords: 2-dimensional gas chromatography; Conjugated linoleic acid (CLA); Fatty acid; Ovine milk; Sheep breed

Journal of Dairy Science

Volume 98, Issue 4, Pages 2088-2102

Use of Fourier transform infrared (FTIR) spectroscopy to predict VFA and ammonia from *in vitro* rumen fermentation

Tagliapietra, F., Rossi, G., Schiavon, S., Ferragina, A., Cipolat-Gotet, C., Bittante, G.

University of Padova, DAFNAE Department, Viale dell'Università 16, 35020 Legnaro (PD), Italy

Abstract

The aim of the present study was to develop a FTIR method to quantify amounts and proportions of volatile fatty acids (VFA) and ammonia nitrogen (N-NH_3) in fermentation fluids collected *in vitro* using innovative Bayesian models as chemometric technique. A set of 170 fluids, collected before and after 4 *in vitro* incubations of 8 diets in 5 replication plus 5 blanks, were analysed for VFA, N-NH_3 and scanned using the MilcoScan FT2 (Foss Electric, Hillerød, Denmark) in the spectral range between 5000 and 900 cm^{-1}. A Bayes B model was used to calibrate equations for each fermentative trait. The calibration equation predicts well VFA and N-NH_3 amounts in calibration and also in validation (R^2_{VAL} ranged from 0.93 to 0.83 for isovaleric and n-butyric acid, respectively). However, the prediction of VFA expressed as proportions of total amount was much less accurate (R^2_{VAL} ranged from 0.81 to 0.52 for iso-valeric and n-butyric acid, respectively). In conclusion, FTIR and Bayesian models can be used as tools to accurately predict VFA amounts *in vitro*.

Keywords: Bayesian regression model; *In vitro* rumen fermentation; Mid infrared spectroscopy

Poljoprivreda

Volume 21, Issue 1 (Supplement), Pages 130-134

Wolf (*Canis lupus*) predation on dairy cattle in eastern Italian Alps

Faccioni, G.[a], Sturaro, E.[a], Calderola, S.[b], Ramanzin, M.[a]

[a] DAFNAE - Università di Padova, Via dell'Università 16, 35020 Legnaro (PD), Italy
[b] Regione del Veneto - Sezione Caccia e Pesca, via Torino 110, 30172 Mestre (VE), Italy

Abstract

Natural wolf recolonization of the Alps brings the challenge to reduce livestock losses and social conflicts. The uncommon impact of a wolf pack on the cattle farming systems of the "Lessinia", in the eastern Italian Alps was examined in this study. Dairy cattle farming predominates there using summer pastures (June-September) and grazing on lowland meadows out of summer. Grazing is organized with aim to minimize labour and costs. Animals are usually left unattended during the day and night in unprotected pastures. Since the return of the wolf in 2012, which formed a pack in 2013, attacks to livestock increased rapidly. Predations peaked during the summer, and they also were extended into the preceding and following months, especially during 2014. Cattle were the predominant species predated (79% of events and 71% of individual losses), with a strong selection towards young age classes. To prevent attacks, livestock should be grouped and kept protected by electric fences or in stables during the night, but this is in contrast with the freegrazing management that farmers have adopted for reducing costs. We suggest that management costs and introduction of protection measures changes should be taken into account for a future economic valorisation of the cattle farming sector.

Keywords: Dairy cattle; Depredation; Livestock systems; Mountain; Wolf

Poljoprivreda

Volume 21, Issue 1 (Supplement), Pages 138-141

Ecology and environmental sciences

Assessment of energy potential from wetland plants along the minor channel network on an agricultural floodplain

Pappalardo, S.E.[a], Prosdocimi, M.[b], Tarolli, P.[b], Borin, M.[a]

[a] Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padua-Agripolis Campus, Viale dell'Università, Legnaro, PD, Italy
[b] Department of Land, Environment, Agriculture and Forestry (T.e.S.A.F.), University of Padua-Agripolis Campus, Viale dell'Università, Legnaro, PD, Italy

Abstract

Renewable energy sources such as biomasses can play a pivotal role to ensure security of energy supply and reduce greenhouse gases through the substitution of fossil fuels. At present, bioenergy is mainly derived from cultivated crops that mirror the environmental impacts from the intensification of agricultural systems for food production. Instead, biomass from perennial herbaceous species growing in wetland ecosystems and marginal lands has recently aroused interest as bioenergy for electricity and heat, methane and 2nd-generation bioethanol. The aim of this paper is to assess, at local scale, the energy potential of wetland vegetation growing along the minor hydrographic network of a reclamation area in Northeast Italy, by performing energy scenarios for combustion, methane and 2nd-generation ethanol. The research is based on a cross-methodology that combines survey analyses in the field with a GIS-based approach: the former consists of direct measurements and biomass sampling, the latter of spatial analyses and scaling up simulations at the minor channel network level. Results highlight that biomass from riparian zones could represent a significant source of bioenergy for combustion transformation, turning the disposal problem to cut and store in situ wetland vegetation into an opportunity to produce sustainable renewable energy at local scale.

Keywords: Bioenergy; Biomass; Energy security; GIS; Renewable resources; Wetland

Environmental Science and Pollution Research

Volume 22, Issue 4, Pages 2479-2490

Comparison of carbon balance in Mediterranean pilot constructed wetlands vegetated with different C4 plant species

Barbera, A.C.[a]*, Borin, M.*[b]*, Cirelli, G.L.*[c]*, Toscano, A.*[c]*, Maucieri, C.*[ab]

[a] Department of Agriculture and Food Science, DISPA, University of Catania, Via Valdisavoia, Catania, Italy
[b] Department of Agronomy, Food, Natural Resources, Animals and Environment, DAFNAE, University of Padua, Agripolis Campus, Viale dell'Università, Legnaro, PD, Italy
[c] Department of Agri-food and Environmental Systems Management, DiGeSA, University of Catania, Via S. Sofia, Catania, Italy

Abstract

This study investigates carbon dioxide (CO_2) and methane (CH_4) emissions and carbon (C) budgets in a horizontal subsurface flow pilot-plant constructed wetland (CW) with beds vegetated with *Cyperus papyrus* L., *Chrysopogon zizanioides* (L.) Roberty, and *Mischantus* x *giganteus* Greef et Deu in the Mediterranean basin (Sicily) during the 1st year of plant growing season. At the end of the vegetative season, *M. giganteus* showed the higher biomass accumulation (7.4 kg m^{-2}) followed by *C. zizanioides* (5.3 kg m^{-2}) and *C. papyrus* (1.8 kg m^{-2}). Significantly higher emissions of CO_2 were detected in the summer, while CH_4 emissions were maximum during spring. Cumulative CO_2 emissions by *C. papyrus* and *C. zizanioides* during the monitoring period showed similar trends with final values of about 775 and 1,074 g m^{-2}, respectively, whereas *M. giganteus* emitted 3,395 g m^{-2}. Cumulative CH_4 bed emission showed different trends for the three C4 plant species in which total gas release during the study period was for C. papyrus 12.0 g m^{-2} and ten times higher for *M. giganteus*, while *C. zizanioides* bed showed the greatest CH_4 cumulative emission with 240.3 g m^{-2}. The wastewater organic carbon abatement determined different C flux in the atmosphere. Gas fluxes were influenced both by plant species and monitored months with an average C-emitted-to-C-removed ratio for *C. zizanioides*, *C. papyrus*, and *M. giganteus* of 0.3, 0.5, and 0.9, respectively. The growing season C balances were positive for all vegetated beds with the highest C sequestered in the bed with M. giganteus (4.26 kg m^{-2}) followed by *C. zizanioides* (3.78 kg m^{-2}) and *C. papyrus* (1.89 kg m^{-2}). To our knowledge, this is the first paper that presents preliminary results on CO_2 and CH_4 emissions from CWs vegetated with C4 plant species in Mediterranean basin during vegetative growth.

Keywords: Biomass production; *Chrysopogon zizanioides* (L.) Roberty; *Cyperus papyrus* L; Greenhouse gases; Horizontal subsurface flow constructed wetland; *Mischantus* × *giganteus* Greef et Deu

Environmental Science and Pollution Research

Volume 22, Issue 4, Pages 2372-2383

Crop management modifies the benefits of insect pollination in oilseed rape

Marini, L.[a], Tamburini, G.[a], Petrucco-Toffolo, E.[a], Lindström, S.A.M.[bc], Zanetti, F.[d], Mosca, G.[a], Bommarco, R.[b]

[a] DAFNAE-Entomology, University of Padova, Viale dell'Università 16, Legnaro, Padova, Italy
[b] Swedish University of Agricultural Sciences, Department of Ecology, P.O. Box 7044, Uppsala, Sweden
[c] Rural Economy and Agricultural Societies/Hushallningssallskapet Kristianstad, P.O. Box 9084, Kristianstad, Sweden [d] DIPSA, University of Bologna, Viale Fanin 44, Bologna, Italy

Abstract

In a factorial field plot experiment, high and low levels of inorganic nitrogen and of insect pollinators visiting the crop were manipulated and their combined effects on oilseed rape yield were quantified. A third factor was also included, testing whether different cultivars responded differently to the tested factors. Insect pollination was required to reach high yield and seed quality (oil content). Final benefits of pollination service were, however, greatly modified by cultivar, where the seed yield of the open-pollinated cultivar largely depended on insect pollination whereas the two hybrid cultivars did not. A near significant interaction between nitrogen input and insect pollination was also found, i.e. benefits to crop yield from insect pollination seemed to increase with decreased nitrogen levels. The differential response of the three cultivars suggested opportunities to use cultivars that are less dependent on insect pollination in landscapes where this service has been deteriorated. Increased access of nitrogen seems to partly compensate yield losses from poor insect pollination. Integrating conservation, environmental and agronomic sciences is therefore crucial to sustain agriculture productions through optimized management of agronomic inputs and biodiversity-based ecosystem services.

Keywords: Cultivar; Ecological intensification; Ecosystem service; Fertilization; Honey bee; Interaction

Agriculture, Ecosystems and Environment

Volume 207, Pages 61-66

Epiphytic lichen diversity along elevational gradients: Biological traits reveal a complex response to water and energy

Nascimbene, J.[a], *Marini, L.*[b]

[a] Natural Sciences Museum of South Tyrol, Bolzano, Italy; Department of Life Sciences, University of Trieste, Trieste, Italy;
[b] Department of Agronomy, Food, Natural Resources, Animals and the Environment (DAFNAE), University of Padova, Legnaro, Padova, Italy

Abstract

Aim: Patterns of epiphytic lichen diversity along elevational gradients covering the range of Norway spruce forests were analysed. The roles of water and energy variables in shaping the observed species-elevation relationship were tested, as well as how growth form and photobiont type distribution varied along the gradient. Location: South Tyrol, northern Italy. Methods: Eight sites were selected spanning the elevational range of spruce forests (900-1900 m a.s.l.) and the regional rainfall gradient. At each site, a pair of forest stands (one mature even-aged and one multilayered) was selected at three elevation steps. Epiphytic lichens were surveyed according to European guidelines for lichen diversity monitoring. Explanatory variables indicative of both forest structure and climate were included in the models. Results: A positive relationship was found between number of species and elevation. This reflected the physiological response of epiphytic lichens to the main climatic factors, trait selection being the mechanism that determined the response at community level. Nonlinear species-temperature and trait-temperature relationships predicted that major changes may be expected in the intermediate part of the gradient. Lichens with a *Trentepohlia* algal partner were more frequent at lower elevations and proved to be sensitive to environmental factors indicative of forest structure. Lichen growth forms had contrasting patterns related to temperature, crustose species richness being enhanced by increasing values, and alectorioid and foliose lichens by decreasing values. Alectorioid lichens were also negatively influenced by rainfall. Main conclusions: In a climate change scenario, lichen diversity in alpine regions will probably not benefit from an increase in air temperature in the same way as flowering plants. Monitoring variations in the proportions of growth form and photobiont type may represent a tool for detecting the effects of climate change on lichen species.

Keywords: Alpine spruce forests; Climate change; Elevational diversity gradient; Energy; Forest structure; Italy; Lichen growth form; Photobiont type; Species richness; Trait selection

Journal of Biogeography

Volume 42, Issue 7, Pages 1222-1232

Investigation on 'bois noir' epidemiology in north-eastern Italian vineyards through a multidisciplinary approach

Mori, N.[a], *Quaglino, F.*[b], *Tessari, F.*[c], *Pozzebon, A.*[a], *Bulgari, D.*[b], *Casati, P.*[b], *Bianco, P.A.*[b]

[a] Dipartimento di Agronomia Animali Alimenti Risorse Naturali e Ambiente, Università degli Studi di Padova, Legnaro, Italy
[b] Dipartimento di Scienze Agrarie e Ambientali - Produzione, Territorio e Agroenergia, Università degli Studi di Milano, Via Celoria 2, Milan, Italy
[c] AGREA Centro Studi, San Giovanni Lupatoto, Italy

Abstract

A multidisciplinary approach, based on field surveys, molecular biology techniques, and spatial data analyses, was utilised to investigate the Bois noir (BN) epidemiology in north-eastern Italian vineyards during the years 2010-12. Symptomatic grapevines, weeds and specimens of the insect vector *Hyalesthes obsoletus* were monitored and mapped. Leaf samples from symptomatic grapevines and weeds, and captured insect specimens were analyzed by real-time PCR to identify BN phytoplasma (BNp; '*Candidatus* Phytoplasma solani' species), the etiological agent of BN. Data spatial distribution was analyzed using SADIE (Spatial Analysis by Distance IndicEs). Bois noir phytoplasma strains identified in weed candidates for an epidemiological role were characterised by RFLP-based analyses of tuf gene amplicons. Results highlighted that, in the examined areas, the host systems *Convolvulus arvensis* - *H. obsoletus* and *Urtica dioica* - *H. obsoletus* play the main role in BN diffusion. It was also evidenced that other weeds (i.e. *Chenopodium album* and *Malva sylvestris*) spatially associated with symptomatic grapevines and/or insect vectors and infected by the same tuf type identified in grapevines and insects, could play a role in BN diffusion. On the other hand, some weeds (i.e. *Trifolium repens*) were uninfected and not associated with symptomatic grapevines and/or insect vectors. The synergic application of our multidisciplinary approach improved the knowledge of BN epidemiology, and provided helpful indication for designing experimental plans to contain BN spreading in vineyards through weed management. The approach described in the present work could be used to investigate the complex epidemiology of other phytoplasma diseases.

Keywords: *Candidatus* Phytoplasma solani; Grapevine yellows; Host plants; *Hyalesthes obsoletus*; Real-time PCR; Spatial analysis

Annals of Applied Biology

Volume 166, Issue 1, Pages 75-89

Landscape composition affects parasitoid spillover

Inclán, D.J.[a], Cerretti, P.[ab], Marini, L.[a]

[a] DAFNAE-Entomology, University of Padova, Viale dell'Università 16, Legnaro, Padova, Italy
[b] Department of Biology and Biotechnology 'Charles Darwin', Sapienza University of Rome, Piazzale A. Moro 5, Rome, Italy

Abstract

The intensification of agriculture has led to a severe simplification of agricultural landscapes, resulting in a marked reduction in the diversity of insect natural enemies. However, how this simplification shapes the movement of insect parasitoids between crop and non-crop habitats (i.e., spillover) is still unclear. We examined the potential spillover of tachinid parasitoids from semi-natural habitats into apple orchards across different landscapes. We sampled commercial apple orchards localized in three landscape types (forest-, grassland- or apple-dominated landscapes) to first evaluate if landscape composition affects the local species richness in apple orchards. Second, we tested whether the contribution of forest and grassland habitats to the local tachinid community composition of apple orchards changes according to landscape composition. We found that landscape composition did not affect local tachinid species richness in apple orchards, while it affected the species spillover. Independently of the landscape, we found highly nested communities of tachinids between apple orchards and forest habitats suggesting a strong spillover of tachinids between these habitats. In contrast, tachinids in apple orchards were nested with grassland habitats only in landscapes dominated by apple orchards. Our results have important implications for the conservation of insect parasitoids in agricultural landscapes, as the spillover of species in the crop can be affected by the type and the area of semi-natural habitats in the surrounding landscape.

Keywords: Agricultural intensification; Habitat fragmentation; Nestedness; Species movement; *Tachinidae*

Agriculture, Ecosystems and Environment

Volume 208, Pages 48-54

Municipal wastewater treatment with *Phragmites australis* L. and *Typha latifolia* L. for irrigation reuse. Boron and heavy metals

Morari, F., Dal Ferro, N., Cocco, E.

Department of Agronomy, Food, Natural Resources, Animals and Environment,
Agripolis, University of Padova, Viale Dell'Università 16, Legnaro, Padova, Italy

Abstract

In this work, we compared the performance of *Phragmites australis* and *Typha latifolia* for depurating primary-treated urban wastewater and evaluated their suitability for irrigation reuse. Macrophytes were planted in two pilot-scale constructed wetland systems (CWs) and monitored during a 2-year experiment (2002-2003). CW efficiency was evaluated in terms of both mass removal and water quality considering boron (B) and the following heavy metals: aluminium (Al), arsenic (As), beryllium (Be) manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), cadmium (Cd), lead (Pb), selenium (Se) and vanadium (V). The accumulation of the elements, both in plant tissues and sandy substrate layer, and their offtake with the macrophyte harvest were also measured. In quantitative terms, the established CW systems showed high removal efficiency for Al (96 %), Cu (91 %), Pb (88 %) and Zn (85 %), while lower efficiencies were observed for Fe (44 %), Co (31 %) and B (40 %). The sediment played a strategic role in the adsorption and accumulation of wastewater pollutants, while plants acted as phytostabilizers since element root concentrations were generally from one to two orders of magnitude higher than those observed in the other parts. The results were less favourable in terms of water quality because the high evapotranspiration counteracted the depuration process by concentrating the elements in the outflow water. Outflow water contained more B (68 %), Mn (196 %) and, in the case of CW managed with *Phragmites*, also Fe (73 %) than inflow water, breaking the Italian guidelines for irrigation reuse. Integrating solutions to reduce the high evapotranspiration of CWs with more efficient pre-cleaning systems are necessary to obtain better removal efficiencies that reduce the effect of ET on water quality.

Keywords: Boron; Constructed wetland; Evapotranspiration; Heavy metals; Irrigation; Wastewater reuse

Water, Air, and Soil Pollution

Volume 226, Issue 3, Article number 56

Organic farming enhances parasitoid diversity at the local and landscape scales

Inclán, D.J.[ab], Cerretti, P.[ac], Gabriel, D.[d], Benton, T.G.[e], Sait, S.M.[e], Kunin, W.E.[e], Gillespie, M.A.K.[e], Marini, L.[a]

[a] DAFNAE-Entomology, University of Padova, Viale dell'Università 16, Legnaro, Padova, Italy

[b] Research Associate, Museo Ecuatoriano de Ciencias Naturales, Sección Invertebrados, Rumipamba 341 y Av. de los Shyris, Quito, Ecuador

[c] Department of Biology and Biotechnology 'Charles Darwin', Sapienza University of Rome, Piazzale A. Moro 5, Rome, Italy

[d] Institute of Crop and Soil Science, Julius Kühn-Institut (JKI), Federal Research Centre for Cultivated Plants, Bundesallee 50, Braunschweig, Germany

[e] School of Biology, Faculty of Biological Sciences, University of Leeds, Leeds, United Kingdom

Abstract

Summary: The magnitude of the benefits derived from organic farming within contrasting managed landscapes remains unclear and, in particular, the potential scale-dependent response of insect parasitoids is relatively unexplored. Identifying the scale at which parasitoids are affected by organic farming will be an important step to enhance their conservation. We sampled tachinid parasitoids at the centre and margin of arable and grassland fields on paired organic and conventional farms located in landscapes with different proportions of organic land. A total of 192 fields were sampled in two biogeographical regions of the UK. We found that the positive effect of organic farming on tachinid parasitoid diversity can be observed at multiple spatial scales. At the local scale, we found higher abundance and species richness of tachinid parasitoids on organic than on conventional farms and on field margins than on field centres. At the landscape scale, the diversity of tachinids was higher in landscapes with higher proportions of organic land. At both scales, the positive effect of organic farming was clear for arable fields, while it was almost neutral for grasslands. Synthesis and applications. Any attempt to enhance parasitoid diversity in agricultural landscapes needs to consider the local management in relation to the habitat type, location within the field and agricultural management in the surrounding landscape. To restore parasitoid diversity, the promotion of organic agriculture should aim to increase both the total extent of organic farming and the connectivity of individual farms. As the benefits of organic farming to biodiversity clearly spread beyond individual farm boundaries, any assessment of organic farming should consider these positive externalities. Any attempt to enhance parasitoid diversity in agricultural landscapes needs to consider the local management in relation to the habitat type, location within the field and agricultural management in the surrounding landscape. To restore parasitoid diversity, the promotion of organic agriculture should aim to increase both the total extent of organic farming and the connectivity of individual farms. As the benefits of organic farming to biodiversity

clearly spread beyond individual farm boundaries, any assessment of organic farming should consider these positive externalities.

Keywords: Agri-environment schemes; Agricultural intensification; Biocontrol; Conventional farming; Management; Natural enemies; Scale dependence; Tachinida

Journal of Applied Ecology

Volume 52, Issue 4, Pages 1102-1109

Performance of a wall cascade constructed wetland treating surfactant-polluted water

Tamiazzo, J.[a], Breschigliaro, S.[b], Salvato, M.[c], Borin, M.[ac]

[a] Department of Agronomy, Food, Natural Resources, Animals and Environment-DAFNAE, University of Padua, Agripolis Campus, Viale dell'Università 16, Legnaro, PD, Italy
[b] LANDLAB Studio associato, Via Quintarello 12/A, Quinto Vicentino, VI, Italy
[c] PAN srl, Via della Croce Rossa 112, Padua, Italy

Abstract

Carwashes are highly water-consuming processes that require wastewater treatment before discharge into a sewer system due to the complex composition of leachate. Anionic surfactants (AS) are the main constituents of this wastewater because of their cleaning and solubilization properties; they can be potentially dangerous for the environment if not adequately treated. Constructed wetlands (CWs) are low-cost systems increasingly used to treat different types of wastewater; however, there are few studies on their use for the treatment of carwash wastewater. In this study, an innovative constructed wetland arranged in a "cascade" to simulate a wall system (WCCW) was experimented in 2010 and 2011 to treat AS. Three plant species were tested at different AS inlet concentrations (10, 50, and 100 mg L^{-1}) with two hydraulic retention times (HRTs; 3 and 6 days): ribbon grass (*Typhoides arundinacea* (L.) Moench (syn. *Phalaris arundinacea* L.) var. picta; Ta), water mint (*Mentha aquatica* L.; Ma), and divided sedge (*Carex divisa* Hudson; Cd). All plant species grew constantly over the experimental period, showing a capacity to tolerate even the highest AS concentration. Using the HRT of 6 days, raising the inlet concentration increased the AS outlet concentration, with similar values for the treatments (median values of 0.13-0.15, 0.47-0.78, and 1.19-1.46 mg L^{-1} at inlet concentrations in the order 10, 50, and 100 mg L^{-1}). The shorter HRT led to significant differences among treatments in the reduction of outlet concentration, the best result being given by the tanks vegetated with Ma (A = 97.7 % with outlet concentration 0.35 mg L^{-1}). After treatments of the WCCW, the AS content was reduced almost completely, with removal in the ranges 0.07-10.2 g m^{-2} day^{-1} for tanks planted with Ta, 0.10-9.1 g m^{-2} day^{-1} for Ma tanks, and 0.11-9.5 g m^{-2} day^{-1} for Cd tanks depending on the inlet concentration.

Keywords: Anionic surfactants; Carwash wastewater; Constructed wetlands; Treatment wetlands; Wastewater treatment; Wetland plant species

Environmental Science and Pollution Research

Volume 22, Issue 17, Pages 12816-12828

Recovery of plant diversity in restored semi-natural pastures depends on adjacent land use

Winsa, M.[a], *Bommarco, R.*[a], *Lindborg, R.*[b], *Marini, L.*[c], *Öckinger, E.*[a]

[a] Department of Ecology, Swedish University of Agricultural Sciences, Uppsala, Sweden;
[b] Department of Physical Geography and Quaternary Geology, Stockholm University, Stockholm, Sweden;
[c] DAFNAE - Department of Agronomy, Food, Natural Resources, Animals and the Environment, University of Padova, Legnaro (Padova), Italy

Abstract

Questions: Does restoration success of formerly abandoned semi-natural pastures depend on adjacent land use? Is species richness higher in restored pastures adjacent to an intact semi-natural pasture than in restored pastures adjacent to arable land? Does community similarity between a restored and an adjacent intact pasture decrease with distance from the border between the two pastures? Do differences in species richness and community similarity decrease over time? Location: Agricultural landscapes in south-central Sweden. Methods: The plant community in previously abandoned but now restored semi-natural pastures was surveyed along a distance gradient from the border between the restored pastures and adjacent fields towards the centre of the pastures. The restored pastures were located adjacent to either a crop field (N = 8) or a continuously grazed pasture (N = 6), and differed in time since restoration (1-13 yr). Results: The total species richness was higher in pastures adjoining continuously grazed pastures compared to crop fields. Richness of both total and specialist species increased with time since restoration. Irrespective of adjacent land use, richness of specialist species decreased with increasing distance from the edge, an effect that became weaker with increasing time since restoration. The similarity in species composition compared to that in adjacent continuously grazed pasture also decreased towards the centre of the restored pasture. Conclusions: Our results suggest that restoration of biodiversity in semi-natural pastures benefits from adjacent pastures that can act as source habitats. The most likely mechanism is step-wise short-distance dispersal, but also other processes, such as more long-distance dispersal, seed bank dynamics and historical legacies are probably involved. To best succeed in habitat restoration in fragmented landscapes, the spatial location of source populations must be considered. We assessed how adjacent land use influences restoration success in previously abandoned semi-natural pastures. Our results indicate that restoration success, in terms of plant community recovery, is higher in pastures situated adjacent to an intact grassland that can act as a population source than in pastures surrounded by crop fields.

Keywords: Abandonment; Colonization; Community composition; Habitat fragmentation; Habitat restoration; Isolation; Semi-natural grassland

Applied Vegetation Science

Volume 18, Issue 3, Pages 413-422

Screening of 18 species for digestate phytodepuration

Pavan, F., Breschigliaro, S., Borin, M.

Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padua, Agripolis Campus, Viale dell'Università 16, Legnaro, PD, Italy

Abstract

This experiment assesses the aptitude of 18 species in treating the digestate liquid fraction (DLF) in a floating wetland treatment system. The pilot system was created in NE Italy in 2010 and consists of a surface-flow system with 180 floating elements (Tech-IA®) vegetated with ten halophytes and eight other wetland species. The species were transplanted in July 2011 in basins filled with different proportions of DLF/water (DLF/w); periodic increasing of the DLF/w ratio was imposed after transplanting, reaching the worst conditions for plants in summer 2012 (highest EC value 7.3 mS cm/L and NH_4-N content 225 mg/L). It emerged that only *Cynodon dactylon*, *Typha latifolia*, *Elytrigia atherica*, *Halimione portulacoides*, *Salicornia fruticosa*, *Artemisia caerulescens*, *Spartina maritima* and *Puccinellia palustris* were able to survive under the system conditions. Halophytes showed higher dry matter production than other plants. The best root development (up to 40-cm depth) was recorded for *Calamagrostis epigejos*, *Phragmites australis*, *T. latifolia* and *Juncus maritimus*. The highest nitrogen (10–15 g/m^2) and phosphorus (1–4 g/m^2) uptakes were obtained with *P. palustris*, *Iris pseudacorus* and *Aster tripolium*. In conclusion, two halophytes, *P. palustris* and *E. atherica*, present the highest potential to be used to treat DLF in floating wetlands.

Keywords: Anaerobic digestion wastewater; ECw; Floating wetlands; Nutrient removal; Salt tolerance halophytes

Environmental Science and Pollution Research

Volume 22, Issue 4, Pages 2455-2466

Seed production of a subalpine *Festuca nigrescens–Agrostis capillaris* semi-natural grassland in the eastern Italian Alps

Scotton, M., Piccinin, L., Coraiola, M.

Department of Agronomy Food Natural Resources Animals and Environment, University of Padova, Agripolis, Viale dell'Università 16, Legnaro (PD), Italy

Abstract

Studying the seed production of semi-natural grasslands helps to understand their regeneration processes and to evaluate the possibility of collecting seed useful for ecological restoration. In a subalpine *Festuca nigrescens–Agrostis capillaris* meadow, the development of the main grasses' seed production and the standing seed yield at meadow maturity were studied. The inflorescence size was in the lower part of the variation range reported in previous studies. The seed viability was average to high and durable. The biological floret site utilization was mostly above 50%. With 458 fertile stems m^{-2}, the seeds produced (all seeds produced up to the time of the meadow maturity) by the main grasses were 21,000 m^{-2}, of which 77% were of *A. capillaris* and 81% were viable. On a 3-year average, the standing seed yield (seeds present on the plants, i.e. produced minus shed seeds) at meadow maturity was 6953 seeds m^{-2}. Amount and composition of the produced seeds were affected mainly by the variability among years of the fertile stems density of the perennial grasses and the annual hemiparasite *Rhinanthus freynii*. The percentage of produced seeds which formed the standing seed yield was affected mainly by the collecting date.

Keywords: Fertile stem density; floret site utilization; germinability; seed production; standing seed yield; viability

Plant Biosystems

Volume 149, Issue 2, Pages 404-414

Spatial spread and infestation risk assessment in the Asian longhorned beetle, *Anoplophora glabripennis*

Favaro, R.[a], *Wichmann, L.*[b], *Ravn, H.P.*[c], *Faccoli, M.*[a]

[a] Department of Agronomy, Food, Natural resources, Anial and Environment, University of Padova, Legnaro, Italy
[b] Stensebyvejen 28, Nexø, Denmark
[c] Department of Forest and Landscape, University of Copenhagen, Copenhagen, Denmark

Abstract

The Asian longhorned beetle (ALB), *Anoplophora glabripennis* (Motschulsky) (Coleoptera: *Cerambycidae*), is recognised as potentially one of the most damaging invasive insects in Europe and North America. International trade has increased the risk of accidental introduction of ALB. An eradication programme was initiated in Northeast Italy in June 2009, when an ALB infestation was discovered. The infestation was monitored by annual surveys of all host-tree species growing in the eradication area. Infested trees were cut down and chipped. This study analyses the spatiotemporal distribution of infested trees for a 5-year period from 2008 to 2012 using a generalised linear model approach. The results show that spread and infestation risk were significantly affected by (1) distance of suitable hosts from the nearest infested trees, (2) number of infested trees in the surroundings, and (3) annual variation. The significant differences in beetle dispersal between years reflect to some extent the onset of the eradication programme. The model allowed the estimation of arbitrary probability-based management boundaries surrounding ALB-infested trees. For example, the model estimated a 0.1% probability of attack on a suitable host tree 1 910 m from an existing attack.

Keywords: Binomial; *Cerambycidae*; Coleoptera; Distance-dependent attack index; Generalised linear model; Probability of attack; Probit link function; Spatial distribution

Entomologia Experimentalis et Applicata

Volume 155, Issue 2, Pages 95-101

Spray drift reduction techniques for vineyards in fragmented landscapes

Otto, S.[a], Loddo, D.[a], Baldoin, C.[b], Zanin, G.[c]

[a] Institute of Agro-environmental and Forest Biology - CNR, Agripolis, Viale dell'Università 16, 35020 Legnaro, PD, Italy
[b] Department of Land, Environment, Agriculture and Forestry (TESAF), University of Padova, Agripolis, Viale dell'Università 16, 35020 Legnaro, PD, Italy
[c] Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Agripolis, Viale dell'Università 16, 35020 Legnaro, PD, Italy

Abstract

In intensive agricultural systems spray drift is one of the major potential diffuse pollution pathways for pesticides and poses a risk to the environment. There is also increasing concern about potential exposure to bystanders and passers-by, especially in fragmented landscapes like the Italian pre-Alps, where orchards and vineyards are surrounded by residential houses. There is thus an urgent need to do field measurements of drift generated by air-blast sprayer in vineyards, and to develop measures for its reduction (mitigation). A field experiment with an "event method" was conducted in north-eastern Italy in no-wind conditions, in the hilly area famed for Prosecco wine production, using an air-blast sprayer in order to evaluate the potential spray drift from equipment and the effectiveness of some practical mitigation measures, either single or in combination. A definition of mitigation is proposed, and a method for the calculation of total effectiveness of a series of mitigation measures is applied to some what-if scenarios of interest. Results show that low-drift equipment reduced potential spray drift by 38% and that a fully developed vine curtain mitigated it by about 70%; when the last row was treated without air-assistance mitigation was about 74%; hedgerows were always very effective in providing mitigation of up to 98%. In conclusion, spray drift is not inevitable and can be markedly reduced using a few mitigation measures, most already available to farmers, that can be strongly recommended for environmental regulatory schemes and community-based participatory research.

Keywords: Hedgerow; Low-drift add-on equipment; Mitigation calculation; Mitigation measures; Prosecco wine; Spray drift

Journal of Environmental Management

Volume 162, Pages 290-298

Temperature influence on nitrogen removal in a hybrid constructed wetland system in Northern Italy

Mietto, A., Politeo, M., Breschigliaro, S., Borin, M.

Department of Agronomy, Food, Natural Resources, Animals, Environment-DAFNAE
Agripolis, University of Padova, Viale Dell'Università 16, Legnaro, Padova, Italy

Abstract

The objective of this research was to investigate the efficiency and seasonal performance of a full-scale hybrid constructed wetland system (HCW) in reducing total nitrogen (TN), ammonia nitrogen (NH_4-N) and nitrate nitrogen (NO_3-N). HCW with a total area of about 130 m^2 and hydraulic load of 2 m^3/day was composed of three subsurface flow vertical systems (VF), working in parallel and one horizontal (HF) connected in series. The system was loaded daily with synthetic wastewater having an average concentration of TN of 250 mg/L (about 125 mg/L of NH_4-N and 125 mg/L of NO_3-N). Water samples were collected and analyzed from May to July 2011 and from January 2012 to July 2012. Variations were observed in nutrient removal performance related to temperature. During the whole monitoring period median reduction efficiency (RE) in the HCW was TN 95%, NH_4-N 95% and NO_3-N 93%, although three sub-periods characterized by different performances have been observed. During the first period (from May to July 2011) the RE was positive for the three nitrogen forms considered, whereas from January to the end of March 2012 the RE was lower, particularly for TN and NO_3-N. From April 2012, when the temperature rose above 14.8 °C, there was an increase in the performance that reached the 2011 values. Internal production of NO_3-N was observed, mainly in the VF systems between January and March 2012. The median removals of mass pollutants per m^2 of HCW per day were TN 3.1 g/m^2/d, NH_4-N 1.5 g/m^2/d, NO_3-N 1.5g/m^2/d. Segmented regression analysis identified a breakpoint at 14.2 °C for wastewater temperature that caused variations in TN and NO_3-N concentration reduction performances. According to this approach the abatement was always positively correlated with temperature, but different regression slopes were obtained below and above the breakpoint. In particular, with lower temperature the abatement of NO_3-N and TN increased by 1.7 and 2.0% per °C of temperature increase; with temperature higher than 14.2 °C the increase in abatement due to increased temperature was sharper, especially for NO_3-N.

Keywords: *Canna indica*; Horizontal flow (HF); *Phragmites australis*; Temperature; Vertical flow (VF); VF operation mode

Ecological Engineering

Volume 75, Pages 291-302

Vertical stratification of ichneumonid wasp communities: The effects of forest structure and life-history traits

Di Giovanni, F.[abc], *Cerretti, P.*[abc], *Mason, F.*[b], *Minari, E.*[b], *Marini, L.*[c]

[a] Dipartimento di Biologia e Biotecnologie Charles Darwin, Università di Roma La Sapienza, Piazzale A. Moro 5, Rome, Italy
[b] Centro Nazionale per lo Studio e la Conservazione della Biodiversità Forestale, Corpo Forestale dello Stato, via Carlo Ederle 16/A, Verona, Italy
[c] DAFNAE, Università di Padova, viale dell'Università 16, Legnaro, Padua, Italy

Abstract

Parasitoid wasp communities of the canopy of temperate forests are still largely unexplored. Very little is known about the community composition of parasitoids between canopy and understory and how much of this difference is related to forest structure or parasitoid biological strategies. In this study we investigated upon the difference in the community composition of the parasitic wasps *Ichneumonidae* between canopy and understory in a lowland temperate forest in northern Italy. We used general linear models to test whether parasitic strategy modifies species vertical stratification and the effect of forest structure. We also tested differences in β-diversity between canopy and understory traps and over time within single forest layers. We found that stand basal area was positively related to species richness, suggesting that the presence of mature trees can influence local wasp diversity, providing a higher number of microhabitats and hosts. The ichneumonid community of the canopy was different from that of the understory, and the β-diversity analysis showed higher values for the canopy, due to a higher degree of species turnover between traps. In our analyses, the vertical stratification was different between groups of ichneumonids sharing different parasitic strategies. Idiobiont parasitoids of weakly or deeply concealed hosts were more diverse in the understory than in the canopy while parasitoids of spiders were equally distributed between the two layers. Even though the ichneumonid community was not particularly species-rich in the canopy of the temperate forests, the extension of sampling to that habitat significantly increased the number of species recorded.

Keywords: Biological traits; Canopy; Forest structure; *Ichneumonidae*; Vertical stratification; β-diversity

Insect Science

Volume 22, Issue 5, Pages 688-699

Development, assessment and evaluation of a biopile for hydrocarbons soil remediation

Baldan, E.[ac]*, Basaglia, M.*[a]*, Fontana, F.*[a]*, Shapleigh, J.P.*[b]*, Casella, S.*[a]

[a] Department of Agronomy Food Natural Resources Animals and Environment, University of Padova, Viale dell'Università 16, Legnaro PD, Italy
[b] Department of Microbiology Cornell University, Wing Hall 123 Wing Drive, Ithaca, NY, United States
[c] Department of Biology, University of Padova, via U Bassi 58/B, Padova, Italy

Abstract

Soil at a site located in North-Eastern Italy has been impacted by a persistent, long term exposure to diesel fuel. The concentration of organic contaminants in soil exceeded the acceptable limits indicated by the present regulations concerning the specific use-destination of the sites and its reclamation. The contamination involved several thousands square meters of soil surface resulting in some thousand cubic meters of soil. Approximately 650 cubic meter of the most contaminated soil was moved to a nearby site where a biopile was assessed with the aim to enhance the activities of the soil community to reduce the oil concentration. In order to verify the correct operation of the system it was continuously monitored in terms of chemical concentration, biological activity and community structure. The main remediation occurred within the first 300 days of treatment allowing the soil to be back within the threshold defined by current laws for industrial and commercial activities. An additional 200 days treatment was needed to further reduce the oil content to the limits required for public parks and residential areas. Lab scale biopiles were also assessed to evaluate the role of commercial inoculants in the remediation process.

Keywords: ARDRA community; Bioaugmentation; Biodegradation; Biopile; DGGE; Hydrocarbons; Inoculation

International Biodeterioration and Biodegradation

Volume 98, Pages 66-72

Potential spread of forest soil-borne fungi through earthworm consumption and casting

Montecchio, L.[a], Scattolin, L.[a], Squartini, A.[b], Butt, K.R.[c]

[a] TeSAF Department, University of Padova, v.le dell'Università 16, Legnaro, PD, Italy
[b] DAFNAE Department, University of Padova, v.le dell'Università 16, Legnaro, PD, Italy
[c] School of Built and Natural Environment, University of Central Lancashire, Preston, United Kingdom

Abstract

To test if forest soil-borne fungi concerned with plant health can be selectively dispersed by earthworms, 10 fungal species isolated from 5 forests were presented at 2 concentrations to 3 ecologically distinct earthworm species in laboratory trials. Between 5 and 13 days after introduction, casts were collected (where possible) from each earthworm species fed with a different fungus. These casts were analysed using molecular methods for the presence of the given fungus and its vitality verified through traditional plating techniques. The research confirmed that earthworms have an important role in dispersal of soil fungi in forests, and that such activity can depend on the taxonomical position of the fungus, ecological category of the earthworm species involved and the fungal concentration. In certain instances there is a suggestion that some fungi may be toxic to some earthworms at the given concentrations, which equated to those within and outside of the rhizosphere.

Keywords: Dispersal; Earthworms; Ecology; Fungal inoculum; Soil-borne fungi

IForest

Volume 8, Pages 295-301

Snow vole (*Chionomys nivalis* Martins) affects the redistribution of soil organic matter and hormone-like activity in the alpine ecosystem: Ecological implications

Pizzeghello, D.[a]*, Cocco, S.*[b]*, Francioso, O.*[c]*, Ferrari, E.*[d]*, Cardinali, A.*[a]*,*
Nardi, S.[a]*, Agnelli, A.*[e]*, Corti, G.*[b]

[a] Dipartimento di Agronomia, Animali, Alimenti, Risorse Naturali e Ambiente (DAFNAE), Università di Padova, Legnaro, Padova, Italy
[b] Dipartimento di Scienze Agrarie, Alimentari e Ambientali, Università Politecnica delle Marche, Ancona, Italy
[c] Dipartimento di Scienze Agrarie, Università di Bologna, Bologna, Italy
[d] Dipartimento di Scienze Chimiche e Geologiche, Università di Modena e Reggio Emilia, Modena, Italy
[e] Dipartimento di Scienze Agrarie, Alimentari ed Ambientali, Università di Perugia, Perugia, Italy

Abstract

In alpine environments, colonies of snow vole (*Chionomys nivalis* Martins) cause strong pedoturbation, which may affect humification process and soil organic matter (SOM) cycling, with repercussions on the hormone-like activity of organics. We investigated the effect of snow vole pedoturbation on the chemical and spectroscopic features of soil organic fractions, and the potential hormone-like activity of humic and fulvic acids (HA, FA). The study site was located on the high-mountain environment of the Majella massif (central Italy). Pedoturbated and regular soils were morphologically described and characterized for pH and content of total organic carbon, total extractable carbon, HA, and FA. Both HA and FA were extracted and investigated using attenuated total reflectance/Fourier transform infrared (ATR/FTIR), nuclear magnetic resonance with high-resolution magic angle spinning (HRMAS-NMR), and 1H-13C heteronuclear single quantum coherence (HSQC). HA and FA were also tested for their auxin-like and gibberellin-like activities. Results provide evidences that bioturbated and regular soils contain a poorly decomposed SOM, but HA and FA with a well-defined molecular structure. The HA and FA from both bioturbated and regular soils show a hormone-like activity with a different allocation along the soil profile. In the regular soil, the highest auxin-like activity was shown by HA and FA from Oe1 horizon, while gibberellin-like activity was expressed by FA from Oe2 horizon. Burrowing activity determines a redistribution of organics throughout the profile with a relatively high auxin-like activity in the FA from straw tunnel wall (STW) and gibberellin-like activity in the HA from vole feces (VF). The relative high presence of carboxylic acids, amides, proteins, and amino acids in the FA from STW and the aromatic moieties in the HA from VF put evidences for their different behavior. The fact that snow vole activity has modified the chemical and biological properties of SOM in these soils otherwise considered governed only by low temperature has important ecological implications such as the preservation of soil fertility and vegetal biodiversity.

Keywords: ATR/FTIR; Fulvic and humic acids; Functional ecology; HRMAS-NMR; Italian Long-Term Ecological Research site; Pedoturbation

Ecology and Evolution

Volume 5, Issue 20, Pages 4542-4554

Soil bacterial community response to sulfadiazine in the soil-root zone

Reichel, R.[a], Michelini, L.[b], Ghisi, R.[b], Thiele-Bruhn, S.[a]

[a] Soil Science, Faculty of Regional and Environmental Sciences, University of Trier, Behringstraße 21, Trier, Germany
[b] D.A.F.N.A.E. Department, University of Padova, Agripolis, Viale dell'Università 16, Legnaro (PD), Italy

Abstract

Sulfonamide antibiotics reach soil via manure and adversely affect microbial diversity. Cleareffects of these bacteriostatic, growth-inhibiting antibiotics occur in the presence of a parallel input of microbial activity stimulating manure. Natural hot spots with already increased soil microbial activity are located in the rhizosphere, comprising microorganism such as Pseudomonas with plant growth promoting and pathogenic strains. The hypothesis was therefore that the antibiotic activity of sulfonamides is promoted in the rhizosphere even in the absence of manure, followed by shifts of the natural plant-specific microbial community structure. This was evaluated by a laboratory experiment with *Salix fragilis* L. and *Zea mays* L. After 40 d of incubation, sub-areas such as non-rhizosphere soil, rhizosphere soil and plant roots were sampled. Effects on microbial community structure were analyzed using 16S rRNA gene fragment patterns of total bacteria community and Pseudomonas. Selected exoenzymes of N-, P-, and C-cycling were used to test effects on microbial functions. Compared to the factors soil sub-area and sulfadiazine (SDZ) content, plant species had the largest influence on the bacterial community structure and soil exoenzyme activity pattern. This was also reflected by an up to 1.5-fold higher acid phosphatase activity in samples from maize- compared to willow-planted soil. We conclude that antibiotic effects on the bacterial community structures are influenced by the antibiotic concentration and root influence.

Keywords: Exoenzymes; Pseudomonas; *Salix fragilis* L.; Sulfadiazine; *Zea mays* L

Journal of Plant Nutrition and Soil Science

Volume 178, Issue 3, Pages 499-506

Entomology

A fundamental step in IPM on grapevine: Evaluating the side effects of pesticides on predatory mites

Pozzebon, A., Tirello, P., Moret, R., Pederiva, M., Duso, C.

Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, Legnaro, Padova, Italy

Abstract

Knowledge on side effects of pesticides on non-target beneficial arthropods is a key point in Integrated Pest Management (IPM). Here we present the results of four experiments conducted in vineyards where the effects of chlorpyrifos, thiamethoxam, indoxacarb, flufenoxuron, and tebufenozide were evaluated on the generalist predatory mites *Typhlodromus pyri* Scheuten and *Amblyseius andersoni* (Chant), key biocontrol agents of herbivorous mites on grapevines. Results show that indoxacarb and tebufenozide had a low impact on the predatory mites considered here, while a significant impact was observed for chlorpyrifos, flufenoxuron, and thiamethoxam. The information obtained here should be considered in the design of IPM strategies on grapevine.

Keywords: *Amblyseius andersoni*; Chemical treatments; Phytoseiidae; Side-effects; *Typhlodromus pyri*; *Vitis vinifera*

Insects

Volume 6, Issue 4, Pages 847-857

A single nucleotide polymorphism in the acetylcholinesterase gene of the predatory mite *Kampimodromus aberrans* (Acari: Phytoseiidae) is associated with chlorpyrifos resistance

Cassanelli, S.[a], *Ahmad, S.*[b], *Duso, C.*[b], *Tirello, P.*[b], *Pozzebon, A.*[b]

[a] Department of Life Sciences, University of Modena and Reggio Emilia, Via A. Amendola 2, Reggio Emilia, Italy
[b] Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, Legnaro, Padova, Italy

Abstract

The predatory mite *Kampimodromus aberrans* (Oudemans) (Acari: Phytoseiidae) is one of the most important biocontrol agents of herbivorous mites in European perennial crops. The use of pesticides, such as organophosphate insecticides (OPs), is a major threat to the success of biocontrol strategies based on predatory mites in these cropping systems. However, resistance to OPs in K. aberrans has recently been reported. The present study investigated the target site resistance mechanisms that are potentially involved in OP insensitivity. In the herbivorous mite *Tetranychus urticae* Koch (Acari: Tetranychidae). , resistance to OPs is due to a modified and insensitive acetylcholinesterase (AChE; EC: 3.1.1.7) that bears amino acid substitution F331W (AChE Torpedo numbering). To determine whether the predators and prey have evolved analogous molecular mechanisms to withstand the same selective pressure, the AChE cDNA from a putative orthologous gene was cloned and sequenced from susceptible and resistant strains of *K. aberrans*. No synonymous mutation coding for a G119S substitution was determined to be strongly associated with the resistant phenotype instead of the alternative F331W. Because the same mutation in *T. urticae* AChE was not associated with comparable levels of chlorpyrifos resistance, the role of the G119S substitution in defining insensitive AChE in *K. aberrans* remains unclear. G119S AChE genotyping can be useful in ecological studies that trace the fate of resistant strains after field release or in marker-assisted selection of improved populations of *K. aberrans* to achieve multiple resistance phenotypes through gene pyramiding. The latent complexity of the target site resistance in *K. aberrans* vs. that of *T. urticae* is also discussed in the context of data from the genome project of the predatory mite *Metaseiulus occidentalis* (Nesbitt) (Acari: Phytoseiidae).

Keywords: Insecticide resistance; Molecular marker; Mutated acetylcholinesterase

Biological Control

Volume 90, Pages 75-82

Check list and zoogeographic analysis of the scale insect fauna (Hemiptera: Coccomorpha) of Greece

Pellizzari, G.[a], Chadzidimitriou, E.[a], Milonas, P.[b], Stathas, G.J.[c], Kozár, F.[d]

[a] University of Padova, Department of Agronomy, Food, Natural Resources, Animals and Environment DAFNAE, viale dell'Università 16, Legnaro, Italy
[b] Laboratory of Biological Control, Department of Entomology and Agricultural Zoology, Benaki Phytopathological Institute, Athens, Greece
[c] Technological Educational Institute of Peloponnese, Department of Agricultural Technology, Laboratory of Agricultural Entomology and Zoology, Antikalamos, Greece
[d] Department of Zoology, Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Herman Otto 15, Budapest, Hungary

Abstract

This paper presents an updated checklist of the Greek scale insect fauna and the results of the first zoogeographic analysis of the Greek scale insect fauna. According to the latest data, the scale insect fauna of the whole Greek territory includes 207 species; of which 187 species are recorded from mainland Greece and the minor islands, whereas only 87 species are known from Crete. The most rich families are the Diaspididae (with 86 species), followed by Coccidae (with 35 species) and Pseudococcidae (with 34 species). In this study the results of a zoogeographic analysis of scale insect fauna from mainland Greece and Crete are also presented. Five species, four from mainland Greece and one from Crete are considered to be endemic. Comparison with the scale insect fauna of other countries is provided.

Keywords: Biogeography; Chorotypes; Crete; Mainland Greece

Zootaxa

Volume 4012, Issue 1, Pages 57-77

Contrasting patterns of host adaptation in two egg parasitoids of the pine processionary moth (Lepidoptera: Thaumetopoeidae)

Ruschioni, S.[a], *Riolo, P.*[a], *Isidoro, N.*[a], *Romani, R.*[b], *Petrucco-Toffolo, E.*[c],
Zovi, D.[c], *Battisti, A.*[c]

[a] Dipartimento di Scienze Agrarie, Alimentari Ed Ambientali, Università Politecnica Delle Marche, Via Brecce Bianche, Ancona, Italy
[b] Dipartimento di Scienze Agrarie, Alimentari, Università Degli Studi di Perugia, Borgo XX Giugno, 74, Perugia, Italy
[c] DAFNAE - Entomologia, Università Degli Studi di Padova, Via dell'Università 16, Legnaro (PD), Italy

Abstract

Adaptation of parasitoids to their phytophagous host is often mediated by environmental conditions and by the food plant of the phytophagous host. Therefore, the host food plant can indirectly affect the survival and fitness of parasitoids that also attack quiescent host stages, such as eggs, in which the resources available to the immature parasitoid stages are limited. Our aim was to investigate how two egg parasitoid species of the pine processionary moth, *Thaumetopoea pityocampa* (Denis & Schiffermü ller), respond to variations in egg traits at the extremes of a west-to-east geographic gradient in northern Italy. We considered one specialist [*Baryscapus servadeii* (Domenichini)] and one generalist [*Ooencyrtus pityocampae* (Mercet)] parasitoid, which reproduce mainly by thelytokous parthenogenesis and are common throughout the whole range of this pest. The size and shell structure of the pine processionary moth eggs were studied under light microscopy and tested experimentally under controlled conditions. We can conclude that 1) the pine processionary moth egg shell thickness is inversely proportional to the parasitism performance; 2) the larger eggs from the pine processionary moth eastern population produce parasitoid females of a larger size, which have greater realized fecundity; 3) the generalist parasitoid performs successfully with either the "home" or "away" (i.e., from both extremes of the geographic gradient) pine processionary moth eggs, which is not the case for the specialist parasitoid. The implications of these responses in the regulation of phytophagous populations are numerous and should be considered in population dynamics studies and pest management programs.

Keywords: *Baryscapus servadeii*; host adaptation; *Ooencyrtus pityocampae*; *Thaumetopoea pityocampae*

Environmental Entomology

Volume 44, Issue 3, Pages 480-487

Efficacy of two common methods of application of residual insecticide for controlling the Asian tiger mosquito, *Aedes albopictus* (Skuse), in urban areas

Marini, L.[a], Baseggio, A.[b], Drago, A.[c], Martini, S.[c], Manella, P.[d], Romi, R.[e], Mazzon, L.[a]

[a] Department of Agronomy, Food, Natural Resources, Animals, and Environment (DAFNAE), University of Padua, Legnaro (PD), Italy
[b] India Srl, Arre (PD), Italy
[c] Entostudio Snc, Brugine (PD), Italy
[d] ACTIVA Srl, Milano, Italy
[e] Department of Infectious, Parasitic and Immuno-mediated Diseases, Vector-Borne Diseases and International Health Unit, Istituto Superiore di Sanitaà (ISS), Roma, Italy

Abstract

After its first introduction in the 1980's the Asian tiger mosquito, *Aedes albopictus* (Skuse), has spread throughout Southern Europe. Ae. albopictus is considered an epidemiologically important vector for the transmission of many viral pathogens such as the yellow fever virus, dengue fever and Chikungunya fever, as well as several filarial nematodes such as *Dirofilaria immitis* or *D. repens*. It is therefore crucial to develop measures to reduce the risks of disease transmission by controlling the vector populations. The aim of the study was to compare the efficacy of two application techniques (mist vs. stretcher sprayer) and two insecticides (Etox based on the nonester pyrethroid Etofenprox vs. Microsin based on the pyrethroid type II Cypermetrin) in controlling adult tiger mosquito populations in highly populated areas. To test the effect of the two treatments pre- and post-treatment human landing rate counts were conducted for two years. After one day from the treatment we observed a 100% population decrease in mosquito abundance with both application methods and both insecticides. However, seven and 14 days after the application the stretcher sprayer showed larger population reductions than the mist sprayer. No effect of insecticide type after one day and 14 days was found, while Etox caused slightly higher population reduction than Microsin after seven days. Emergency measures to locally reduce the vector populations should adopt adulticide treatments using stretcher sprayers. However, more research is still needed to evaluate the potential negative effects of adulticide applications on non-target organisms.

PLoS ONE

Volume 10, Issue 8, e0134831

Feeding habits of overwintered predatory mites inhabiting European vineyards

Lorenzon, M., Pozzebon, A., Duso, C.

Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padua, Viale dell'Università 16, Padua, Legnaro, Italy

Abstract

Biological parameters, prey consumption and prey conversion into egg biomass of overwintered females of the phytoseiids *Kampimodromus aberrans*, *Amblyseius andersoni* and *Typhlodromus pyri* fed with varying numbers of *Eotetranychus carpini* females or pollen were studied in the laboratory. We found that pollen and *E. carpini* females proved to be optimal foods for overwintered phytoseiids. In most comparisons pollen induced performances comparable to those recorded when predatory mites were supplied with 4–8 prey per day. *Typhlodromus pyri* and *A. andersoni* females survived longer when fed on pollen and the percentage of *K. aberrans* ovipositing females was higher with pollen than with spider mite prey. For *K. aberrans* and *T. pyri*, periods to diapause termination were comparable when pollen or eight prey per predator per day were provided. Oviposition times were long for *T. pyri* and *A. andersoni* fed with pollen. Low numbers (1–2) of *E. carpini* drastically reduced predatory mite oviposition, particularly in *A. andersoni* that showed high requirements in terms of food biomass. Results highlighted the importance of pollen for overwintered predatory mites. The results obtained can help in understanding some aspects of the biology and behaviour of the three predatory mites in vineyards.

Keywords: Biological control; Phytoseiidae; Pollen; Spider mites; Tetranychidae; *Vitis vinifera* L

BioControl

Volume 60, Issue 5, Pages 605-615

Improving the early detection of alien wood-boring beetles in ports and surrounding forests

Rassati, D., Faccoli, M., Petrucco Toffolo, E., Battisti, A., Marini, L.

Department of Agronomy, Food, Natural Resources, Animals and Environment
(DAFNAE), University of Padova, Viale dell' Università, Legnaro Padova, Italy

Abstract

International ports are generally considered the most likely points of entry for alien wood-boring beetles. A better understanding of the factors affecting their arrival and establishment at ports and their surrounding areas is of utmost importance to improve the efficacy and the cost-effectiveness of early detection programmes. Our work aimed at understanding how port size and the characteristics of the landscape surrounding the port, in terms of forest cover and forest composition, influence the occurrence of alien wood-boring beetles. From May to September 2012, 15 Italian international ports and the surrounding forests were monitored with multi-funnel traps baited with a multi-lure blend (-pinene, ethanol, ipsdienol, ipsenol, methyl-butenol), three in each port and three in forests located 3-5 km away from the port. We identified both alien and native Scolytinae, Cerambycidae and Buprestidae beetles. Fourteen alien species, among which four are new to Italy, were trapped. Alien species richness was positively related to the amount of imported commodities at the port scale. Broadleaf forests surrounding ports received larger number of alien species than conifer forests. By contrast, total forest cover in the landscape surrounding ports was positively related to the occurrence of native but not alien species. The alien and native species richness was higher in the surrounding forests than in the ports. Synthesis and applications. The simultaneous use of traps in ports with large volume of imported commodities and in their surrounding broadleaf forests can strongly increase the probability of alien wood-boring beetle interceptions. The identification of sites where the arrival and establishment of alien species is more probable, combined with an efficient trapping protocol, can substantially improve the efficacy of early detection. Similar approaches may be used in other countries as early warning systems to implement timely measures to eradicate or contain alien invasions at the European scale. The simultaneous use of traps in ports with large volume of imported commodities and in their surrounding broadleaf forests can strongly increase the probability of alien wood-boring beetle interceptions. The identification of sites where the arrival and establishment of alien species is more probable, combined with an efficient trapping protocol, can substantially improve the efficacy of early detection. Similar approaches may be used in other countries as early warning systems to implement timely measures to eradicate or contain alien invasions at the European scale.

Keywords: Bark beetles; Exotic species; Forest pests; Invasion; Jewel beetles; Landscape; Longhorn beetles; Monitoring; Species interception; Surveillance

Journal of Applied Ecology

Volume 52, Issue 1, Pages 50-58

Life history of the asian longhorn beetle *Anoplophora glabripennis* (Coleoptera Cerambycidae) in southern Europe

Faccoli, M.[a], Favaro, R.[a], Smith, M.T.[b], Wu, J.[c]

[a] Department of Agronomy, Food, Natural Resources, Animals and Environment, University of Padova, viale dell'Università 16, Legnaro (PD), Italy
[b] Beneficial Insects Introduction Research Unit, United States Department of Agriculture, Agricultural Research Service, 501 South Chapel Street, Newark, United States
[c] Department of Entomology and Wildlife Ecology, University of Delaware, 250 Townsend Hall, Newark, DE, United States

Abstract

The Asian longhorn beetle Anoplophora glabripennis is highly polyphagous and widely spread over regions with different climates. Determining the key life-history traits is important for understanding how local conditions affect its successful establishment and to develop adaptive management strategies. Field and laboratory studies were conducted from 2010 to 2012 on an A. glabripennis infestation in Northern Italy, aiming to determine its seasonal phenology, adult beetle longevity, density of successful emergence, infestation age and overwintering life history. Adult beetle emerged from infested trees from 22 May to 28 June. Ninety percent of emergence was reached around 20 July. The first 1% of emergence was accurately predicted by an accumulated degree-day model. In the laboratory, the mean longevity of males and females developed under natural conditions was 27.8±1.7 and 24.9±1.8days, respectively. In northern Italy, A. glabripennis largely overwinter as mature larvae in the xylem. The mean density of exit holes was 24.0±2.7 holes/ m^2 of bark, with successful emergence from branches as small as 3.2cm in diameter. Although the infestation was discovered in June 2009, the oldest exit hole found in infested trees dated from 2005.

Keywords: Asian longhorn beetle; Biology; Invasive species; Life history; Phenology; Survival

Agricultural and Forest Entomology

Volume 17, Issue 2, Pages 188-196

Predation on heterospecific larvae by adult females of *Kampimodromus aberrans, Amblyseius andersoni, Typhlodromus pyri* and *Phytoseius finitimus* (Acari: Phytoseiidae)

Ahmad, S.[ab], *Pozzebon, A.*[a], *Duso, C.*[a]

[a] Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, Legnaro, Padova, Italy
[b] CABI Central and West Asia, Opposite 1-A Data Gunj Bakhsh Road, Satellite Town, Rawalpindi, Pakistan

Abstract

The predatory mites *Kampimodromus aberrans* (Oudemans), *Amblyseius andersoni* (Chant), *Typhlodromus pyri* Scheuten and *Phytoseius finitimus* Ribaga are important biological control agents in orchards and vineyards in Europe and elsewhere. They can coexist in the same habitat and engage in intraguild predation (IGP). In the laboratory we evaluated the longevity, fecundity and prey consumption of females of these predatory mites fed with heterospecific larvae considered as intraguild prey (IG-prey). The survival and age-specific oviposition curves of predatory mites fed with pollen were compared with those obtained on different IG-prey. We assessed the prey conversion rate into eggs expressed by the different IG-predator as an indicator of their capacity to persist when prey is diminishing. Results suggest that *A. andersoni* should be considered the superior intraguild predator but the least efficient in food conversion. *Phytoseius finitimus* appeared to suffer from intraguild predation, and its efficiency in food conversion was not superior to that of *K. aberrans* and *T. pyri*. The profiles of *K. aberrans* and *T. pyri* were less definite. The comparison between pollen and IG-prey diets confirmed the positive effect of pollen on the fecundity of all four predatory mite species. Fecundity was higher on pollen than on IG-prey. We can suggest that *A. andersoni* have the potential to exclude the other predatory mites only at high food resource availability, whereas low levels of food availability can favor the other species in IGP.

Keywords: Generalist predatory mites; Interspecific predation; Intraguild predation; Phytoseiidae; Predatory mites

Experimental and Applied Acarology

Volume 67, Issue 1, 20p

Role of supplemental foods and habitat structural complexity in persistence and coexistence of generalist predatory mites

Pozzebon, A.[a], *Loeb, G.M.*[b], *Duso, C.*[a]

[a] Department of Agronomy, Food, Natural Resources, Animals and Environment, University of Padova, Italy
[b] Department of Entomology, New York State Agricultural Experiment Station, Cornell University, Geneva, NY, United States

Abstract

Plant traits can influence the interactions between herbivore arthropods and their natural enemies. In these interactions generalist predators are often present, preying on herbivores and also on other arthropods in the same trophic guild. Variation in the strength of intraguild predation (IGP) may be related to habitat structural complexity and to additional resources outside the narrow predator-prey relationship. In this paper we study the food web interactions on grape, which involves two generalist predatory mites. We evaluated the effects of grape powdery mildew (GPM) as supplemental food, and habitat structural complexity provided by domatia. The inoculation of GPM resulted in higher predatory mite densities and reduced the negative impact of unfavorable leaf structure for one species. Access to domatia was the main factor in promoting population abundance and persistence of predatory mites. Access to domatia and GPM availability favored the coexistence of predatory mites at a low density of the intraguild prey. Our findings suggest that structural and nutritional diversity/complexity promote predatory mite abundance and can help to maintain the beneficial mites - plants association. The effect of these factors on coexistence between predators is influenced by the supplemental food quality and relative differences in body size of interacting species.

Scientific Reports

Volume 5, Article number 14997.

Seasonal and daily activity patterns of mosquito (Diptera: Culicidae) vectors of pathogens in Northeastern Italy

Montarsi, F.[a], Mazzon, L.[b], Cazzin, S.[a], Ciocchetta, S.[a], Capelli, G.[a]

[a] Istituto Zooprofilattico Sperimentale delle Venezie, Viale dell'Università, 10, Legnaro (Padua), Italy
[b] Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padua, Viale dell'Università 16, Legnaro, Padua, Italy

Abstract

The seasonal and daily activity of mosquito vectors of pathogens affecting animals and humans were studied in northeastern Italy at a site within the Po River Delta Park. A CDC-CO_2 trap and a gravid trap were operated at 2-h intervals for 24 h every 15 d from May to October 2010. Overall, 5,788 mosquitoes comprising six species were collected, namely *Culex pipiens* L. (75.1% of total), *Aedes caspius* (Pallas) (15.2%), *Aedes vexans* (Meigen) (6.9%), *Anopheles maculipennis* s.l. Meigen (2.6%), *Culiseta annulata* (Schrank) (0.2%), and *Culex modestus* Ficalbi (<0.1%). The relative abundance of these species increased from May until the beginning of July and then decreased, disappearing at the beginning of October. The diel host-seeking patterns and oviposition site-seeking patterns were species specific and were differentially affected by the ecological variables recorded at the day and hour of mosquito collection or two weeks before collection. Knowledge of the seasonal and daily host-seeking patterns of mosquitoes highlights the time periods of the day and the seasons of potential exposure for animals and humans to mosquito-borne pathogens, therefore delineating the best time for the application of preventive measures. Furthermore, knowledge of the oviposition site-seeking activity of the mosquitoes optimizes the capture of gravid females, thereby enhancing the likelihood of detecting pathogens.

Keywords: Daily activity; Host-seeking activity; Mosquito-borne pathogen; Northeastern Italy; Oviposition activity

Journal of Medical Entomology

Volume 52, Issue 1, Pages 56-62

Single and combined releases of biological control agents against canopy- and soil-dwelling stages of *Frankliniella occidentalis* in cyclamen

Pozzebon, A., Boaria, A., Duso, C.

Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padua, Viale dell'Università 16, Legnaro, Padua, Italy

Abstract

Frankliniella occidentalis is a major pest in agriculture. Problems with its control are mainly due to pupation in the soil and resistance to insecticides. Biological control agents (BCA) can be used against thrips pests as an alternative to conventional insecticides. We evaluated the effectiveness of a number of BCA for control of *F. occidentalis* in cyclamen under greenhouse conditions. Three BCA (*Amblyseius swirskii*, *Neoseiulus californicus* and *Orius laevigatus*) were applied to the canopy and two (*Macrocheles robustulus* and *Steinernema feltiae*) to the soil in single and combined releases. Results showed that applications of predatory mites and nematodes achieved good control of *F. occidentalis*. Unsatisfactory control was obtained using *O. laevigatus*. The use of nematodes determined the extinction of thrips, but with a delay. The combination of nematodes in the soil and predatory mites in the canopy caused a more prompt reduction of thrips in flowers.

Keywords: *Amblyseius swirskii*; Biological control; *Frankliniella occidentalis*; *Macrocheles robustulus*; *Neoseiulus californicus*; *Steinernema feltiae*

BioControl

Volume 60, Issue 3, Pages 341-350

The allergenic protein Tha p 2 of processionary moths of the genus *Thaumetopoea* (Thaumetopoeinae, Notodontidae, Lepidoptera): Characterization and evolution

Berardi, L.[a], *Battisti, A.*[a], *Negrisolo, E.*[b]

[a] Department of Agronomy Food Natural Resources Animals and Environment (DAFNAE), University of Padova, Agripolis, Legnaro, PD, Italy
[b] Department of Comparative Biomedicine and Food Science (BCA), University of Padova, Agripolis, Legnaro, PD, Italy

Abstract

The allergenic Tha p 2 protein has been extracted recently from the urticating setae of the pine processionary moth *Thaumetopoea pityocampa*. In the present paper, we test for the occurrence of this protein in other Thaumetopoeinae, with a particular focus on members of the genus *Thaumetopoea*, as well as unrelated moth species, to better understand the physicochemical properties of the protein, the nature of encoding genes and their evolutionary history. Tha p 2 is encoded by the intronless gene *Tha p 2* that is restricted to the processionary moths (Thaumetopoeinae, Notodontidae, Lepidoptera). Most of the species present two isoforms of Tha p 2 that can be interpreted as the result of heterozygosity in the single gene. The only exception is represented by *Thaumetopoea wilkinsoni*, in which 20 different isoforms occur in a single specimen, leading to the conclusion that, at least in this species, multiple copies of *Tha p 2* exist. Serine, glycine, cysteine and leucine are abundant in Tha p 2, a protein well conserved among processionary moths. The predicted secondary structures of Tha p 2 indicate the presence of 3 -helices and six -barrels. Finally, the evolution of the gene and the protein was characterized by a combination of positive and negative selection, with the latter being more evident.

Keywords: Allergenic protein Tha p 2; Molecular evolution; Processionary moths; Secondary structure prediction; Thaumeotopoeinae; *Thaumetopoea*

Gene

Volume 574, Issue 2, Pages 317-324

Two new species of *Coccidohystrix lindinger* (Hemiptera: Coccomorpha: Pseudococcidae) with notes on the related genus *Artemicoccus* Balachowsky

Kaydan, M.B.[a], *Pellizzari, G.*[b], *Szita, É.*[c]

[a] Imamoglu Vocational School, ÇUkurova University, Adana, Turkey
[b] Department of Agronomy, Food, Natural Resources, Animals and Environment DAFNAE, University of Padova, Italy
[c] Centre for Agricultural Research, Plant Protection Institute, Hungarian Academy of Sciences, Herman Ottó út 15, Budapest, Hungary

Abstract

Scale insect (Hemiptera: Coccomorpha: Pseudococcidae) samples were collected in Turkey (Hakkari- Yüksekova) and Bulgaria (Kresna Gorge) between 2007 and 2012. Two new *Coccidohystrix Lindinger* species, are described and illustrated. An identification key including the new species is also provided. Additionaly, the type material of *C. zangherii* was studied and the species is transferred into the genus *Artemicoccus* Balachowsky. A new identification key for species in the genus *Artemicoccus* is also provided.

Keywords: Eriococcidae; Lacking ostiole; Phenacoccinae

Turkiye Entomoloji Dergisi

Volume 39, Issue 4, Pages 355-366

Food science and technology

Profiling chicory sesquiterpene lactones by high resolution mass spectrometry

Graziani, G.[a], Ferracane, R.[a], Sambo, P.[b], Santagata, S.[b], Nicoletto, C.[b], Fogliano, V.[c]

[a] Department of Agricultural and Food Science, University of Napoli Federico II, Parco Gussone, Portici, Napoli, Italy
[b] Department of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE), Agripolis - University of Padova, Viale dell'Università 16, Legnaro, PD, Italy
[c] Food Quality and Design Group, Wageningen University, PO Box 8129, Wageningen, Netherlands

Abstract

Sesquiterpene lactones (SLs) are the main determinants of radicchio bitterness and the control of their concentrations is a key point for the market value of this product. An innovative analytical approach based on two complementary mass spectrometers, Orbitrap-HRMS and MS/MS, was used for quantitative analysis of SLs in aerial part of four different varieties of chicory. Data highlighted the presence of eight SLs: 11β,13-dihydrolactucin, lactucin, 8-deoxy-lactucin, dihydro-8-deoxylactucin, dihydrolactucopicrin, lactucopicrin, lactuside C (jaquinellin glucoside) and dihydro-lactucopicrin oxalate. Significant varietal differences were found. The highest amount of SLs was found in the radicchio "Treviso Precoce" variety (189.71 μg/g), the lowest amount in "Treviso Tardivo" variety (45.78 μg/g). Lactucopicrin was the most abundant compound with concentration ranged between 99.36 in "Treviso Precoce" and 13.50 μg/g in "Treviso Tardivo" while dihydro-lactucopicrin oxalate was the less abundant in all analyzed varieties with an average concentration of about 1% on the total amount of SLs.

Keywords: Bitterness; Chicory; High resolution mass spectrometry; Radicchio; Sesquiterpene lactones; Tandem mass spectrometry

Food Research International

Volume 67, Pages 193-198

Advantages of the KDS/BCA assay over the bradford assay for protein quantification in white wine and grape juice

Gazzola, D.[ab], *Vincenzi, S.*[ab], *Pasini, G.*[ab], *Lomolino, G.*[ab], *Curioni, A.*[ab]

[a] Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE)

[b] Centro Interdipartimentale per la Ricerca in Viticoltura ed Enologia (CIRVE), Università di Padova, via dell'Università 16, Legnaro, PD, Italy

Abstract

The present study compared the performance of two colorimetric protein assays, the Bradford and the potassium dodecyl sulfate/bicinchoninic acid (KDS/BCA) assays, for use in wine and grape juice analysis. The Bradford assay was affected by protein type, whereas the KDS/BCA assay had lower protein-to-protein variation. Bovine serum albumin and lysozyme yielded an absorbance (562 nm) vs. protein concentration slope (dose–response curve) similar to that of wine proteins. In the Bradford assay, the presence of 12% ethanol and 200 mg/L of wine polyphenols decreased the protein absorbance by 28 and 16%, respectively, whereas in the KDS/BCA assay such interference was not significant. Among 64 white wines, the correlation between protein haze potential, determined by a heat test, and protein content was better for the KDS/BCA assay. This study confirmed the superiority of the KDS/BCA assay over the Bradford assay for quantifying protein in white grape juice and wine, and it yielded better predictive value with respect to the risk of white wine protein instability.

Keywords: Colorimetric assays; Protein quantification; White wine; Wine haze

American Journal of Enology and Viticulture

Volume 66, Issue 2, Pages 227-233

‘Artificial sensory analysis’ for sensory classification of Prosecco sparkling wines

Franceschi, D.[a], Tiranno, M.[a], Vincenzi, S.[a], Boatto, V.[a], Bravi, M.[ab]

[a] Centro Interdipartimentale per la Ricerca in Viticoltura Ed Enologia, Università di Padova, Padova, Italy
[b] Dipartimento Ingegneria Chimica Materiali Ambiente, Sapienza Università di Roma, Rome, Italy

Abstract

Real-time in-process reference to a consolidated standard during sparkling wine production would be beneficial for reducing product loss and/or allowing a timely diagnosis of intervention needs (correction etc). Instead, end-point control by assaying by the oenologist supervising batch production is the only form of (sensory) control normally carried out in wineries. Afterwards, samples from production batches must pass the evaluation step at the Commission’s desk. The present experimental study was carried out to assess whether the responses of an “electronic nose”, i.e. a non-specific, gas-phase analytical instrument, is capable to draw an outline of the sensory profile of Conegliano Valdobbiadene Prosecco Superiore DOCG and Prosecco DOC in a way that is objective, repeatable and that can be simply related to the verdict of a group of expert judges.

Keywords: Electronic Noses; Fermentations

Chemical Engineering Transactions

Volume 43, Pages 181-186

Characterization of crude esterase activity from two plants used in cheese making: *Cynara cardunculus* L. and *Ficus carica* L.

Lomolino, G., Zannoni, S., Di Pierro, G.

Department of Agronomy, Food, Natural Resources, Animals, and Environment-DAFNAE, Università degli Studi di Padova, Padova, Italy

Abstract

For centuries, extracts of *Cynara cardunculus* and the latex of *Ficus carica* have been used in cheesemaking, thanks to their proteases that clot milk casein. Since esterase activity is important to release odorous compounds in cheese, in this study we characterized the esterase activity of an extract of *Cynara cardunculus* and the latex of *Ficus carica*. The results, obtained by titration, spectrophotometric techniques, zymograms, and tests on agar plates, show the presence of esterase activity in the samples tested, with affinity towards the esters of short chain fatty acids, such as acetic, propionic, and butyric acid. In conclusion, this study provides a background information on esterases of two plants used in cheesemaking and represents a starting point for further investigations of their applications, not only in dairying but also in other industries involved in the processing and transformation of lipids.

Keywords: *Cynara cardunculus*; Esterase; *Ficus carica*; Latex; Plant coagulants; Zymogram

Food Biotechnology

Volume 29, Issue 4, Pages 297-316

Citrus hydrosols as useful by-products for tyrosinase inhibition

Lante, A., Tinello, F.

Department of Agronomy, Food, Natural Resources, Animals and Environment,
DAFNAE Agripolis, University of Padova, Viale Universitá, 16, Legnaro, PD, Italy

Abstract

Hydrosols are coproduced during water or steam distillation of plant material. Three kinds of citrus hydrosols (CIHSs) were assessed for anti-tyrosinase (TYR) activity using spectrophotometric assays and terpene content as quantified by gas chromatography (GC). All of the distillate waters were found to inhibit commercial mushroom tyrosinase at varying levels (21.8-68.9%) depending on substrate type and concentration. The GC analysis indicated that a number of known tyrosinase inhibitors including myrcene, sabinene, geraniol and citral were present in CIHS, which behave as mixed-type inhibitors towards tyrosinase. *Industrial relevance*: Citrus hydrosols have great potential to meet the demands of the food and cosmetic industries, since they are not only easy and inexpensive to produce but also without any perceivable hazard for humans. In addition, since citrus hydrosols can be extracted from the discarded peels of citrus fruits, their use as anti-browning agents would allow the repurposing of what has typically been considered a biological waste product.

Keywords: Citrus; Gas chromatography; Hydrosols; Inhibitors; Tyrosinase

Innovative Food Science and Emerging Technologies

Volume 27, Issue , Pages 154-159

Digestibility of pasta made with three wheat types: A preliminary study

Simonato, B.[a], *Curioni, A.*[b], *Pasini, G.*[b]

[a] Department of Biotechnology, University of Verona, Strada Le Grazie 15, Verona, Italy
[b] DAFNAE (Department of Agronomy, Natural Resource, Animals and Environment), University of Padova, Legnaro, PD, Italy

Abstract

The aim of this study was to assess the digestibility of the protein and starch in pasta made with different cereals, i.e. *Triticum durum*, *Triticum polonicum* and *Triticum dicoccum*, and to measure the glycemic index (GI) of the different types of pasta. The digestibility of the starch in *T. polonicum* pasta differed significantly from the others. It seemed to be less digested than *dicoccum* and *durum* wheat pasta. *T. polonicum* pasta also had a lower glycemic index, while there were no significant differences in the protein digestibility of the three types of pasta.

Keywords: Glycemic index; Protein digestibility; Starch digestibility; Wheat pasta

Food Chemistry

Volume 174, Issue , Pages 219-225

Foaming properties of potato (*Solanum tuberosum*) proteins: A study by the gas sparging method

Lomolino, G., Vincenzi, S., Gazzola, D., Crapisi, A., Curioni, A.

Department of Agronomy, Food Natural Resources, Animal and Enviroment DAFNAE,
University of Padua, Viale dell'Università 16, Legnaro, Padova, Italy

Abstract

The proteins used in food industry to make foams are mostly of animal origin, thus arising ethical and health concerns. For this reasons, protein of plant origin, such as those of potato, may represent an interesting alternative.In this research, the foaming behavior of potato proteins has been studied, using a commercial sample of potato proteins enriched in patatin (Laffort), intended for use as a fining agent in wine.The potato proteins, solubilized in buffers at different pH (3-7), were subjected to foaming by sparging N_2, air and CO_2, in the same conditions of pressure and time.The results showed a significant foam expansion (400%) in samples in which N_2 and CO_2 were sparged. However, some differences were observed in foam stability. In particular, foam was very stable when produced with N_2 (40%), less stable with air (30%) and very unstable with CO_2 (10%). Image analysis carried out on foams by a stereomicroscope and d_{32} (Sauter mean diameter) measurements showed significant differences related to the nature of the sparged gas and the pH. In particular, the foam obtained with N_2 produced very small bubbles, with low polydispersity. Electrophoretic analyses of the proteins migrating into the foam revealed differences in protein patterns, especially in relation to the sparged gas, indicating that the nature of the dissolved gas affects the behavior of the different protein components.In conclusion, the patatin preparation here tested produces foams with stability and expansion which depend not only on the pH but also on the type of gas sparged in the liquid.

Keywords: Foam; Foam expansion; Foam stability; Gas sparging; Image analysis; Patatin

Colloids and Surfaces A: Physicochemical and Engineering Aspects

Volume 475, Issue 1, Pages 75-83

Investigating the use of gradient boosting machine, random forest and their ensemble to predict skin flavonoid content from berry physical-mechanical characteristics

Brillante, L.[a], Gaiotti, F.[a], Lovat, L.[a], Vincenzi, S.[b], Giacosa, S.[c], Torchio, F.[c], Segade, S.R.[c], Rolle, L.[c], Tomasi, D.[a]

[a] CRA-VIT Council for Agricultural Research and Economics, Viticulture Research Center, Conegliano, TV, Italy
[b] University of Padova, Centro Interdipartimentale per la Ricerca in Viticoltura ed Enologia, Legnaro, PD, Italy
[c] University of Turin, Dipartimento di Scienze Agrarie, Forestali e Alimentari, Grugliasco, TO, Italy

Abstract

Flavonoids are a class of bioactive compounds largely represented in grapevine and wine. They also affect the sensory quality of fruits and vegetables, and derived products. Methods available for flavonoid measurement are time-consuming, thus a rapid and cost-effective determination of these compounds is an important research objective. This work tests if applying machine learning techniques to texture analysis data allows to reach good performances for flavonoid estimation in grape berries. Whole berry and skin texture analysis was applied to berries from 22 red wine grape cultivars and linked to the total flavonoid content. Three machine-learning techniques (regression tree, random forest and gradient boosting machine) were then applied. Models reached a high accuracy both in the external and internal validation. The R^2 ranged from 0.75 to 0.85 for the external validation and from 0.65 to 0.75 for the internal validation, while RMSE (Root Mean Square Error) went from 0.95 mg g^{-1} to 0.7 mg g^{-1} in the external validation and from 1.3 mg g^{-1} to 1.1mg g^{-1} in the internal validation.

Keywords: Flavonoids; Gradient Boosting Machine (GBM); Random forest; Texture analysis; Wine-grape

Computers and Electronics in Agriculture

Volume 117, Issue , Pages 186-193

Short communication: Characterization of soluble thiols in bovine milk

Niero, G., De Marchi, M., Masi, A., Penasa, M., Cassandro, M.

Department of Agronomy, Food, Natural resources, Animals and Environment,
University of Padova, Viale dell'Università 16, Legnaro (PD), Italy

Abstract

Antioxidants are molecules essential for the maintenance of cell homeostasis and their intake through the diet has positive effects on human health. Among antioxidants, low-molecular-weight (LMW) thiols represent an important class of compounds. The aim of this study was to identify LMW thiols in bovine milk. A total of 96 individual milk samples from Brown Swiss, Holstein-Friesian, Alpine Grey, and Simmental cattle breeds were collected in 8 herds. The LMW thiols were extracted from the soluble fraction of milk and, following a derivatization protocol, they were separated by reverse phase HPLC and detected fluorimetrically. Six thiol species were detected and 2, glutathione (GSH) and cysteine-glycine (Cys-Gly), were identified and quantified. Regardless of the breed, the average concentration of Cys-Gly in milk was greater than that of GSH. Overall, milk from dual-purpose breeds (Simmental and Alpine Grey) was richer in LMW thiols than milk from dairy cows (Holstein-Friesian and Brown Swiss). Glutathione and Cys-Gly, closely linked metabolically, were strongly correlated. Pearson correlations of Cys-Gly with protein and casein contents were moderately low, and no relationship was found between GSH and milk chemical composition. Future research should focus on the identification of all detected LMW thiol species.

Keywords: Antioxidant; Cattle breed; Milk; Thiol

Journal of Dairy Science

Volume 98, Issue 9, Pages 6014-6017

Structural and Nutritional Properties of Pasta from *Triticum monococcum* and *Triticum durum* Species. A Combined ^{1}H NMR, MRI, and Digestibility Study

Pasini, G.[a], Greco, F.[b], Cremonini, M.A.[c], Brandolini, A.[d], Consonni, R.[b], Gussoni, M.[be]

[a] DAFNAE, Dipartimento di Agronomia, Animali, Alimenti, Risorse Naturali Ed Ambiente, Università Degli Studi di Padova, Legnaro (PD), Italy
[b] Laboratorio NMR, Istituto per Lo Studio Delle Macromolecole, Consiglio Nazionale Delle Ricerche, Milano, Italy
[c] Dipartimento di Scienze Degli Alimenti, Università di Bologna, Cesena, Italy
[d] Unità per la Selezione Dei Cereali e la Valorizzazione Delle Varietà Vegetali, Consiglio per la Ricerca e la Sperimentazione in Agricoltura (CRA-SCV), Via Forlani 3, S. Angelo Lodigiano (LO), Italy
[e] Dipartimento di Fisiopatologia Medico Chirurgica e Dei Trapianti, Università Degli Studi di Milano, Via F.lli Cervi, 93, Segrate (MI), Italy

Abstract

The aim of the present study was to characterize the structure of two different types of pasta, namely *Triticum turgidum* ssp. *durum* (cv. Saragolla) and *Triticum monococcum* ssp. *monococcum* (cv. Monlis), under different processing conditions. MRI analysis and NMR spectroscopy (i.e., T_1 and T_2 NMR relaxation times and diffusion parameters) were conducted on pasta, and ^{1}H NMR spectroscopic analysis of the chemical compounds released by pasta samples during the cooking process was performed. In addition, starch digestibility (enzimatically determined) was also investigated. The NMR results indicated that Saragolla pasta has a more compact structure, ascribed to pasta network and in particular to different technological gluten properties, that mainly determine the lower ability of Monlis pasta in binding water. These results correlate well with the lower rate of starch hydrolysis measured for Monlis pasta compared to Saragolla when both are dried at high temperature.

Keywords: ^{1}H NMR spectroscopy; diffusion; in vitro starch digestibility; MRI; pasta microstructure; T_1/T_2 relaxation times

Journal of Agricultural and Food Chemistry

Volume 63, Issue 20, Pages 5072-5082

Study of combined effect of proteins and bentonite fining on the wine aroma loss

Vincenzi, S.[a], *Panighel, A.*[b], *Gazzola, D.*[a], *Flamini, R.*[b], *Curioni, A.*[a]

[a] Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale XXVIII Aprile 14, Conegliano, TV, Italy
[b] Cons. per la Ric. in Agricoltura e l'Analisi dell'Economia Agraria-Ctro. di Ric. per la Viticoltura, Viale XXVIII Aprile 26, Conegliano, TV, Italy

Abstract

The wine aroma loss as a consequence of treatments with bentonite is due to the occurrence of multiple interaction mechanisms. In addition to a direct effect of bentonite, the removal of aroma compounds bound to protein components adsorbed by the clay has been hypothesized but never demonstrated. We studied the effect of bentonite addition on total wine aroma compounds (extracted from Moscato wine) in a model solution in the absence and presence of total and purified (thaumatin-like proteins and chitinase) wine proteins. The results showed that in general bentonite alone has a low effect on the loss of terpenes but removed ethyl esters and fatty acids. The presence of wine proteins in the solution treated with bentonite tended to increase the loss of esters with the longest carbon chains (from ethyl octanoate to ethyl decanoate), and this was significant when the purified proteins were used. The results here reported suggest that hydrophobicity can be one of the driving forces involved in the interaction of aromas with both bentonite and proteins.

Keywords: bentonite; ethyl esters; terpenes; wine aroma; wine proteins

Journal of Agricultural and Food Chemistry

Volume 63, Issue 8, Pages 2314-2320

Total phenolic content, antioxidant and antimicrobial activity of *Haberlea rhodopensis* extracts obtained by pressurized liquid extraction

Mihaylova, D.[a], *Lante, A.*[b], *Krastanov, A.*[a]

[a] Department of Biotechnology, University of Food Technologies, 26 Maritza Blvd., Plovdiv, Bulgaria
[b] Department of Agronomy Food, Natural Resources, Animals and Environment, Università di Padova, Viale dell'Universitá 16, Agripolis, Legnaro, Padova, Italy

Abstract

The present study was designed to investigate the antioxidant and antimicrobial activities of pressurized liquid extracts from *Haberlea rhodopensis* Friv. The total phenolic content was performed using the Folin-Ciocalteu phenol reagent. To determine the antioxidant activities of the extracts, several complementary tests were used: ABTS and DPPH radical scavenging activities, oxygen radical absorbance capacity, and ferric-reducing antioxidant power assay. The phenolic concentration was 15.98±0.09 and 9.42±0.06 mg GAE g^{-1} DW for 70 and 85% ethanol extracts, respectively. Of all the performed methods, the highest antioxidant activity values were measured by the ORAC assay - 224.6±6.6 and 154.0±9.9 M TE g^{-1} DW for 70 and 85% ethanol extracts, respectively. Results also showed that both extracts exhibited very weak antimicrobial activity against the examined microorganisms. However, the 70% ethanol extract possessed higher inhibition ability, which correlated with higher total phenolic content and antioxidant activity.

Keywords: Antimicrobial activity; Antioxidant activity; *Haberlea rhodopensis*; Pressurized liquid extract

Acta Alimentaria

Volume 44, Issue 3, Pages 326-332

Effect of microparticulated whey protein concentration and protein-to-fat ratio on Caciotta cheese yield and composition

Sturaro, A.[a], *De Marchi, M.*[a], *Zorzi, E.*[b], *Cassandro, M.*[a]

[a] Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, Legnaro, PD, Italy
[b] Dairy Cooperative Latteria di Soligo, Via 1 Settembre 32, Soligo, TV, Italy

Abstract

The dairy industry exploits different processes to increase the value of whey protein (WP). Microparticulated whey proteins (MWP), which are colloidal particles formed by controlled aggregation of WP, are widely used in low-fat products. The aim of the present study was to evaluate the effects of MWP concentration (0.0-4.0%, v/v) and different protein-to-fat ratios (PFRs) on milk coagulation process, cheese yield and composition of Caciotta cheese. Samples of cheese were analysed after 10d of ripening. The increment of PFR affected rennet coagulation time. Moreover, cheese yield decreased as the level of fat decreased, and it was greater in low-fat cheese (high PFR) with 4.0% MWP than in low-fat cheese with 3.0% MWP. No differences were detected for cheese yield in standard and high-fat cheese (standard and low PFR) across MWP concentrations. The stable composition of low-fat Caciotta suggests the possibility to include MWP as fat replacer.

International Dairy Journal

Volume 48, Pages 46-52

Effectiveness of mid-infrared spectroscopy for prediction of the contents of calcium and phosphorus, and titratable acidity of milk and their relationship with milk quality and coagulation properties

Toffanin, V.[a], De Marchi, M.[a], Lopez-Villalobos, N.[b], Cassandro, M.[a]

[a] Department of Agronomy, Food, Natural Resources, Animals and Environment, University of Padova, Viale dell'Università16, Legnaro, PD, Italy
[b] Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Private Bag 11-222, Palmerston North, New Zealand

Abstract

Individual milk samples from Holstein Friesian cows were collected and analysed by inductively coupled plasma optical emission spectrometry (ICP-OES) and titration for the determination of calcium (Ca), phosphorus (P) and titratable acidity (TA) contents, respectively. Prediction models were obtained using partial least squares (PLS) regression analyses using two statistical packages. The average Ca, P and TA were 1156 mg kg-1, 934 mg kg^{-1} and 3.42 °SH 50 mL^{-1}, respectively. Pearson's correlations between Ca and P and other milk traits were significant (P <0.05) and ranged from 0.16 to 0.53 for chemical composition traits and from 0.17 to-0.35 for milk coagulation properties (MCP). Results from the two statistical packages were comparable. Prediction models using MIR spectroscopy were satisfactory for Ca, P and TA, with coefficients of correlation of cross-validation greater than 0.73. Moreover, the study highlighted favourable relationships of these traits with milk coagulation properties.

International Dairy Journal

Volume 41, Pages 68-73

Milk skimming, heating, acidification, lysozyme, and rennet affect the pattern, repeatability, and predictability of milk coagulation properties and of curd-firming model parameters: A case study of Grana Padano

Stocco, G.[a], *Cipolat-Gotet, C.*[a], *Cecchinato, A.*[a], *Calamari, L.*[b], *Bittante, G.*[a]

[a] Department of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE), University of Padova, Legnaro, Italy
[b] Istituto di Zootecnica, Università Cattolica del sacro Cuore, Piacenza, Italy

Abstract

Milk coagulation properties are used to evaluate the cheesemaking aptitude of milk samples. No international standard procedure exists, although laboratories often mimic the production of a full-fat fresh cheese for milk coagulation properties. Questions have arisen about the predictability of such a procedure for different types of cheese production. The aim of this study was to establish a procedure mimicking the production conditions of a long-ripened hard cheese, taking Protected Designation of Origin Grana Padano as a case study. With respect to the traditional conditions (standard procedure; SP), the Grana Padano procedure (GP) modifications were the use of standardized milk, coagulation lower temperature, previous milk acidification, lysozyme addition, and rennet type. Each modification was tested in turn versus the SP and also all together in the GP. Another 3 tests were carried out: SP on naturally creamed milk, SP with double the quantity of rennet, and a simplified GP on a full-fat milk sample. The 10 procedures were tested on 2 subsamples with 2 replicates each and were repeated using individual milk samples from 15 dual-purpose Simmental cows in 4 sessions for a total of 600 tests. Two Formagraph instruments (Foss Electric A/S, Hillerød, Denmark) measuring curd firmness every 15 s were used, prolonging test duration to 60. min to obtain 5 traditional single-point milk coagulation properties and 3 parameters of the curd firming model using all 240 points recorded for each replicate. The 8 traits of each replicate were analyzed according to a mixed model with fixed effects of 4 sessions, 10 treatments, 2 instruments, and 16. microvats, and random effects of 15 animals and 300 subsamples. Compared with the SP, the coagulation and curd firming was slowed by low temperature and was accelerated by acidification and by adding a double amount of rennet; natural creaming, fat standardization, and rennet with 5% pepsin affected only some traits, whereas lysozyme addition affected none. Combination of all modifications tended to compensate for each of their effects, resulting in similar average patterns between GP and SP. Modifications to repeatability were found for all traits with the exception of 2. The ability of the SP to predict GP, tested through correlations between procedures, was not very high. Whereas SP is used for both research and in the dairy industry, better results for Grana Padano cheesemaking can only be achieved by adopting specific, more complex, and labor-intensive procedures at the research level or, possibly, by specific calibrations through Fourier-transform infrared spectroscopy at the industry level.

Keywords: Cheese-making; Curd firming modeling; Grana Padano; Milk coagulation properties

Journal of Dairy Science

Volume 98, Issue 8, Pages 5052-5067

Phenotypic analysis of cheese yields and nutrient recoveries in the curd of buffalo milk, as measured with an individual model cheese-manufacturing process

Cipolat-Gotet, C., Bittante, G., Cecchinato, A.

Department of Agronomy, Food Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università, Legnaro (PD), Italy

Abstract

Traits associated with cheese yield and milk nutrient recovery in curd are used to describe the efficiency of the cheese-making process. This is fundamental for all dairy species, including the Italian Mediterranean buffalo, which is largely used for milk production aimed at the dairy industry. To assess cheese-making traits among buffalo, a model cheese-manufacturing process was tested; it was capable of processing 24 samples per run, using 0.5-L samples of milk from individual buffalo. In total, 180 buffalo reared in 7 herds located in Northeast Italy were sampled once. Briefly, each sample was weighed and heated (35°C for 30min), inoculated with starter culture (90min), and mixed with rennet (51.2 international milk-clotting units/L of milk). After 10min of gelation, the curd was cut; 5min after the cut, the curd was separated from the whey, and the curd was subjected to draining (for 30min) and pressing (18h). The curd and whey were weighed, analyzed for pH and the total solid, fat, lactose, and protein contents, and subjected to estimation of the energy content. Three measures of cheese yield (%CY), $\%CY_{CURD}$, $\%CY_{SOLIDS}$, and $\%CY_{WATER}$, were computed as the ratios between the weight of the curd, the curd dry matter, and the water retained in the curd, respectively, and the weight of the milk processed. These traits were multiplied by the daily milk yield to define the 3 corresponding measures of daily cheese yield (dCY, kg/d). The milk component recoveries (REC) in the curd, REC_{FAT}, $REC_{PROTEIN}$, and REC_{SOLIDS}, represented the ratios between the weights of the fat, protein, and total solids in the curd, respectively, and the corresponding components in the milk. Finally, energy recovery (REC_{ENERGY}) was estimated. The values for $\%CY_{CURD}$, $\%CY_{SOLIDS}$, $\%CY_{WATER}$, $REC_{PROTEIN}$, REC_{FAT}, REC_{SOLIDS}, and REC_{ENERGY} averaged 25.6, 12.7, 12.9, 80.4, 95.1, 66.7, and 79.3%, respectively, indicating that buffalo milk has a higher aptitude to cheese-making than bovine milk. The effect of days in milk was the most important source of variation for %CY, $REC_{PROTEIN}$, and the overall recoveries (which showed higher values toward the end of lactation), whereas parity did not appear to influence any of the investigated traits. The cheese-making procedure tested allowed us to assess the variability of and relationships among different cheese yield traits, recovery traits, daily milk production traits, and milk components at the individual level.

Keywords: Buffalo milk; Cheese-making trait; Individual cheese yield; Whey loss

Journal of Dairy Science

Volume 98, Issue 1, Pages 633-645

Prediction of bovine milk technological traits from mid-infrared spectroscopy analysis in dairy cows

Visentin, G.[ab], McDermott, A.[ab], McParland, S.[a], Berry, D.P.[a], Kenny, O.A.[c], Brodkorb, A.[c], Fenelon, M.A.[c], De Marchi, M.[a]

[a] Animal and Grassland Research and Innovation Center, Teagasc, Moorepark, Fermoy, Co. Cork, Ireland
[b] Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, Legnaro (PD), Italy
[c] Teagasc Food Research Center, Moorepark, Fermoy, Co. Cork, Ireland

Abstract

Rapid, cost-effective monitoring of milk technological traits is a significant challenge for dairy industries specialized in cheese manufacturing. The objective of the present study was to investigate the ability of mid-infrared spectroscopy to predict rennet coagulation time, curd-firming time, curd firmness at 30 and 60min after rennet addition, heat coagulation time, casein micelle size, and pH in cow milk samples, and to quantify associations between these milk technological traits and conventional milk quality traits. Samples (n=713) were collected from 605 cows from multiple herds; the samples represented multiple breeds, stages of lactation, parities, and milking times. Reference analyses were undertaken in accordance with standardized methods, and mid-infrared spectra in the range of 900 to 5,000cm^{-1} were available for all samples. Prediction models were developed using partial least squares regression, and prediction accuracy was based on both cross and external validation. The proportion of variance explained by the prediction models in external validation was greatest for pH (71%), followed by rennet coagulation time (55%) and milk heat coagulation time (46%). Models to predict curd firmness 60min from rennet addition and casein micelle size, however, were poor, explaining only 25 and 13%, respectively, of the total variance in each trait within external validation. On average, all prediction models tended to be unbiased. The linear regression coefficient of the reference value on the predicted value varied from 0.17 (casein micelle size regression model) to 0.83 (pH regression model) but all differed from 1. The ratio performance deviation of 1.07 (casein micelle size prediction model) to 1.79 (pH prediction model) for all prediction models in the external validation was <2, suggesting that none of the prediction models could be used for analytical purposes. With the exception of casein micelle size and curd firmness at 60min after rennet addition, the developed prediction models may be useful as a screening method, because the concordance correlation coefficient ranged from 0.63 (heat coagulation time prediction model) to 0.84 (pH prediction model) in the external validation.

Keywords: Casein micelle size; Grass; Milk acidity; Milk coagulation properties; Milk heat stability

Journal of Dairy Science

Volume 98, Issue 9, Pages 6620-6629

Proton transfer reaction time-of-flight mass spectrometry: A high throughput and innovative method to study the influence of dairy system and cow characteristics on the volatile compound fingerprint of cheeses

Bergamaschi, M.[ab], Biasioli, F.[b], Cappellin, L.[b], Cecchinato, A.[a], Cipolat-Gotet, C.[a], Cornu, A.[c], Gasperi, F.[b], Martin, B.[c], Bittante, G.[a]

[a] Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, Legnaro (PD), Italy
[b] Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach (FEM), Via E. Mach 1, San Michele all'Adige (TN), Italy
[c] INRA, UMR 1213 Herbivores, Saint-Genès Champanelle, France; Clermont Université, VetAgro Sup, BP 10448, Clermont-Ferrand, France

Abstract

The aim of this work was to study the effect of dairy system and individual cow-related factors on the volatile fingerprint of a large number of individual model cheeses analyzed by proton transfer reaction time-offlight mass spectrometry (PTR-ToF-MS). A total of 1,075 model cheeses were produced using milk samples collected from individual Brown Swiss cows reared in 72 herds located in mountainous areas of Trento province (Italy). The herds belonged to 5 main dairy systems ranging from traditional to modern and the cows presented different daily milk yields (24.6 ± 7.9 kg x d^{-1}), stages of lactation (199 ± 138 d in milk), and parities (2.7 ± 1.8). The PTR-ToF-MS revealed 619 peaks, of which the 240 most intense were analyzed, and 61 of these were tentatively attributed to relevant volatile organic compounds on the basis of their fragmentation patterns and data from the literature. Principal component analysis was used to convert the multiple responses characterizing the PTR-ToF-MS spectra into 5 synthetic variables representing 62% of the total information. These principal components were related to groups of volatile compounds tentatively attributed to different peaks and used to investigate the relationship of the volatile compound profile obtained by PTR-ToF-MS to animal and farm characteristics. Lactation stage is related to 4 principal components which brought together 52.9% of the total variance and 57.9% of the area of analyzed peaks. In particular, 2 principal components were positively related to peaks tentatively attributed to aldehydes and ketones and negatively related to alcohols, esters, and acids, which displayed a linear increase during lactation. The second principal component was affected by dairy system; it was higher in the modern system in which cows received total mixed rations. The third principal component was positively related to daily milk production. In summary, we report the first application of this innovative, high-throughput technique to study the effects of dairy system and individual animal factors on volatile organic compounds of model cheeses. Individual cheesemaking procedures together with this spectrometric technique open new avenues for genetic selection of dairy species with respect to both milk and cheese quality.

Keywords: Cheese smell; Dairy system; New phenotypes; Proton transfer reaction time of flight mass spectrometry (PTR-ToF-MS); Volatile compound fingerprint

Journal of Dairy Science

Volume 98, Issue 12, Pages 8414-8427

Reproducibility and repeatability of milk coagulation properties predicted by mid-infrared spectroscopy

Penasa, M., De Marchi, M., Ton, S., Ancilotto, L., Cassandro, M.

Department of Agronomy, Food, Natural Resources, Animals and Environment,
University of Padova, Viale dell'Università 16, Legnaro, PD, Italy

Abstract

The objective of this study was to assess reproducibility and repeatability of milk coagulation properties (MCP) predicted by mid-infrared spectroscopy (MIRS). Individual milk samples from a single dairy herd were collected during 8 sampling dates, added with preservative (Bronopol) and analysed in two laboratories. Ten cows were randomly selected within each sampling date. Rennet coagulation time (RCT, min) and curd firmness (a_{30}, mm) were predicted in the two laboratories using MIRS. Besides predicting MCP through MIRS, RCT and a_{30} were measured by Formagraph. Reproducibilities of RCT and a_{30} predicted by MIRS were 67.3 and 71.9%, respectively. Repeatabilities of RCT and a_{30} predicted by MIRS and determined by Formagraph were 92.8 and 95.8%, and 98.6 and 95.8%, respectively. Results indicated that MIRS predictions of MCP are repeatable, but not highly reproducible. Further efforts are needed to improve the standardisation among laboratories to enhance reproducibility.

International Dairy Journal

Volume 47, Pages 1-5

Short communication: Predictive ability of Fourier-transform mid-infrared spectroscopy to assess CSN genotypes and detailed protein composition of buffalo milk

Bonfatti, V.[a], Cecchinato, A.[b], Carnier, P.[a]

[a] Department of Comparative Biomedicine and Food Science (BCA), University of Padova, Legnaro, Padova, Italy

[b] Department of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE), University of Padova, Legnaro, Padova, Italy

Abstract

The aim of this work was to test the applicability of Fourier-transform mid-infrared spectroscopy (FT-MIR) for the prediction of the contents of casein (CN) and whey protein fractions in buffalo milk. Buffalo milk samples spectra were collected using a MilkoScan FT2 (Foss, Hillerød, Denmark) over the spectral range from 5,000 to 900 wavenumber x cm^{-1}. Contents of protein fractions, as well as CSN1S1 and CSN3 genotypes, were assessed by reversed phase HPLC. The highest coefficients of determination in cross-validation (1 - VR) were obtained for the contents (g/L of milk) of total protein and CN (1 - VR=0.92), followed by the content of β-CN, total whey protein, and αS2-CN (1 - VR of 0.87, 0.77, and 0.63, respectively). Conversely, contents of αS1-CN, γ-CN, glycosylated-κ-CN, total κ-CN, and whey protein fractions were predicted with poor accuracy (1 - VR <0.51). When protein fractions were expressed as percentages to total protein, 1 - VR values were never greater than 0.61 (β-CN). Only 56 and 70% of the observations were correctly classified by discriminant analysis in each of 2 groups of CSN1S1 and CSN3 genotypes, respectively. Results showed that FT-MIR spectroscopy is not applicable when prediction of detailed milk protein composition with high accuracy is required. Predictions may play a role as indicator traits in selective breeding, if the genetic correlation between FT-MIR predictions and measures of milk protein composition are high enough and predictions of protein fraction contents are sufficiently independent from the predicted total protein content. © 2015 American Dairy Science Association.

Keywords: Buffalo milk; Casein fractions; Protein composition; Spectroscopy

Journal of Dairy Science

Volume 98, Issue 9, Pages 6583-6587

Technical note: Improving the accuracy of mid-infrared prediction models by selecting the most informative wavelengths

Gottardo, P., De Marchi, M., Cassandro, M., Penasa, M.

Department of Agronomy, Food, Natural Resources, Animals and Environment,
University of Padova, Viale dell'Università 16, Legnaro, (PD), Italy

Abstract

Mid-infrared spectroscopy (MIRS) is widely used to collect milk phenotypes at the population level. The aim of this study was to test the ability of the uninformative variable elimination (UVE) method to select and remove uninformative wavelength variables before partial least squares (PLS) analysis. Milk titratable acidity (TA) and Ca content were used as examples to illustrate the procedure. Reference values and MIRS spectra (n. = 208) of TA and Ca were retrieved from an existing database. The data set was randomly divided into calibration (70% of data) and validation (30% of data) sets, and PLS analysis was carried out before and after the UVE procedure. The UVE procedure selected 244 and 113 informative wavelengths for TA and Ca, respectively, from a total of 1,060. The elimination of uninformative variables before PLS regression increased the accuracy of MIRS prediction models, and it substantially reduced the computation time. Dealing with fewer variables is expected to enhance the efficiency of MIRS models to predict phenotypes at population level.

Keywords: Calcium content; Mid-infrared spectroscopy (MIRS); Partial least squares regression; Titratable acidity; Uninformative variable selection

Journal of Dairy Science

Volume 98, Issue 6, Pages 4168-4173

Use of near infrared technology to predict fatty acid groups in commercial ground meat products

Ton, S.[a], De Marchi, M.[a], Manfrin, D.[b], Meneghesso, M.[b], Cassandro, M.[a], Penasa, M.[a]

[a] Department of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Universitá16, Legnaro, PD, Italy
[b] FOSS Italia, Corso Stati Uniti 1/77, Padova, Italy

Abstract

Near infrared transmittance (NIT, 850 to 1048 nm) spectroscopy was used to predict groups of fatty acids (FA), namely saturated FA (SFA), monounsaturated FA (MUFA) and polyunsaturated FA (PUFA), in commercial ground meat samples aiming to develope a fast and reliable method for their determination in support of label declaration by the new EC Regulation 1169/2011. Dataset was built using 81 samples of commercial ground meat from different species: beef, pork, chicken and turkey. In some samples, meat was mixtured with different ingredients such as bread, cheese, spices and additives. Samples were first analysed by NIT instrument for spectral information and reference FA values were obtained by gas chromatographic analysis. Prediction models for SFA, MUFA and PUFA expressed on total FA exhibited coefficients of determination of calibration of 0.822, 0.367 and 0.780 on intact samples, and 0.879, 0.726 and 0.908 on minced samples, respectively. Good results were also obtained when FA groups were expressed as g/100g of fresh meat: the coefficient of determination of calibration increased to values larger than 0.915. Moreover, comparing the slightly lower coefficient of determination in cross validation of intact compared with minced meat suggested that equations developed for minced samples were more accurate than those built for intact products. Results highlighted the effectiveness of NIT spectroscopy to predict the major FA groups in commercial meat products.

Keywords: Fatty acid; Ground meat; Infrared spectroscopy

Poljoprivreda

Volume 21, Issue 1, Pages 232-236

Genetics and genomics

A method for genotyping elite breeding stocks of leaf chicory (*Cichorium intybus* L.) by assaying mapped microsatellite marker loci

Ghedina, A.[a], *Galla, G.*[a], *Cadalen, T.*[b], *Hilbert, J.-L.*[b], *Tiozzo Caenazzo, S.*[c], *Barcaccia, G.*[a]

[a] DAFNAE, Laboratory of Plant Genetics and Breeding, University of Padua, Campus of Agripolis, 35020 Legnaro, PD, Italy
[b] Agro-food and Biotechnology Laboratory, Charles Viollette Research Institute, Université Lille1 Sciences et Technologies, GIS CARTOCHIC, Cité Scientifique, 59655 Villeneuve d'Ascq, France
[c] T&T Produce, S.S. Romea 77/bc, 30015 Sant'Anna Di Chioggia, VE, Italy

Abstract

Background: Leaf chicory (*Cichorium intybus* subsp. *intybus* var. *foliosum* L.) is a diploid plant species ($2n = 18$) of the Asteraceae family. The term "chicory" specifies at least two types of cultivated plants: a leafy vegetable, which is highly differentiated with respect to several cultural types, and a root crop, whose current industrial utilization primarily addresses the extraction of inulin or the production of a coffee substitute. The populations grown are generally represented by local varieties (*i.e.*, landraces) with high variation and adaptation to the natural and anthropological environment where they originated, and have been yearly selected and multiplied by farmers. Currently, molecular genetics and biotechnology are widely utilized in marker-assisted breeding programs in this species. In particular, molecular markers are becoming essential tools for developing parental lines with traits of interest and for assessing the specific combining ability of these lines to breed F1 hybrids.

Results: The present research deals with the implementation of an efficient method for genotyping elite breeding stocks developed from old landraces of leaf chicory, Radicchio of Chioggia, which are locally dominant in the Veneto region, using 27 microsatellite (SSR) marker loci scattered throughout the linkage groups. Information on the genetic diversity across molecular markers and plant accessions was successfully assessed along with descriptive statistics over all marker loci and inbred lines. Our overall data support an efficient method for assessing a multi-locus genotype of plant individuals and lineages that is useful for the selection of new varieties and the certification of local products derived from Radicchio of Chioggia.

Conclusions: This method proved to be useful for assessing the observed degree of homozygosity of the inbred lines as a measure of their genetic stability; plus it allowed an estimate of the specific combining ability (SCA) between maternal and paternal inbred lines on the basis of their genetic diversity and the predicted degree of heterozygosity of their F1 hybrids. This information could be exploited for planning crosses and predicting plant vigor traits (*i.e.*, heterosis) of experimental F1 hybrids on the basis of the genetic distance and allelic divergence between parental inbred lines. Knowing the parental genotypes would allow us not only to protect newly registered varieties but also to assess the genetic purity and identity of the seed stocks of commercial F1 hybrids, and to certificate the origin of their food derivatives.

Keywords: Genotyping; Inbred lines; MAB; Radicchio; SSR markers

BMC Research Notes

Volume 8, 831

A novel set of microsatellite markers for the European Grapevine Moth *Lobesia botrana* isolated using next-generation sequencing and their utility for genetic characterization of populations from Europe and the Middle East

Reineke, A.[a], *Assaf, H.A.*[ab], *Kulanek, D.*[a], *Mori, N.*[b], *Pozzebon, A.*[b], *Duso, C.*[b]

[a] Department of Phytomedicine, Geisenheim University, D-65366 Geisenheim, Germany
[b] Department of Agronomy, Food, Natural Resources, Animals and the Environment, University of Padova, 35020 Legnaro (Padova), Italy

Abstract

Using a high-throughput 454 pyrosequencing approach a novel set of microsatellite markers was developed for one of the key grapevine insect pests, the European grapevine moth *Lobesia botrana* (Lepidoptera: Tortricidae). 20 primer pairs flanking a microsatellite motif were designed based on the sequences obtained and were subsequently evaluated in a sample of 14 *L. botrana* populations from Europe and the Middle East. 11 markers showed stable and reproducible amplification patterns; however, one of the 11 markers was monomorphic in all *L. botrana* populations analysed. Estimated frequencies of null alleles of more than 20% were evident for two of the markers tested, but varied substantially depending on the respective *L. botrana* population. In 12 of the 14 *L. botrana* populations observed heterozygosities were lower to those expected under Hardy-Weinberg equilibrium, indicating a deficiency of heterozygotes in the respective populations. The overall F_{ST} value of 0.075 suggested a moderate but significant genetic differentiation between the *L. botrana* populations included in this study. In addition, a clear geographic structure was detected in the set of samples, evident through a significant isolation by distance and through results from structure analysis. In structure analysis, *L. botrana* populations were grouped in two clearly separated clusters according to their European (Spain, Italy, Germany) or Middle Eastern (Israel, Syria, Turkey) origin. This novel set of microsatellite markers can now be applied to study the evolutionary ecology of this species including host shifts and host adaptation as well as spread of individuals across worldwide viticulture.

Keywords: 454 pyrosequencing; *Lobesia botrana*; Microsatellites; Population genetics; SSR markers

Bulletin of Entomological Research

Volume 105, Issue 4, Pages 408-416

Analysis of gene expression changes in peach leaves in response to *Plum pox virus* infection using RNA-Seq

Rubio, M.[a], *Rodríguez-Moreno, L.*[b], *Ballester, A.R.*[c], *de Moura, M.C.*[d],
Bonghi, C.[e], *Candresse, T.*[fg], *Martínez-Gómez, P.*[a]

[a] Department of Plant Breeding, Centro de Edafología y Biología Aplicada del Segura (CEBAS-CSIC), PO Box 164, E-30100 Espinardo-Murcia, Spain
[b] Department of Stress Biology and Plant Pathology, Centro de Edafología y Biología Aplicada del Segura (CEBAS-CSIC), PO Box 164, E-30100 Espinardo-Murcia, Spain
[c] Department of Food Science, Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), Avda. Agustín Escardino 7, 46980 Paterna (Valencia), Spain
[d] aScidea Computational Biology Solutions, S.L. Parc de Reserca UAB, Edifici Eureka, 08193 Bellaterra (Cerdanyola del Vallés), Barcelona, Spain
[e] Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padua, I-35020 Padua, Italy
[f] INRA, UMR 1332 BFP, CS20032, 33882 Villenave d'Ornon Cedex, France
[g] UMR 1332 BFP, CS20032, Université de Bordeaux, 33882 Villenave d'Ornon Cedex, France

Abstract

Differences in gene expression were studied after *Plum pox virus* (PPV, sharka disease) infection in peach GF305 leaves with and without sharka symptoms using RNA-Seq. For each sample, more than 80% of 100-nucleotide paired-end (PE) Illumina reads were aligned on the peach reference genome. In the symptomatic sample, a significant proportion of reads were mapped to PPV reference genomes (1.04% compared with 0.00002% in non-symptomatic leaves), allowing for the ultra-deep assembly of the complete genome of the PPV isolate used (9775 nucleotides, missing only 11 nucleotides at the 5' genome end). In addition, significant alternative splicing events were detected in 359 genes and 12 990 single nucleotide polymorphisms (SNPs) were identified, 425 of which could be annotated. Gene ontology annotation revealed that the high-ranking mRNA target genes associated with the expression of sharka symptoms are mainly related to the response to biotic stimuli, to lipid and carbohydrate metabolism and to the negative regulation of catalytic activity. A greater number of differentially expressed genes were observed in the early asymptomatic phase of PPV infection in comparison with the symptomatic phase. These early infection events were associated with the induction of genes related to pathogen resistance, such as jasmonic acid, chitinases, cytokinin glucosyl transferases and Lys-M proteins. Once the virus had accumulated, the overexpression of Dicer protein 2a genes suggested a gene silencing plant response that was suppressed by the virus HCPro and P1 proteins. These results illustrate the dynamic nature of the peach-PPV interaction at the transcriptome level and confirm that sharka symptom expression is a complex process that can be understood on the basis of changes in plant gene expression.

Keywords: Plant-virus interaction; PPV; *Prunus*; RNA-Seq; Sharka disease

Molecular Plant Pathology

Volume 16, Issue 2, Pages 164-176

De novo sequencing of the *Hypericum perforatum* L. flower transcriptome to identify potential genes that are related to plant reproduction *sensu lato*

Galla, G.[a]*, Vogel, H.*[b]*, Sharbel, T.F.*[c]*, Barcaccia, G.*[a]

[a] Laboratory of Plant Genetics and Genomics, DAFNAE – University of Padova, Campus of Agripolis, Viale dell'Università 16, 35020 Legnaro, Italy
[b] Max Planck Institute for Chemical Ecology, Hans-Knöll-Straße 8, 07745 Jena, Germany
[c] Apomixis Research Group; Department of Cytogenetics and Genome Analysis, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstraße 3, 06466 Gatersleben, Germany

Abstract

Background: St. John's wort (*Hypericum perforatum* L.) is a medicinal plant that produces important metabolites with antidepressant and anticancer activities. Recently gained biological information has shown that this species is also an attractive model system for the study of a naturally occurring form of asexual reproduction called apomixis, which allows cloning plants through seeds. In aposporic gametogenesis, one or multiple somatic cells belonging to the ovule nucellus change their fate by dividing mitotically and developing functionally unreduced embryo sacs by mimicking sexual gametogenesis. Although the introduction of apomixis into agronomically important crops could have revolutionary implications for plant breeding, the genetic control of this mechanism of seed formation is still not well understood for most of the model species investigated so far. We used Roche 454 technology to sequence the entire *H. perforatum* flower transcriptome of whole flower buds and single flower verticils collected from obligately sexual and unrelated highly or facultatively apomictic genotypes, which enabled us to identify RNAs that are likely exclusive to flower organs (*i.e.*, sepals, petals, stamens and carpels) or reproductive strategies (*i.e.*, sexual *vs.* apomictic).

Results: Here we sequenced and annotated the flower transcriptome of *H. perforatum* with particular reference to reproductive organs and processes. In particular, in our study we characterized approximately 37,000 transcripts found expressed in male and/or female reproductive organs, including tissues or cells of sexual and apomictic flower buds. Ontological annotation was applied to identify major biological processes and molecular functions involved in flower development and plant reproduction. Starting from this dataset, we were able to recover and annotate a large number of transcripts related to meiosis, gametophyte/gamete formation, and embryogenesis, as well as genes that are exclusively or preferentially expressed in sexual or apomictic libraries. Real-Time RT-qPCR assays on pistils and anthers collected at different developmental stages from accessions showing alternative modes of reproduction were used to identify potential genes that are related to plant reproduction *sensu lato* in *H. perforatum*.

Conclusions: Our approach of sequencing flowers from two fully obligate sexual genotypes and two unrelated highly apomictic genotypes, in addition to different flower parts dissected from a facultatively apomictic accession, enabled us to analyze the complexity of the flower transcriptome according to its main reproductive organs as well as for alternative reproductive behaviors. Both annotation and expression data provided original results supporting the hypothesis that apomixis in *H. perforatum* relies upon spatial or temporal mis-expression of genes acting during female sexual reproduction. The present analyses aim to pave the way toward a better understanding of the molecular basis of flower development and plant reproduction, by identifying genes or RNAs that may differentiate or regulate the sexual and apomictic reproductive pathways in *H. perforatum*.

Keywords: Apomixis; Apospory; Flower; Hypericum perforatum; Reproductive organs

BMC Genomics

Volume 16, 254

Development of microsatellite loci in *Pityophthorus juglandis*, a vector of thousand cankers disease in *Juglans* spp.

Hadziabdic, D.[a], Wadl, P.A.[a], Staton, M.E.[a], Klingeman, W.E.[b], Moulton, J.K.[a], Pscheidt, J.W.[c], Wiggins, G.J.[a], Grant, J.F.[a], Lambdin, P.L.[a], Windham, M.T.[a], Faccoli, M.[d], Merten, P.R.[e], Trigiano, R.N.[a]

[a] Department of Entomology and Plant Pathology, The University of Tennessee, 370 Plant Biotechnology Building, 2505 E.J. Chapman Dr., Knoxville, TN 37996, USA
[b] Department of Plant Sciences, The University of Tennessee, 2431 Joe Johnson Dr., Knoxville, TN 37996, USA
[c] Department of Botany and Plant Pathology, Oregon State University, 1089 Cordley Hall, Corvallis, OR 97331, USA
[d] Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padua, Viale dell'Università, 16, 35020 Legnaro, PD, Italy
[e] USDA Forest Service, Forest Health Protection, 200 W.T. Weaver Boulevard, Asheville, NC 28804, USA

Abstract

Using next-generation sequencing, 18 microsatellite loci were developed and characterized for walnut twig beetle, *Pityophthorus juglandis*, a vector of thousand cankers disease (TCD) affecting *Juglans* spp. Although all *Juglans* species are susceptible to TCD infection, native populations of *J. nigra* and *J. cinerea*, which is endangered in Canada, are most susceptible and threatened by habitat loss. Novel primers amplified di-, tri-, and tetra nucleotide repeats and detected 4–14 alleles per locus. Averaged observed and expected heterozygosity was 0.22 and 0.67, respectively. Our results indicate that *P. juglandis* microsatellite loci can be used to investigate genetic diversity and population structure of this vector across a widespread geography. These markers will be useful tools for evaluating genetic structure of *P. juglandis* population outbreaks and developing appropriate conservation strategies. Microsatellite loci obtained in this study can also be utilized to determine relationships of *P. juglandis* to other closelys related *Pityophthorus* spp.

Keywords: Genetic diversity; *Juglans cinerea*; *Juglans nigra*; Microsatellite loci; *Pityophthorus juglandis*; Thousand cankers disease; Walnut twig beetle

Conservation Genetics Resources

Volume 7, Issue 2, Pages 431-433

DNA analysis of dust particles sampled from the Turin Shroud

Barcaccia, G.[a], *Galla, G.*[a], *Achilli, A.*[b], *Olivieri, A.*[c], *Torroni, A.*[c]

[a] Laboratorio di Genetica e Genomica, DAFNAE - Università di Padova, Viale Università 16, 35020 Legnaro, Italy
[b] Dipartimento di Chimica, Biologia e Biotecnologie, Università di Perugia, Via Elce di Sotto 8, 06123 Perugia, Italy
[c] Dipartimento di Biologia e Biotecnologie "L. Spallanzani", Università di Pavia, Via Ferrata 9, 27100 Pavia, Italy

Abstract

The Turin Shroud is traditionally considered the burial cloth in which the body of Jesus Christ was enveloped after his dead about 2000 years ago. Here we report the main findings from the analysis of genomic DNA extracted from dust particles, which were vacuumed from the backside of Turin Shroud corresponding to internal parts of the body image and the lateral edge used for its radiocarbon dating. Specific plant chloroplast DNA (cpDNA) and human mitochondrial DNA (mtDNA) target regions were analyzed to identify plant taxonomic entities and human genetic lineages. Plant species native to the Mediterranean countries and widespread in the Middle East (Vavilov's centers of origin V and IV, respectively) were identified, in addition to others living in temperate and boreal regions of the northern hemisphere or having their primary center of origin and distribution in central and eastern Asia (mainly China, I) or native only to the Americas. Since many of these species were introduced into Europe after the Marco Polo travels and Christopher Columbus voyages, our findings suggest a geographic scenario for which only some of the detected plant cpDNAs are compatible with the supposed origin and trail of the relic, whereas others are likely from a historical interval later than the Medieval period. As for human mtDNAs, our analyses allowed the detection of sequences from multiple subjects, which clustered into a number of western Eurasian haplogroups, including some known to be typical of western Europe (H1 and H3), the Near East (H13 and H33), the Arabian Peninsula (R0a) and the Indian sub-continent (M56 and R8). Such mitogenome diversity could be due to contacts with subjects of different ethnic origins in recent centuries, but it is also compatible with the historic path followed by the Turin Shroud during its supposed 2000-year journey from the Near East. Furthermore it raises the possibility of an Indian manufacture of the linen cloth.

MATEC Web of Conferences

Volume 36, 03001

Pattern of association between endemic Hawaiian fruit flies (Diptera, Tephritidae) and their symbiotic bacteria: Evidence of cospeciation events and proposal of "*Candidatus* Stammerula trupaneae"

Viale, E.[a]*, Martinez-Sañudo, I.*[a]*, Brown, J.M.*[b]*, Simonato, M.*[a]*, Girolami, V.*[a]*,*
Squartini, A.[a]*, Bressan, A.*[c]*, Faccoli, M.*[a]*, Mazzon, L.*[a]

[a] Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova - Agripolis, Viale dell'Università, 16, 35020 Legnaro, Padova, Italy

[b] Department of Biology, Grinnell College, Grinnell, IA 50012, USA

[c] Bayer Crop Science LP, R&D Trait Research, 3500 Paramount Parkway, Morrisville, NC 27560, USA

Abstract

Several insect lineages have evolved mutualistic association with symbiotic bacteria. This is the case of some species of mealybugs, whiteflies, weevils, tsetse flies, cockroaches, termites, carpenter ants, aphids and fruit flies. Some species of Tephritinae, the most specialized subfamily of fruit flies (Diptera: Tephritidae), harbour co-evolved vertically transmitted, bacterial symbionts in their midgut, known as "*Candidatus* Stammerula spp.". The 25 described endemic species of Hawaiian tephritids, plus at least three undescribed species, are taxonomically distributed among three genera: the cosmopolitan genus *Trupanea* (21 described spp.), the endemic genus *Phaeogramma* (2 spp.) and the Nearctic genus *Neotephritis* (2 spp.). We examined the presence of symbiotic bacteria in the endemic tephritids of the Hawaiian Islands, which represent a spectacular example of adaptive radiation, and tested the concordant evolution between host and symbiont phylogenies. We detected through PCR assays the presence of specific symbiotic bacteria, designated as "*Candidatus* Stammerula trupaneae", from 35 individuals of 15 species. The phylogeny of the insect host was reconstructed based on two regions of the mitochondrial DNA (16S rDNA and COI-tRNALeu-COII), while the bacterial 16S rRNA was used for the symbiont analysis. Host and symbiont phylogenies were then compared and evaluated for patterns of cophylogeny and strict cospeciation. Topological congruence between Hawaiian Tephritinae and their symbiotic bacteria phylogenies suggests a limited, but significant degree of host-symbiont cospeciation. We also explored the character reconstruction of three host traits, as island location, host lineage, and host tissue attacked, based on the symbiont phylogenies under the hypothesis of cospeciation.

Keywords: *Candidatus* Stammerula; Cospeciation; Endosymbiosis; Fruit fly; Hawaiian Tephritinae

Molecular Phylogenetics and Evolution

Volume 90, Pages 67-79

Prunus transcription factors: breeding perspectives

Bianchi, V.J.[a], Rubio, M.[b], Trainotti, L.[c], Verde, I.[d], Bonghi, C.[e], Martínez-Gómez, P.[b]

[a] Department of Plant Physiology, Instituto de Biologia, Universidade Federal de Pelotas, Pelotas-RS, Brazil
[b] Department of Plant Breeding, Centro de Edafología y Biología Aplicada del Segura, Consejo Superior de Investigaciones Científicas, Murcia, Spain
[c] Department of Biology, University of Padua, Padova, Italy
[d] Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria (CRA) - Centro di ricerca per la frutticoltura, Roma, Italy
[e] Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padua, Padova, Italy

Abstract

Many plant processes depend on differential gene expression, which is generally controlled by complex proteins called transcription factors (TFs). In peach, 1533 TFs have been identified, accounting for about 5.5% of the 27,852 protein-coding genes. These TFs are the reference for the rest of the *Prunus* species. TF studies in *Prunus* have been performed on the gene expression analysis of different agronomic traits, including control of the flowering process, fruit quality, and biotic and abiotic stress resistance. These studies, using quantitative RT-PCR, have mainly been performed in peach, and to a lesser extent in other species, including almond, apricot, black cherry, Fuji cherry, Japanese apricot, plum, and sour and sweet cherry. Other tools have also been used in TF studies, including cDNA-AFLP, LC-ESI-MS, RNA, and DNA blotting or mapping. More recently, new tools assayed include microarray and high-throughput DNA sequencing (DNA-Seq) and RNA sequencing (RNA-Seq). New functional genomics opportunities include genome resequencing and the well-known synteny among *Prunus* genomes and transcriptomes. These new functional studies should be applied in breeding programs in the development of molecular markers. With the genome sequences available, some strategies that have been used in model systems (such as SNP genotyping assays and genotyping-by-sequencing) may be applicable in the functional analysis of *Prunus* TFs as well. In addition, the knowledge of the gene functions and position in the peach reference genome of the TFs represents an additional advantage. These facts could greatly facilitate the isolation of genes via QTL (quantitative trait loci) map-based cloning in the different *Prunus* species, following the association of these TFs with the identified QTLs using the peach reference genome.

Keywords: Abiotic stress; Biotic stress; Breeding; Flowering time; Fruit quality; Gene regulation; *Prunus* spp; Transcription factors

Frontiers in Plant Science

Volume 6, 443

Transcriptomic signatures in seeds of apple (*Malus domestica* L. Borkh) during fruitlet abscission

Ferrero, S.[a], Carretero-Paulet, L.[b], Mendes, M.A.[a], Botton, A.[c], Eccher, G.[c], Masiero, S.[a], Colombo, L.[a]

[a] Dipartimento di BioScienze, Università degli Studi di Milano, Milan, Italy
[b] Department of Biological Sciences, University at Buffalo, Buffalo, New York, United States of America
[c] Department of Agronomy, Food, Natural Resources, Animals, and Environment, University of Padova, Agripolis, Legnaro, Italy

Abstract

Abscission is the regulated process of detachment of an organ from a plant. In apple the abscission of fruits occurs during their early development to control the fruit load depending on the nutritional state of the plant. In order to control production and obtain fruits with optimal market qualities, the horticultural procedure of thinning is performed to further reduce the number of fruitlets. In this study we have conducted a transcriptomic profiling of seeds from two different types of fruitlets, according to size and position in the fruit cluster. Transcriptomic profiles of central and lateral fruit seeds were obtained by RNAseq. Comparative analysis was performed by the functional categorization of differentially expressed genes by means of Gene Ontology (GO) annotation of the apple genome. Our results revealed the overexpression of genes involved in responses to stress, hormone biosynthesis and also the response and/or transport of auxin and ethylene. A smaller set of genes, mainly related to ion transport and homeostasis, were found to be down-regulated. The transcriptome characterization described in this manuscript contributes to unravelling the molecular mechanisms and pathways involved in the physiological abscission of apple fruits and suggests a role for seeds in this process.

PLoS ONE

Volume 10, Issue 3, e0120503

Uncovering the sources of DNA found on the Turin Shroud

Barcaccia, G.[a], *Galla, G.*[a], *Achilli, A.*[b], *Olivieri, A.*[c], *Torroni, A.*[c]

[a] Laboratorio di Genomica, DAFNAE - Università di Padova, Via Università 16, 35020 Legnaro, Italy
[b] Dipartimento di Chimica, Biologia e Biotecnologie, Università di Perugia, Via Elce di Sotto 8, 06123 Perugia, Italy
[c] Dipartimento di Biologia e Biotecnologie "L. Spallanzani", Università di Pavia, Via Ferrata 9, 27100 Pavia, Italy

Abstract

The Turin Shroud is traditionally considered to be the burial cloth in which the body of Jesus Christ was wrapped after his death approximately 2000 years ago. Here, we report the main findings from the analysis of genomic DNA extracted from dust particles vacuumed from parts of the body image and the lateral edge used for radiocarbon dating. Several plant taxa native to the Mediterranean area were identified as well as species with a primary center of origin in Asia, the Middle East or the Americas but introduced in a historical interval later than the Medieval period. Regarding human mitogenome lineages, our analyses detected sequences from multiple subjects of different ethnic origins, which clustered into a number of Western Eurasian haplogroups, including some known to be typical of Western Europe, the Near East, the Arabian Peninsula and the Indian sub-continent. Such diversity does not exclude a Medieval origin in Europe but it would be also compatible with the historic path followed by the Turin Shroud during its presumed journey from the Near East. Furthermore, the results raise the possibility of an Indian manufacture of the linen cloth.

Scientific Reports

Volume 5, 14484

A new polymorphism on chromosome 6 associated with bolting tendency in sugar beet

Broccanello, C.[a], *Stevanato, P.*[a], *Biscarini, F.*[b], *Cantu, D.*[c], *Saccomani, M.*[a]

[a] DAFNAE, Dipartimento di Agronomia Animali Alimenti Risorse Naturali e Ambiente, Università degli Studi di Padova, viale dell'Università 16, 35020 Legnaro, PD, Italy
[b] FPTP, Fondazione Parco Tecnologico Padano, viale Einstein, Loc. Cascina Codazza, 26900 Lodi, Italy
[c] Department of Viticulture and Enology, University of California, Davis, 2146 RMI North Building Davis, Davis, CA 95616, USA

Abstract

Background: Premature flowering or bolting is an undesirable characteristic that causes severe sugar yield losses and interferes with harvesting. Vernalization is a prerequisite for the floral induction, achieved by exposure to low temperatures for 10-14 weeks. This process is also controlled by other environmental factors, such as long daylight photoperiods and a combination of genetic factors. The objective of this study was the identification of new genetic polymorphisms linked to bolting tendency in sugar beet.

Results: Two pollinators characterized by low and high bolting tendency were subjected to RAD-sequencing in order to detect discriminating SNPs between lines. 6,324 putative SNPs were identified. Of these, 192 were genotyped in a set of 19 pollinators, each comprising bolted and non-bolted individuals, for a total of 987 samples. Among the 192 candidate SNPs, the strongest overall association was found for SNP183 on chromosome 6 (*p*-value = $1.246\ 10^{-13}$). The association between SNP183 and bolting tendency was then confirmed in an independent population of 730 plants from 11 breeding lines (*p*-value = 0.0061). SNP183 is located in the intron of *Bv_22330_orky*, a sugar beet homolog of a matrix metalloproteinase (MMP) gene that could be implied in flowering in *Arabidopsis thaliana*.

Conclusion: Our data support a significant association between an intronic SNP in the MMP gene located on chromosome 6 and the regulation of bolting tendency in sugar beet. The newly identified locus supports the polygenic nature of flowering control. The associated marker can be used to design SNP panels for the discrimination of bolters and non-bolters, to be used in sugar beet breeding programs for the development of improved germplasm with low bolting tendency.

Keywords: *Beta vulgaris*; Bolting tendency; Molecular breeding; RAD sequencing; SNP association

BMC Genetics

Volume 16, 142

Selection and validation of reference genes for quantitative real-time PCR studies during *Saccharomyces cerevisiae* alcoholic fermentation in the presence of sulfite

Nadai, C.[a], Campanaro, S.[b], Giacomini, A.[ac], Corich, V.[ac]

[a] Department of Agronomy Food Natural Resources Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, Legnaro, PD 35020, Italy
[b] Department of Biology, University of Padova, Via Ugo Bassi 58/b, Padova 35121, Italy
[c] Interdepartmental Centre for Research in Viticulture and Enology (CIRVE), University of Padova, Via XXVIII Aprile 14, Conegliano, TV 31015, Italy

Abstract

Sulfur dioxide is extensively used during industrial fermentations and contributes to determine the harsh conditions of winemaking together with low pH, high sugar content and increasing ethanol concentration. Therefore the presence of sulfite has to be considered in yeast gene expression studies to properly understand yeast behavior in technological environments such as winemaking. A reliable expression pattern can be obtained only using an appropriate reference gene set that is constitutively expressed regardless of perturbations linked to the experimental conditions. In this work we tested 15 candidate reference genes suitable for analysis of gene expression during must fermentation in the presence of sulfite. New reference genes were selected from a genome-wide expression experiment, obtained by RNA sequencing of four *Saccharomyces cerevisiae* wine strains grown in enological conditions. Their performance was compared to that of the most common genes used in previous studies. The most popular software based on different statistical approaches (geNorm, NormFinder and BestKeeper) were chosen to evaluate expression stability of the candidate reference genes. Validation was obtained using other wine strains by comparing normalized gene expression data with transcriptome quantification both in the presence and absence of sulfite. Among 15 reference genes tested *ALG9*, *FBA1*, *UBC6* and *PFK1* appeared to be the most reliable while *ENO1*, *PMA1*, *DED1* and *FAS2* were the worst. The most popular reference gene *ACT1*, widely used for *S. cerevisiae* gene expression studies, showed a stability level markedly lower than those of our selected reference genes. Finally, as the expression of the new reference gene set remained constant over the entire fermentation process, irrespective of the perturbation due to sulfite addition, our results can be considered also when no sulfite is added to the must.

Keywords: Fermentation; Gene expression; RNA-seq; *Saccharomyces cerevisiae*; SO_2; Wine yeasts

International Journal of Food Microbiology

Volume 215, Pages 49-56

Causal relationships between milk quality and coagulation properties in Italian Holstein-Friesian dairy cattle

Tiezzi, F.[a], *Valente, B.D.*[b], *Cassandro, M.*[c], *Maltecca, C.*[a]

[a] Department of Animal Science, North Carolina State University, Raleigh, NC 27695, USA
[b] Department of Animal Science, University of Wisconsin, Madison, WI 53706, USA
[c] Department of Agronomy, Food, Natural Resources, Animals and Environment,
University of Padova, 35020 Legnaro, (PD), Italy

Abstract

Background: Recently, selection for milk technological traits was initiated in the Italian dairy cattle industry based on direct measures of milk coagulation properties (MCP) such as rennet coagulation time (RCT) and curd firmness 30 min after rennet addition (a_{30}) and on some traditional milk quality traits that are used as predictors, such as somatic cell score (SCS) and casein percentage (CAS). The aim of this study was to shed light on the causal relationships between traditional milk quality traits and MCP. Different structural equation models that included causal effects of SCS and CAS on RCT and a_{30} and of RCT on a_{30} were implemented in a Bayesian framework.

Results: Our results indicate a non-zero magnitude of the causal relationships between the traits studied. Causal effects of SCS and CAS on RCT and a_{30} were observed, which suggests that the relationship between milk coagulation ability and traditional milk quality traits depends more on phenotypic causal pathways than directly on common genetic influence. While RCT does not seem to be largely controlled by SCS and CAS, some of the variation in a_{30} depends on the phenotypes of these traits. However, a_{30} depends heavily on coagulation time. Our results also indicate that, when direct effects of SCS, CAS and RCT are considered simultaneously, most of the overall genetic variability of a_{30} is mediated by other traits.

Conclusions: This study suggests that selection for RCT and a_{30} should not be performed on correlated traits such as SCS or CAS but on direct measures because the ability of milk to coagulate is improved through the causal effect that the former play on the latter, rather than from a common source of genetic variation. Breaking the causal link (e.g. standardizing SCS or CAS before the milk is processed into cheese) would reduce the impact of the improvement due to selective breeding. Since a_{30} depends heavily on RCT, the relative emphasis that is put on this trait should be reconsidered and weighted for the fact that the pure measure of a_{30} almost double-counts RCT.

Genetics Selection Evolution

Volume 47, 45

Comparison between different statistical models for the prediction of direct genetic component on embryo establishment and survival in Italian Brown Swiss dairy cattle

Tiezzi, F.[b], Maltecca, C.[b], Cecchinato, A.[a], Bittante, G.[a]

[a] Department of Agronomy, Food, Natural resources, Animals and Environment, University of Padova, Viale dell'Università 16, 35020 Legnaro, PD, Italy
[b] Department of Animal Science, North Carolina State University, Raleigh 27695, NC, USA

Abstract

The aims of this study were to infer variance components and heritability for the direct component on embryo establishment and survival related traits and to compare different statistical models in terms of goodness-of-fit and predictive ability. Embryo establishment and survival (EES) was defined as the outcome of an AI event, its direct effect was represented as the effect of the service sire from which semen was taken. Indicators of EES were calving per service (CS) and non-return at 56 d after service (NR56). Insemination records from the Italian Brown Swiss population reared in the Alps were used. Data included 124,206 inseminations performed by 86 technicians on 28,873 cows in 1400 herds. Services were recorded from 1999 to 2008. Linear-sire, linear-animal, threshold-sire, and threshold-animal models were used to estimate (co) variance components for CS and NR56. Four levels of complexity within each model were tested, so that 16 different models were compared for each of the two fertility traits. Comparison was assessed on the basis of the goodness-of-fit and predictive ability. Paternal half-sibs groups were created as average outcome of the inseminations from a given service sire. Goodness-of-fit was evaluated by regressing the service sire estimated breeding value from each model to paternal half-sibs average CS or NR56. Predictive ability was assessed through sums of chi-squared and percentage of wrong predictions. Predictors were the respective service sire's estimated breeding values constructed on a reduced (independent) training dataset, including years from 1999 to 2005, and predictands were the paternal half-sibs means for every bull in the remaining years (2006-2008). Prediction of EES was considered differently according to whether service sires had observations in the training dataset (prediction of proven bulls) or they had not (prediction of young bulls). Estimates of heritability ranged from 0.011 to 0.119 for CS, and from 0.005 to 0.054 for NR56. In general, threshold models explained a larger proportion of additive genetic variance than linear models, and animal models yielded higher heritabilities than sire models. Calving per service was much more predictable than NR56, but no significant differences were found among models. Although heritabilities were low, the prediction of future EES of a paternal half-sib group is feasible.

Keywords: Bull fertility; Heritability; Model comparison; Threshold model

Livestock Science

Volume 180, Pages 6-13

Evolution of increased competitiveness in cows trades off with reduced milk yield, fertility and more masculine morphology

Sartori, C., Mazza, S., Guzzo, N., Mantovani, R.

Department of Agronomy, Food, Natural Resources, Animals and Environment-DAFNAE, University of Padua, Viale dell'Università 16, 35020 Legnaro, PD, Italy

Abstract

In some species females compete for food, foraging territories, mating, and nesting sites. Competing females can exhibit morphological, physiological, and behavioral adaptations typical of males, which are commonly considered as secondary sexual traits. Competition and the development of traits increasing competitiveness require much energy and may exert adverse effects on fecundity and survival. From an evolutionary perspective, positive selection for increased competitiveness would then result in evolution of reduced values for traits related to fitness such as fecundity and survival. There is recent evidence for such evolutionary trade-offs involving male competition, but no study has considered competing females so far. Using data from competitions for dominance in cows (*Bos taurus*), we found negative genetic correlations between traits providing success in competition, that is, fighting ability and fitness traits related to milk production and with fertility (the inverse of parity-conception interval). Fighting ability also showed low but positive genetic correlations with "masculine" morphological traits, and negative correlations with "feminine" traits. A genetic change in traits over time has occurred due to selection on competitiveness, corresponding to an evolutionary process of "masculinization" counteracting the official selection for milk yield. Similar evolutionary trade-off between success in competition and fitness components may be present in various species experiencing female competition.

Keywords: Female contest; Fitness; Genetic correlations; Life-history evolution; Quantitative genetics; Secondary sexual traits

Evolution

Volume 69, Issue 8, Pages 2235-2245

Factor analysis for genetic evaluation of linear type traits in dual-purpose autochthonous breeds

Mazza, S., Guzzo, N., Sartori, C., Mantovani, R.

Department of Agronomy Food Natural Resources Animals and Environment, University of Padua, Agripolis, 35020 Legnaro, Padova, Italy

Abstract

Factor analysis was applied to individual type traits (TT) scored in primiparous cows belonging to two dual purpose Italian breeds, Rendena (REN; 20 individual type traits evaluated on 11 399 first parity cows), and Aosta Red Pied (ARP; 22 individual type traits evaluated on 36 168 primiparous cows). Six common latent factors (F1 to F6; eigenvalues ≥ 1) which explained 63% (REN) and 58% (ARP) of the total variance were obtained. F1 included TT mainly related to muscularity, and F2 to body size. The F3 and F4 accounted for udder size and conformation, respectively. F5 included rear legs and feet. Biological significance for F6 was not readily obtained. Moderate to low heritability were estimated through REML single-trait analysis from factor scores (from 0.22 to 0.52 in REN, and from 0.08 to 0.37 in ARP). The greatest heritability values were estimated for body size and muscularity (0.52 and 0.37 for body size; and 0.40 and 0.32 for muscularity in REN and ARP, respectively). As expected, rank correlations, obtained considering estimated breeding values derived from best linear unbiased prediction analysis on the individual TT and factor score, showed similar coefficients to those observed in the factor analysis following loading of TT within each latent factor. These results suggest the possibility to implement the factor analysis in the morphological evaluation, simplifying the information given by the type traits into new variables useful for the genetic improvement of dual purpose cattle.

Keywords: Autochthonous breed; Dual purpose; Factor analysis; Type traits

Animal

Volume 10, Issue 3, Pages 372-380

Genetic parameters for milk mineral content and acidity predicted by mid-infrared spectroscopy in Holstein-Friesian cows

Toffanin, V.[a], Penasa, M.[a], McParland, S.[b], Berry, D.P.[c], Cassandro, M.[a], De Marchi, M.[a]

[a] Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, 35020 Legnaro, PD, Italy
[b] Livestock Systems Research Department, Animal and Grassland Research and Innovation Centre, Teagasc, Moorepark, Fermoy, Co. Cork, Ireland
[c] Animal and Bioscience Research Department, Animal and Grassland Research and Innovation Centre, Teagasc, Moorepark, Fermoy, Co. Cork, Ireland

Abstract

The aim of the present study was to estimate genetic parameters for calcium (Ca), phosphorus (P) and titratable acidity (TA) in bovine milk predicted by mid-IR spectroscopy (MIRS). Data consisted of 2458 Italian Holstein-Friesian cows sampled once in 220 farms. Information per sample on protein and fat percentage, pH and somatic cell count, as well as test-day milk yield, was also available. (Co)variance components were estimated using univariate and bivariate animal linear mixed models. Fixed effects considered in the analyses were herd of sampling, parity, lactation stage and a two-way interaction between parity and lactation stage; an additive genetic and residual term were included in the models as random effects. Estimates of heritability for Ca, P and TA were 0.10, 0.12 and 0.26, respectively. Positive moderate to strong phenotypic correlations (0.33 to 0.82) existed between Ca, P and TA, whereas phenotypic weak to moderate correlations (0.00 to 0.45) existed between these traits with both milk quality and yield. Moderate to strong genetic correlations (0.28 to 0.92) existed between Ca, P and TA, and between these predicted traits with both fat and protein percentage (0.35 to 0.91). The existence of heritable genetic variation for Ca, P and TA, coupled with the potential to predict these components for routine cow milk testing, imply that genetic gain in these traits is indeed possible.

Keywords: Genetic parameter; Holstein-Friesian cow; Mid-IR spectroscopy; Milk acidity; Milk mineral

Animal

Volume 9, Issue 5, Pages 775-780

Genetic parameters of cheese yield and curd nutrient recovery or whey loss traits predicted using Fourier-transform infrared spectroscopy of samples collected during milk recording on Holstein, Brown Swiss, and Simmental dairy cows

Cecchinato, A., Albera, A., Cipolat-Gotet, C., Ferragina, A., Bittante, G.

Department of Agronomy, Food, Natural Resources, Animals and Environment
(DAFNAE), University of Padova, Viale dell'Università 16, 35020 Legnaro, Italy

Abstract

Cheese yield is the most important technological parameter in the dairy industry in many countries. The aim of this study was to infer (co)variance components for cheese yields (CY) and nutrient recoveries in curd (REC) predicted using Fourier-transform infrared (FTIR) spectroscopy of samples collected during milk recording on Holstein, Brown Swiss, and Simmental dairy cows. A total of 311,354 FTIR spectra representing the test-day records of 29,208 dairy cows (Holstein, Brown Swiss, and Simmental) from 654 herds, collected over a 3-yr period, were available for the study. The traits of interest for each cow consisted of 3 cheese yield traits (%CY: fresh curd, curd total solids, and curd water as a percent of the weight of the processed milk), 4 curd nutrient recovery traits (REC: fat, protein, total solids, and the energy of the curd as a percent of the same nutrient in the processed milk), and 3 daily cheese production traits (daily fresh curd, total solids, and the water of the curd per cow). Calibration equations (freely available upon request to the corresponding author) were used to predict individual test-day observations for these traits. The (co)variance components were estimated for the CY, REC, milk production, and milk composition traits via a set of 4-trait analyses within each breed. All analyses were performed using REML and linear animal models. The heritabilities of the %CY were always higher for Holstein and Brown Swiss cows (0.22 to 0.33) compared with Simmental cows (0.14 to 0.18). In general, the fresh cheese yield ($\%CY_{CURD}$) showed genetic variation and heritability estimates that were slightly higher than those of its components, $\%CY_{SOLIDS}$ and $\%CY_{WATER}$. The parameter $REC_{PROTEIN}$ was the most heritable trait in all the 3 breeds, with values ranging from 0.32 to 0.41. Our estimation of the genetic relationships of the CY and REC with milk production and composition revealed that the current selection strategies used in dairy cattle are expected to exert only limited effects on the REC traits. Instead, breeders may be able to exploit genetic variations in the %CY, particularly REC_{FAT} and $REC_{PROTEIN}$. This last component is not explained by the milk protein content, suggesting that its direct selection could be beneficial for cheese production aptitude. Collectively, our findings indicate that breeding strategies aimed at enhancing CY and REC could be easily and rapidly implemented for dairy cattle populations in which FTIR spectra are routinely acquired from individual milk samples.

Keywords: Cheese yield; Dairy breed; Genetic parameter; Mid-infrared spectroscopy; Whey loss

Journal of Dairy Science

Volume 98, Issue 7, Pages 4914-4927

Genetic parameters of type traits in two strains of dual purpose autochthonous Valdostana cattle

Mazza, S., Sartori, C., Mantovani, R.

Department of Agronomy, Food, Natural resources, Animals and Environment,
University of Padua, Agripolis, Viale Università, 16, 35020 Legnaro, PD, Italy

Abstract

The main objectives of this study were (i) to investigate the best fitting model for the genetic analysis of type traits and (ii) to estimate the genetic parameters of 4 composite (body size, muscularity, body shape, and udder) and 22 individual type traits between and within the two strains of the Valdostana dual purpose breed: the Aosta Red Pied (ARP; n=25,183 records) and the Aosta Black Pied and Chestnut (ABP-CHES; n=14,701 records). All type traits were scored from 2000 to 2012 on primiparous cows using a 1 to 5-point scale system. A model comparison was undertaken by considering the Akaike Information Criterion values obtained from the analysis, producing a final model that took into account the effects of herd-year-classifier, days in milk, age at calving as fixed and the animal additive genetic effect as random. Heritability estimates obtained through single trait animal model analysis varied from 0.03 for thinness (in both strains) to 0.32 (ARP) and 0.29 (ABP-CHES) for stature. Medium-low heritability estimates were obtained for individual muscularity traits (0.22 for ARP and 0.13 for ABP-CHES), and for individual udder type traits (0.12 on average in both Valdostana strains). The highest genetic correlations between composite traits were for muscularity with body shape in both Valdostana strains (0.55 for ARP and 0.52 for ABP-CHES). Different and opposite values of genetic correlations were found for the composite body shape and the composite udder traits (0.13 for ARP and -0.25 for ABP-CHES; $P < 0.01$), probably due to the different breeding purposes set up for the two Valdostana strains. Regarding the individual type traits, for the ARP strain the highest genetic correlation was 0.97 (between thigh, buttocks side and rear view), whereas for the ABP-CHES strain it was 0.98 (between stature and body length), meaning that improving one trait of each pair led to a positive variation in the other. Most of the genetic correlations between the individual muscularity traits and the individual udder traits were negative, especially those involving udder volume (from -0.19 to -0.42 in ARP, and from -0.17 to -0.41 in ABP-CHES; $P < 0.01$), indicating a substantial antagonistic situation of type traits related to dairy and beef traits. In conclusion, the selection for the dual purpose in local breeds such as in Valdostana cattle implies a thorough consideration of opposite morphological traits.

Keywords: Dual purpose; Genetic parameters; Type traits; Valdostana cattle

Livestock Science

Volume 178, Pages 35-42

Genetic variation and effects of candidate-gene polymorphisms on coagulation properties, curd firmness modeling and acidity in milk from Brown Swiss cows

Cecchinato, A.[a], *Chessa, S.*[b], *Ribeca, C.*[a], *Cipolat-Gotet, C.*[a], *Bobbo, T.*[a], *Casellas, J.*[c], *Bittante, G.*[a]

[a] Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, 35020 Legnaro, Italy
[b] Istituto di Biologia e Biotecnologia Agraria, Consiglio Nazionale delle Ricerche (CNR), via Einstein, 26900 Lodi, Italy
[c] Departament de Ciència Animal i dels Aliments, Grup de Recerca en Remugants, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

Abstract

The aims of this study were to estimate the genetic variation of traditional milk coagulation properties (MCPs), milk acidity, curd firmness (CF) modeled on time t (CF_t; comprising: RCT_{eq}, rennet coagulation time estimated from the equation; CF_P, the asymptotic potential curd firmness; k_{CF}, the curd firming instant rate constant; and k_{SR}, the syneresis instant rate constant) and maximum CF traits (MCF; comprising CF_{max}, the maximum CF value; and t_{max}, the time of attainment). Furthermore, we investigated 96 single nucleotide polymorphisms (SNPs) from 54 candidate genes, testing their associations with the above-listed traits. Milk and blood samples were collected from 1271 cows (each sampled once) from 85 herds. Genotyping was performed using a custom Illumina VeraCode GoldenGate approach. A Bayesian linear animal model (including the effects of herd, days in milk, parity and additive polygenic effects) was used to estimate the genetic parameters of the studied traits. The same model with the addition of the SNP genotype effect was used for our association analysis. The heritability estimates of CF_t and the MCF traits (RCT_{eq} = 0.258; k_{CF} = 0.230; CF_{max} = 0.191; t_{max} = 0.278) were similar to those obtained using traditional MCPs (0.187 to 0.267), except for the lower estimates for CF_P (0.064) and k_{SR} (0.077). A total of 13 of the 51 tested SNPs had relevant additive effects on at least one trait. We observed associations between MCPs and SNPs in the genes encoding ATP-binding cassette sub-family G member 2 (*ABCG2*), chemokine ligand 2 (*CCL2*), growth hormone 1 (*GH1*), prolactin (*PRL*) and toll-like receptor 2 (*TLR2*). Whereas, CF_t and the MCF traits were associated with polymorphisms in the α-s1-casein (*CSN1S1*), β-casein (*CSN2*), *GH1*, oxidized low-density lipoprotein receptor 1 (*OLR1*), phospholipase C β1 (*PLCB1*), *PRL* and signal transducer and activator of transcription 5A (*STAT5A*) genes.

Keywords: Candidate gene; Dairy cow; Heritability; Milk acidity; Milk coagulation properties

Animal

Volume 9, Issue 7, Pages 1104-1112

Genetics of milk fatty acid groups predicted during routine data recording in Holstein dairy cattle

Penasa, M.[a], Tiezzi, F.[b], Gottardo, P.[a], Cassandro, M.[a], De Marchi, M.[a]

[a] Department of Agronomy, Food, Natural resources, Animals and Environment, University of Padova, Viale dell'Università 16, 35020 Legnaro, PD, Italy
[b] Department of Animal Science, North Carolina State University, Raleigh 27695, USA

Abstract

The aim of this paper was to estimate genetic parameters for groups of milk fatty acids (FA), namely saturated (SFA), unsaturated (UFA), monounsaturated (MUFA) and polyunsaturated (PUFA), in Holstein cows. Mid-infrared spectroscopy (MIRS) was used to predict FA groups (g/100 g of milk) of 72,848 samples recorded on 17,873 cows between September 2011 and November 2012. Univariate and multivariate models were implemented in a Bayesian framework to estimate (co) variance components for SFA, UFA, MUFA, PUFA, daily milk yield, milk fat and milk protein. Statistical models included fixed effect of parity by stage of lactation, and random effects of herd-test-date, cow permanent environmental, animal additive genetic and residual. Posterior means of heritability estimates for SFA, UFA, MUFA and PUFA were 0.246, 0.069, 0.082 and 0.078, respectively. Estimates of genetic correlations between FA groups ranged from 0.405 (SFA and PUFA) to 0.952 (MUFA and UFA). The increase of fat content led to an increase of all groups of FA, in particular SFA, with undesirable effects on the healthy quality of the product. The study highlighted the existence of exploitable additive genetic variation for groups of FA routinely predicted by MIRS and thus there is potential to address the selection to healthy milk FA composition.

Keywords: Genetic correlation; Heritability; Holstein; Milk fatty acid

Livestock Science

Volume 173, Pages 9-13

Short communication: Genetic relationships of milk coagulation properties with body condition score and linear type traits in Holstein-Friesian cows

Cassandro, M.[a]*, Battagin, M.*[b]*, Penasa, M.*[a]*, De Marchi, M.*[a]

[a] Department of Agronomy, Food, Natural Resources, Animals and Environment, University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy
[b] Associazione Nazionale Allevatori Frisona Italiana, 26100 Cremona, Italy

Abstract

Milk coagulation properties (MCP) are gaining popularity among dairy cattle producers and the improvement of traits associated with MCP is expected to result in a benefit for the dairy industry, especially in countries with a long tradition in cheese production. The objectives of this study were to estimate genetic correlations of MCP with body condition score (BCS) and type traits using data from first-parity Italian Holstein-Friesian cattle. The data analyzed consisted of 18,460 MCP records from 4,036 cows with information on both BCS and conformation traits. The cows were daughters of 246 sires and the pedigree file included a total of 37,559 animals. Genetic relationships of MCP with BCS and type traits were estimated using bivariate animal models. The model for MCP included fixed effects of stage of lactation, and random effects of herd-test-date, cow permanent environment, additive genetic animal, and residual. Fixed factors considered in the model for BCS and type traits were herd-date of evaluation and interaction between age at scoring and stage of lactation of the cow, and random terms were additive genetic animal, cow permanent environment, and residual. Genetic relationships between MCP and BCS, and MCP and type traits were generally low and significant only in a few cases, suggesting that MCP can be selected for without detrimental effects on BCS and linear type traits.

Keywords: Body condition score; Genetic correlation; Milk coagulation ability; Type trait

Journal of Dairy Science

Volume 98, Issue 1, Pages 685-691

Molecular genotyping of "Rizor" and "Holly" rhizomania resistances in sugar beet

Stevanato, P.[a], De Biaggi, M.[b], Broccanello, C.[a], Biancardi, E.[c], Saccomani, M.[a]

[a] DAFNAE, Dipartimento di Agronomia Animali Alimenti Risorse Naturali e Ambiente,
Università degli Studi di Padova, Legnaro (PD), Italy
[b] Massalombarda (RA), Italy
[c] CRA-CIN Rovigo, Rovigo, Italy

Abstract

Rhizomania is the most damaging and widespread disease of the sugar beet crop and is caused by *Beet necrotic yellow vein virus* vectored by the fungus *Polymyxa betae*. The only disease management tool used is resistant varieties. In the last 30 years, "Rizor" and "Holly" (Rz1) resistances have been the most widely used. Despite the lack of information, a common origin for both types of resistance was hypothesized by some breeders. The aim of this study was to assess the phylogenetic relationship between Rizor and Rz1 by means of SNP analysis. Fifty leaf samples of each were genotyped with a fingerprinting panel of 192 SNPs, using the QuantStudio 12K Flex system coupled with Taqman OpenArray technology. Analysis of molecular variance and principal coordinate analysis confirmed that Rizor and Rz1 cannot be distinguished as separate sources of resistance.

Keywords: Holly; Rhizomania resistance; Rizor; Rz1; SNP genotyping; Sugar beet

Euphytica

Volume 206, Issue 2, Pages 427-431

Microbiology

Severe diseases induced by viruses and phytoplasmas in *Hydrangea* in Italy

Bertaccini, A.[a]*, Paltrinieri, S.*[b]*, Contaldo, N.*[a]*, Cavicchi, L.*[b]*, Mori, N.*[c]*,*
Bellardi, M.G.[a]

[a] DipSA-Plant Pathology, Alma Mater Studiorum, University of Bologna, Via G. Fanin, 42, Bologna, Italy
[b] Plesso Didattico G. Scarabelli (Imola), Alma Mater Studiorum, University of Bologna, Via Ascari, 17, Imola (Bologna), Italy
[c] Dipartimento di Agronomia Animali Alimenti Risorse Naturali e Ambiente, Università degli Studi di Padova, Viale dell'Università, 16, Legnaro (Padova), Italy

Abstract

Epidemiological surveys were carried out in Liguria and Lazio regions to detect virus and phytoplasma infections in *Hydrangea machrophylla*. Plants showing stunting, flower virescence and phyllody, yellowing, necrosis and redness of the leaf edge, collected in "Piana di Albenga" (Liguria region), were infected by *Hydrangea ringspot virus*. One of these hydrangea plants, showing also flower virescence and red edges of leaves, was infected by phytoplasmas belonging to subgroup 16SrI-B, GroELI subgroup III. Almost all hydrangea plants growing in Bolsena city (Lazio region) showed severe leaf and flower symptoms. *Cucumber mosaic virus* was detected in three plants characterized by stunting, leaf mosaic of malformed leaves and flower colour-breaking; *Elm mottle virus* was detected in two plants with mosaic symptoms on the leaves. One plant showing growth reduction, flower virescence and phyllody was found to be infected by phytoplasmas belonging to ribosomal subgroup 16SrXII-A. Leafhoppers present on this latter phytoplasma-infected plant were caught and identified. 16SrI-B phytoplasmas were identified in *L. striatellus*, while 16SrXII-A phytoplasmas were present in *Anaceratogallia* sp.

Keywords: *Hydrangea macrophylla*; Leafhoppers; PCR; RFLP; Symptomatology

Acta Horticulturae

Volume 1072, Issue , Pages 105-112

Bacteriocinogenic LAB from cheeses - Application in biopreservation?

Favaro, L.[a], Barretto Penna, A.L.[b], Todorov, S.D.[bcd]

[a] Department of Agronomy Food Natural resources Animals and Environment (DAFNAE), University of Padova, Agripolis, Viale dell'Università 16, Legnaro, PD, Italy
[b] Department of Food Engineering and Technology, Sao Paulo State University, Rua Cristóvão Colombo, 2265, São José do Rio Preto, SP, Brazil
[c] Department of Food Science and Experimental Nutrition, Faculty of Pharmaceutical Sciences, University of Sao Paulo, Av. Prof. Lineu Prestes 580, Bl. 14, São Paulo, SP, Brazil
[d] Departamento de Veterinária, Universidade Federal de Viçosa, Viçosa, MG, Brazil

Abstract

Over the last decade, there has been an explosion of basic and applied research on lactic acid bacteria bacteriocins, because of their potential as biopreservatives and inhibition of the growth of spoilage bacteria. Although bacteriocins can be produced during cheese production, their titers are much lower than those achieved invitro fermentations under optimal physical and chemical conditions. Safety and technological traits of the bacteriocinogenic lactic acid bacteria (LAB) have to be considered before their wide-spread applications. This review described the perspectives and hurdles to be solved in order to definitively disclose the potential of bacteriocins in the production of safe and healthy cheese commodities.

Trends in Food Science and Technology

Volume 41, Issue 1, Pages 37-48

Consolidated bioprocessing of starchy substrates into ethanol by industrial *Saccharomyces cerevisiae* strains secreting fungal amylases

Favaro, L.[a], Viktor, M.J.[b], Rose, S.H.[b], Viljoen-Bloom, M.[b], van Zyl, W.H.[b], Basaglia, M.[a], Cagnin, L.[a], Casella, S.[a]

[a] Department of Agronomy Food Natural resources Animals and Environment (DAFNAE), Università di Padova, Agripolis, Viale dell'Università 16, Legnaro (PD), Italy
[b] Department of Microbiology, Stellenbosch University, Stellenbosch, South Africa

Abstract

The development of a yeast strain that converts raw starch to ethanol in one step (called Consolidated Bioprocessing, CBP) could significantly reduce the commercial costs of starch-based bioethanol. An efficient amylolytic *Saccharomyces cerevisiae* strain suitable for industrial bioethanol production was developed in this study. Codon-optimized variants of the *Thermomyces lanuginosus* glucoamylase (*TLG1*) and *Saccharomycopsis fibuligera* a-amylase (*SFA1*) genes were d-integrated into two *S. cerevisiae* yeast with promising industrial traits, i.e., strains M2n and MEL2. The recombinant M2n[TLG1-SFA1] and MEL2[TLG1-SFA1] yeast displayed high enzyme activities on soluble and raw starch (up to 8118 and 4461nkat/g dry cell weight, respectively) and produced about 64g/L ethanol from 200g/L raw corn starch in a bioreactor, corresponding to 55% of the theoretical maximum ethanol yield (g of ethanol/g of available glucose equivalent). Their starch-to-ethanol conversion efficiencies were even higher on natural sorghum and triticale substrates (62 and 73% of the theoretical yield, respectively). This is the first report of direct ethanol production from natural starchy substrates (without any pre-treatment or commercial enzyme addition) using industrial yeast strains co-secreting both a glucoamylase and a-amylase.

Keywords: Codon optimization; Consolidated bioprocessing (CBP); Industrial yeast; Raw starch; Sorghum; Triticale

Biotechnology and Bioengineering

Volume 112, Issue 9, Pages 1751-1760

Dimethyl carbonate and switchable anionic surfactants: Two effective tools for the extraction of polyhydroxyalkanoates from microbial biomass

Samorì, C.[a], Basaglia, M.[b], Casella, S.[b], Favaro, L.[b], Galletti, P.[ac], Giorgini, L.[de], Marchi, D.[a], Mazzocchetti, L.[e], Torri, C.[ac], Tagliavini, E.[ac]

[a] Centro Interdipartimentale di Ricerca Industriale Energia Ambiente (CIRI EA), University of Bologna, via S. Alberto 163, Ravenna, Italy
[b] Dipartimento di Agronomia Animali Alimenti Risorse Naturali e Ambiente, University of Padova Agripolis, Viale dell'Università 16, Legnaro (PD), Italy
[c] Dipartimento di Chimica giacomo Ciamician, University of Bologna, via Selmi 2, Bologna, Italy
[d] Dipartimento di Chimica Industriale toso Montanari, University of Bologna, viale del Risorgimento 4, Bologna, Italy
[e] Centro Interdipartimentale di Ricerca Industriale per la Meccanica Avanzata e i Materiali (CIRi MAM), University of Bologna, Viale Risorgimento 2, Bologna, Italy

Abstract

The availability of green and cheap technologies to recover polyhydroxyalkanoates (PHAs) from microbial biomass is crucial for the development of a reliable and sustainable production chain. Here, two novel protocols are proposed to extract PHAs from *Cupriavidus necator*. The first method is based on PHA-extraction with dimethyl carbonate (DMC), a green solvent that is completely biodegradable and less harmful to humans and the environment than most solvents. The procedure can be applied directly to concentrated microbial slurries or to dry biomass, affording very high polymer recovery (>85%) and excellent purity (>95%). No degradation/decomposition of the polymer is observed in both cases. The second protocol uses fatty acid carboxylates as surfactants, which disrupt cell membranes, providing excellent polymer recovery (>99%) and high purity (>90%). Ammonium laurate can be successfully used and easily recycled (98%) by lowering the pH through CO_2 addition. Therefore, both protocols reported here are effective and sustainable: the recovery and purity of the obtained PHAs are very high, the use of toxic chemicals is avoided, and the recycling of various solvents/surfactants used in the processes is optimal.

Green Chemistry

Volume 17, Issue 2, Pages 1047-1056

Identification of ferredoxin II as a major calcium binding protein in the nitrogen-fixing symbiotic bacterium *Mesorhizobium loti*

Moscatiello, R.[a], *Zaccarin, M.*[b], *Ercolin, F.*[a], *Damiani, E.*[c], *Squartini, A.*[d], *Roveri, A.*[b], *Navazio, L.*[a]

[a] Department of Biology, University of Padova, Via U. Bassi 58/B, Padova, Italy
[b] Department of Molecular Medicine, University of Padova, Viale G. Colombo 3, Padova, Italy
[c] Department of Biomedical Sciences, University of Padova, Viale G. Colombo 3, Padova, Italy
[d] Department of Agronomy, Food, Natural Resources, Animals and Environment, DAFNAE, University of Padova, Viale dell'Università 16, Legnaro, Padova, Italy

Abstract

Background: Legumes establish with rhizobial bacteria a nitrogen-fixing symbiosis which is of the utmost importance for both plant nutrition and a sustainable agriculture. Calcium is known to act as a key intracellular messenger in the perception of symbiotic signals by both the host plant and the microbial partner. Regulation of intracellular free Ca^{2+}concentration, which is a fundamental prerequisite for any Ca^{2+}-based signalling system, is accomplished by complex mechanisms including Ca^{2+} binding proteins acting as Ca^{2+} buffers. In this work we investigated the occurrence of Ca^{2+} binding proteins in *Mesorhizobium loti*, the specific symbiotic partner of the model legume *Lotus japonicus*. Results: A soluble, low molecular weight protein was found to share several biochemical features with the eukaryotic Ca^{2+}-binding proteins calsequestrin and calreticulin, such as Stains-all blue staining on SDS-PAGE, an acidic isoelectric point and a Ca^{2+}- dependent shift of electrophoretic mobility. The protein was purified to homogeneity by an ammonium sulfate precipitation procedure followed by anion-exchange chromatography on DEAE-Cellulose and electroendosmotic preparative electrophoresis. The Ca^{2+} binding ability of the *M. loti* protein was demonstrated by $45Ca^{2+}$-overlay assays. ESI-Q-TOF MS/MS analyses of the peptides generated after digestion with either trypsin or endoproteinase AspN identified the rhizobial protein as ferredoxin II and confirmed the presence of Ca^{2+} adducts. Conclusions: The present data indicate that ferredoxin II is a major Ca^{2+} binding protein in *M. loti* that may participate in Ca^{2+} homeostasis and suggest an evolutionarily ancient origin for protein-based Ca^{2+} regulatory systems.

Keywords: Calcium binding proteins; Calcium homeostasis; Ferredoxin II*; Mesorhizobium loti*; Nitrogen fixation; Rhizobium-legume symbiosis

BMC Microbiology

Volume 15, Issue 1, Pages 1-9

Microbial immigration across the Mediterranean via airborne dust

Rosselli, R.[a], *Fiamma, M.*[b], *Deligios, M.*[b], *Pintus, G.*[c], *Pellizzaro, G.*[c], *Canu, A.*[c],
Duce, P.[c], *Squartini, A.*[e], *Muresu, R.*[d], *Cappuccinelli, P.*[b]

[a] Department of Biology, University of Padova, Via Ugo Bassi 58/b, Padova, Italy
[b] Department of Biomedical Sciences, University of Sassari, Italy
[c] Institute of Biometeorology, National Research Council (IBIMET-CNR), Italy
[d] Institute of Animal Production Systems in Mediterranean Environments, National Research Council (ISPAAMCNR), Italy
[e] Department of Agronomy Animals, Food, Natural Resources and Environment, DAFNAE, University of Padova, Viale dell'Università 16, Legnaro (Padova), Italy

Abstract

Dust particles lifting and discharge from Africa to Europe is a recurring phenomenon linked to air circulation conditions. The possibility that microorganisms are conveyed across distances entails important consequences in terms of biosafety and pathogens spread. Using culture independent DNA-based analyses via next generation sequencing of the 16 S genes from the airborne metagenome, the atmospheric microbial community was characterized and the hypothesis was tested that shifts in species diversity could be recorded in relation to dust discharge. As sampling ground the island of Sardinia was chosen, being an ideal cornerstone within the Mediterranean and a crossroad of wind circulation amidst Europe and Africa. Samples were collected in two opposite coastal sites and in two different weather conditions comparing dust-conveying winds from Africa with a control situation with winds from Europe. A major conserved core microbiome was evidenced but increases in species richness and presence of specific taxa were nevertheless observed in relation to each wind regime. Taxa which can feature strains with clinical implications were also detected. The approach is reported as a recommended model monitoring procedure for early warning alerts in frameworks of biosafety against natural spread of clinical microbiota across countries as well as to prevent bacteriological warfare.

Scientific Reports

Volume 5, Article 16306

Outlining a selection procedure for *Saccharomyces cerevisiae* isolated from grape marc to improve fermentation process and distillate quality

Bovo, B.[a], Carlot, M.[b], Fontana, F.[a], Lombardi, A.[c], Soligo, S.[d], Giacomini, A.[ab], Corich, V.[ab]

[a] Dipartimento di Agronomia Animali Alimenti Risorse naturali e Ambiente, Università degli Studi di Padova, Legnaro PD, Italy
[b] Centro Interdipartimentale per la Ricerca in Viticoltura ed Enologia (C.I.R.V.E.), Università degli Studi di Padova, Conegliano TV, Italy
[c] Istituto per la Qualità e le Tecnologie Agroalimentari, Veneto Agricoltura, Thiene VI, Italy
[d] Centro Regionale per la Viticoltura l'Enologia e la Grappa (CeRVEG), Conegliano TV, Italy

Abstract

Nowadays grape marc represents one of the main by-product of winemaking. Many South Europe countries valorize this ligno-cellulosic waste through fermentation and distillation for industrial alcoholic beverage production. The storage of marcs is a crucial phase in the distillation process, due to the physicochemical transformations ascribed to microbial activity. Among the methods adopted by distillers to improve the quality of spirits, the use of selected yeasts has not been explored so far, therefore in this work we evaluated the selection criteria of *Saccharomyces cerevisiae* strains for grape marc fermentation.The proposed selection procedure included three steps: characterization of phenotypical traits, evaluation of selected strains on pasteurised grape marc at lab-scale (100g) and pilot-scale fermentation (350kg). This selection process was applied on 104 strains isolated from grape marcs of different origins and technological treatment. Among physiological traits, -glucosidase activity level as quality trait seems to be only partially involved in increasing varietal flavour. More effective in describing yeast impact on distillate quality is the ratio higher alcohols/esters that indicates strain ability to increase positive flavours. Finally, evaluating grape marc as source of selected yeasts, industrial treatment rather than varietal origin seems to shape strain technological and quality traits.

Keywords: Biodiversity; By-product; GC/MS analysis; Higher alcohols; MtDNA-RFLP

Food Microbiology

Volume 46, Issue , Pages 573-581

Poly(hydroxyalkanoate) production by *Cupriavidus necator* from fatty waste can be enhanced by *phaZ1* inactivation

Povolo, S.[a], *Basaglia, M.*[a], *Fontana, F.*[a], *Morelli, A.*[b], *Casella, S.*[a]

[a] Department of Agronomy Food Natural Resources Animals and Environment (DAFNAE), University of Padova, Agripolis, Viale dell'Università 16, Legnaro, Padova, Italy
[b] Laboratory of Bioactive Polymeric Materials for Biomedical and Environmental Applications (BIOlab), Dept. of Chemistry and Industrial Chemistry, University of Pisa, via Vecchia Livornese, 1291, S. Piero a Grado, Pisa, Italy

Abstract

PHA production from waste oils or fats requires microorganisms that should be both excellent PHA producers and equipped with enzymatic activities allowing hydrolysation of triglycerides. Unfortunately, microbes with the combination of substrate-utilization and PHA production are not currently available, and the strategies to be adopted are the use of costly commercial enzymes, or genetic modification of microorganisms exhibiting high PHA product yields. In the present work, after a general investigation on the ability of *Cupriavidus necator* to grow on a number of fatty substrates, the possibility to enhance PHA production by limiting intracellular depolymerisation, was investigated. By knocking out the related *phaZ1* gene, the construction of *C. necator* recombinant strains impaired in depolymerase (PhaZ1) activity was achieved. The polymer yield of the recombinant strain was finally compared to that of the parental *C. necator* DSM 545.

Keywords: *Cupriavidus necator*; Depolymerase; Glycerol; Lipase activity; Poly(hydroxyalkanoates)

Chemical and Biochemical Engineering Quarterly

Volume 29, Issue 2, Pages 67-74

Utilisation of wheat bran as a substrate for bioethanol production using recombinant cellulases and amylolytic yeast

Cripwell, R.[a], Favaro, L.[b], Rose, S.H.[a], Basaglia, M.[b], Cagnin, L.[b], Casella, S.[b], van Zyl, W.[a]

[a] Department of Microbiology, Stellenbosch University, Private Bag X1, Matieland, Stellenbosch, South Africa
[b] Department of Agronomy Food Natural resources Animals and Environment (DAFNAE), Università di Padova, Agripolis, Viale dell'Università 16, Legnaro, Padova, Italy

Abstract

Wheat bran, generated from the milling of wheat, represents a promising feedstock for the production of bioethanol. This substrate consists of three main components: starch, hemicellulose and cellulose. The optimal conditions for wheat bran hydrolysis have been determined using a recombinant cellulase cocktail (RCC), which contains two cellobiohydrolases, an endoglucanase and a b-glucosidase. The 10% (w/v, expressed in terms of dry matter) substrate loading yielded the most glucose, while the 2% loading gave the best hydrolysis efficiency (degree of saccharification) using unmilled wheat bran. The ethanol production of two industrial amylolytic *Saccharomyces cerevisiae* strains, MEL2[TLG1-SFA1] and M2n[TLG1-SFA1], were compared in a simultaneous saccharification and fermentation (SSF) for 10% wheat bran loading with or without the supplementation of optimised RCC. The recombinant yeast *S. cerevisiae* MEL2[TLG1-SFA1] and M2n[TLG1-SFA1] completely hydrolysed wheat bran's starch producing similar amounts of ethanol (5.3 ± 0.14 g/L and 5.0 ± 0.09 g/L, respectively). Supplementing SSF with RCC resulted in additional ethanol production of about 2.0 g/L. Scanning electron microscopy confirmed the effectiveness of both RCC and engineered amylolytic strains in terms of cellulose and starch depolymerisation. This study demonstrated that untreated wheat bran could be a promising ready-to-use substrate for ethanol production. The addition of crude recombinant cellulases improved ethanol yields in the SSF process and *S. cerevisiae* MEL2[TLG1-SFA1] and M2n[TLG1-SFA1] strains can efficiently convert wheat bran's starch to ethanol.

Keywords: Bioethanol; Industrial engineered amylolytic yeast; Recombinant cellulase cocktail; Simultaneous saccharification and fermentation; Wheat bran

Applied Energy

Volume 160, Issue , Pages 610-617

Plant science

Combined endophytic inoculants enhance nickel phytoextraction from serpentine soil in the hyperaccumulator *Noccaea caerulescens*

Visioli, G.[a], Vamerali, T.[b], Mattarozzi, M.[c], Dramis, L.[a], Sanangelantoni, A.M.[a]

[a] Department of Life Sciences, University of Parma, Parma, Italy
[b] Department of Agronomy, Food, Natural Resources, Animals and the Environment, University of Padova, Padova, Italy
[c] Department of Chemistry, University of Parma, Parma, Italy

Abstract

This study assesses the effects of specific bacterial endophytes on the phytoextraction capacity of the Ni-hyperaccumulator *Noccaea caerulescens*, spontaneously growing in a serpentine soil environment. Five metal-tolerant endophytes had already been selected for their high Ni tolerance (6 mM) and plant growth promoting ability. Here we demonstrate that individual bacterial inoculation is ineffective in enhancing Ni translocation and growth of *N. caerulescens* in serpentine soil, except for specific strains Ncr-1 and Ncr-8, belonging to the *Arthrobacter* and *Microbacterium* genera, which showed the highest indole acetic acid production and 1-aminocyclopropane-1-carboxylic acid-deaminase activity. Ncr-1 and Ncr-8 co-inoculation was even more efficient in promoting plant growth, soil Ni removal, and translocation of Ni, together with that of Fe, Co, and Cu. Bacteria of both strains densely colonized the root surfaces and intercellular spaces of leaf epidermal tissue. These two bacterial strains also turned out to stimulate root length, shoot biomass, and Ni uptake in *Arabidopsis thaliana* grown in MS agar medium supplemented with Ni. It is concluded that adaptation of *N. caerulescens* in highly Ni-contaminated serpentine soil can be enhanced by an integrated community of bacterial endophytes rather than by single strains; of the former, *Arthrobacter* and *Microbacterium* may be useful candidates for future phytoremediation trials in multiple metal-contaminated sites, with possible extension to non-hyperaccumulator plants.

Keywords: *Arabidopsis thaliana*; Nickel; *Noccaea caerulescens*; Phytoextraction; Plant growth-promoting endophytic bacteria (PGPE); Serpentine soil; Tissue colonization

Frontiers in Plant Science

Volume 6, Issue AUG, article 638.

Comprehensive transcript profiling of two grapevine rootstock genotypes contrasting in drought susceptibility links the phenylpropanoid pathway to enhanced tolerance

Corso, M.[ab], Vannozzi, A.[ab], Maza, E.[c], Vitulo, N.[d], Meggio, F.[ab], Pitacco, A.[ab], Telatin, A.[d], D'Angelo, M.[d], Feltrin, E.[d], Negri, A.S.[e], Prinsi, B.[e], Valle, G.[d], Ramina, A.[ab], Bouzayen, M.[e], Bonghi, C.[ab], Lucchin, M.[ab]

[a] Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova Agripolis, Legnaro, Italy
[b] Centro Interdipartimentale per la Ricerca in Viticoltura Ed Enologia (CIRVE), Via XXVIII Aprile, Conegliano (TV), Italy
[c] Genomics and Biotechnology of Fruit (GBF) Laboratory, Institut National Polytechnique de Toulouse, Avenue de l'Agrobiopole, Castanet-Tolosan cedex, Toulouse, France
CRIBI, University of Padova, viale G. Colombo 3, Padova, Italy
[d] Department of Agricultural and Environmental Sciences-Production, Landscape, Agroenergy (DiSAA), University of Milano, Milan, Italy
[e] Laboratoire de Physiologie et de Génétique Moléculaire des Plantes, Campus-Plaine ULB, Bd du Triomphe, Brussels, Belgium

Abstract

In light of ongoing climate changes in wine-growing regions, the selection of drought-tolerant rootstocks is becoming a crucial factor for developing a sustainable viticulture. In this study, M4, a new rootstock genotype that shows tolerance to drought, was compared from a genomic and transcriptomic point of view with the less drought-tolerant genotype 101.14. The root and leaf transcriptome of both 101.14 and the M4 rootstock genotype was analysed, following exposure to progressive drought conditions. Multifactorial analyses indicated that stress treatment represents the main factor driving differential gene expression in roots, whereas in leaves the genotype is the prominent factor. Upon stress, M4 roots and leaves showed a higher induction of resveratrol and flavonoid biosynthetic genes, respectively. The higher expression of *VvSTS* genes in M4, confirmed by the accumulation of higher levels of resveratrol in M4 roots compared with 101.14, was coupled to an up-regulation of several *VvWRKY* transcription factors. Interestingly, *VvSTS* promoter analyses performed on both the resequenced genomes highlighted a significantly higher number of W-BOX elements in the tolerant genotype. It is proposed that the elevated synthesis of resveratrol in M4 roots upon water stress could enhance the plant's ability to cope with the oxidative stress usually associated with water deficit.

Keywords: Flavonoids; Genome re-sequencing; mRNA-Seq; Stilbenes; *Vitis*; Water stress

Journal of Experimental Botany

Volume 66, Issue 19, Pages 5739-5752

Effect of cool storage duration on ripening initiation of 'Angelys®' pear fruit

Jajo, A.[a], Corso, M.[a], Bonghi, C.[a], Dal Molin, A.[a] Avanzato, C.[b], Ferrarini, A.[b], Delledonne, M.[b], Rahim, Md.A.[c], Trainotti, L.[c] Serra, S.[d], Musacchi, S.[d]

[a] Department of Agronomy, Food, Natural Resources, Animals and Environment, University of Padova, Viale dell'Università 16, Legnaro (PD), Ital
[b] Department of Biotechnology, University of Verona, Strada le Grazie 15, Verona, Italy
[c] Department of Biology, University of Padova, Via U. Bassi 58/B, Padova, Italy
[d] Washington State University (TFREC), Wenatchee, WA, United States

Abstract

Winter pears initiate ripening following exposure to room temperature (RT, 20°C), after a chilling period. The number of days required for pear fruit to fully ripen varies mainly depending on cultivar and duration of low temperature storage. However, the longer the time in cold storage, the faster the rate of post-storage ripening. This study aimed to correlate ripening of 'Angelys®' pear fruit with different chilling periods. The correlations of ripening were performed by using measurements of physiologically-related processes (soluble solid concentrations, firmness and titratable acidity) and molecular analyses. Fruit harvested at three different times (185, 192 and 199 days after bloom, DAFB) were separated into distinct lots with the same maturity (with similar chlorophyll content, measured non-destructively using IAD). Each lot was analysed after postharvest ripening for 6 days at RT, following cool storage (0°C) for 0, 1, 2 and 3 months. Results indicated that ripening is halted in the non-chilled pears and initiated in the majority of pears stored for 1, 2 and 3 months independent of the harvest time. Unexpectedly, among the 3 months chilled pears, harvested at 199 DAFB, some fruit were showing a strong delay of ripening (unripe group). The latter lot was compared with the nonchilled pears and those stored for 3 months but showing typical ripening traits (ripe group) by using an RNAseq approach. In the three comparisons, there were 2498 differentially expressed genes. Many of the genes differentially expressed in each comparison showed expression patterns related to cool storage. However, others showed patterns that were related to fruit ripening, such as those encoding for ethylene receptors and cell wall modifying enzymes. In addition, only in the ripe group an up-regulation of genes involved in chromatin remodelling was observed; such genes have been previously associated with the competence to ripen.

Keywords: Chromatin remodelling; Cold-temperature conditioning; *Pyrus communis*; RNAseq; Winter pear

Acta Horticulturae

Volume 1079, Issue, Pages 129-136

Environmental and pathogenic factors inducing brown apical necrosis on fruit of English (Persian) walnut

Scotton, M.[a], *Bortolin, E.*[b], *Fiorin, A.*[c], *Belisario, A.*[d]

[a] Department of Agronomy Foods Natural Resources Animals and Environment,
University of Padova, Viale dell'Università 16, Legnaro, Padova, Italy
[b] Nogalba, Consorzio Produttori di Noce, Pettorazza, Rovigo, Italy
[c] Il Noceto, Società Consortile s.r.l., Chiarano, Treviso, Italy
[d] Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria-Centro di Ricerca per la Patologia Vegetale (CRA-PAV), Via C. G. Bertero 22, Roma, Italy

Abstract

Brown apical necrosis (BAN) is a most recently described disease affecting English (Persian) walnut fruit. BAN was only recorded in intensively managed walnut orchards and was found to be a disease complex mainly caused by *Fusarium* species. All fungi associated with this disease are polyphagous and ubiquitous, not specific to walnut. Consequently, BAN occurrence is more strictly dependent, than generally, on the interaction between pathological features and environmental conditions. Environmental variables identified with regression analysis showed that maximum temperature, angle of main wind direction versus tree row orientation, and orchard distance to the closest river/canal, all representative of climatic conditions occurring in the orchard, were related to fruit drop. The factor displaying the highest influence on severity of BAN fruit drop was maximum temperature and only subordinately factors are associated with relative humidity. BAN symptoms were reproduced with in planta artificial inoculation, and fruit drop of symptomatic fruit was significantly higher than that of the noninoculated trees for each type of inoculum (*Fusarium semitectum*, *F. graminearum*, and *Alternaria* spp.). *F. Semitectum* and *F. graminearum* were more aggressive than *Alternaria* species, and the earliest artificial inoculations in mid-May resulted in the highest fruit drop. The extension of walnut fruit susceptibility and the conducive environmental factors to BAN are discussed.

Phytopathology

Volume 105, Issue 11, Pages 1427-1436

Ethylene negatively regulates transcript abundance of ROP-GAP rheostat-encoding genes and affects apoplastic reactive oxygen species homeostasis in epicarps of cold stored apple fruits

Zermiani, M.[a], *Zonin, E.*[a], *Nonis, A.*[a], *Begheldo, M.*[a], *Ceccato, L.*[a], *Vezzaro, A.*[a], *Baldan, B.*[b], *Trentin, A.*[a], *Masi, A.*[a], *Pegoraro, M.*[a], *Fadanelli, L.*[c], *Teale, W.*[d], *Palme, K.*[defgh] , *Quintieri, L.*[i], *Ruperti, B.*[a]

[a] Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Legnaro, Italy
[b] Department of Biology, University of Padova, Padova, Italy
[c] Edmund Mach Foundation, San Michele all'Adige, Trento, Italy
[d] Institute of Biology II/Molecular Plant Physiology, Faculty of Biology, Albert-Ludwigs-University of Freiburg, Freiburg, Germany
[e] Centre for Biological Systems Analysis, Albert-Ludwigs-University of Freiburg, Freiburg, Germany
[f] Freiburg Institute for Advanced Sciences (FRIAS), Albert-Ludwigs-University of Freiburg, Freiburg, Germany
[g] Centre for Biological Signalling Studies (Bioss), Albert-Ludwigs-University of Freiburg, Freiburg, Germany
[h] Freiburg Initiative for Systems Biology (FRISYS), Albert-Ludwigs-University of Freiburg, Freiburg, Germany
[i] Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Padova, Italy

Abstract

Apple (*Malus x domestica* Borkh) fruits are stored for long periods of time at low temperatures (1 °C) leading to the occurrence of physiological disorders. 'Superficial scald' of Granny Smith apples, an economically important ethylene-dependent disorder, was used as a model to study relationships among ethylene action, the regulation of the ROP-GAP rheostat, and maintenance of H_2O2 homeostasis in fruits during prolonged cold exposure. The ROP-GAP rheostat is a key module for adaptation to low oxygen in *Arabidopsis* through Respiratory Burst NADPH Oxidase Homologs (RBOH)-mediated and ROP GTPase-dependent regulation of reactive oxygen species (ROS) homeostasis. Here, it was shown that the transcriptional expression of several components of the apple ROP-GAP machinery, including genes encoding RBOHs, ROPs, and their ancillary proteins ROP-GEFs and ROP-GAPs, is coordinately and negatively regulated by ethylene in conjunction with the progressive impairment of apoplastic H_2O_2 homeostatic levels. RNA sequencing analyses showed that several components of the known ROP- and ROS-associated transcriptional networks are regulated along with the ROP-GAP rheostat in response to ethylene perception. These findings may extend the role of the ROP-GAP rheostat beyond hypoxic responses and suggest that it may be a functional regulatory node involved in the integration of ethylene and ROS signalling pathways in abiotic stress.

Keywords: Abiotic stress; Ethylene; Fruit senescence; Ionotropic glutamate receptors; NADPH oxidase; RBOH; ROP GTPases; ROS homeostasis

Journal of Experimental Botany

Volume 66, Issue 22, Pages 7255-7270

First report of *fusarium solani* phylogenetic species 25 associated with early stages of thousand cankers disease on *Juglans nigra* and *Juglans regia* in Italy

Montecchio, L.[a]*, Faccoli, M.*[b]*, Short, D.P.G.*[c]*, Fanchin, G.*[a]*, Geiser, D.M.*[d]*,*
Kasson, M.T.[c]

[a] Department of Land, Environment, Agriculture and Forestry (TeSAF), University of Padova, Italy
[b] Department of Agronomy, Food, Natural Resources, Animals, and Environment (DAFNAE), University of Padova, Italy
[c] Division of Plant and Soil Sciences, West Virginia University, Morgantown, United States
[d] Department of Plant Pathology and Environmental Microbiology, The Pennsylvania State University, University Park, United States

Abstract

Thousand cankers disease (TCD) is a disease of *Juglans* spp. resulting from phloem necrosis caused by numerous coalescing *Geosmithia morbida* (*Gm*) branch cankers formed around entrance holes and galleries of its vector, the walnut twig beetle (WTB). Since 2010, TCD has been reported from black walnut (*J. nigra*) in the eastern United States (Griffin 2014) and more recently from Italy (Montecchio and Faccoli 2014) as well as from English walnut (*J. regia*) in both countries (Montecchio et al. 2014; Yaghmour et al. 2014). Members of the *Fusarium solani* species complex (FSSC) are associated with later stage TCD in the United States (Tisserat et al. 2009), but their role in early WTB colonization is unclear. From 2013 to 2014, FSSC-like colonies, in addition to *Gm*, were isolated from cankers on symptomatic *J. nigra* and *J. regia* in Italy. FSSC-like fungi were isolated from 82 and 64% of branch cankers from four *J. nigra* and four *J. regia* and 30% of 186 WTB from *J. nigra*. Symptoms included cankers surrounding WTB galleries as described for *Gm* in Italy (Montecchio and Faccoli 2014; Montecchio et al. 2014). From the necrotic margin of four branch cankers from four *J. nigra*, wood chips were excised and plated. In addition to *Gm*, a fungus with abundant aerial mycelium and sporodochia containing 2 to 3 septa macroconidia grew. Oval to kidney-shaped, aseptate microconidia were produced from elongate monophialides consistent with the FSSC. Four isolates were sent to the Fusarium Research Center (Penn State Univ.) for molecular confirmation. Portions of the nuclear ribosomal RNA gene repeat (rDNA), translation elongation factor 1-α (*EF1-α*), DNA-directed RNA polymerase II subunit 1 (*RPB1*), and DNA-directed RNA polymerase subunit 2 (*RPB2*) were PCR amplified and sequenced to resolve placement within the FSSC. Initial GenBank BLASTn searches revealed isolate S1, S2, and S4 *RPB1* sequences (deposited as KP696752 for S1) were all 99% identical to FSSC 25 (HM347154) and 100% to each other but different from isolate S3, which was 99% similar to FSSC 18 (HM347153). Isolate S1 was used for pathogenicity and multilocus studies. A BLAST search of S1 rDNA sequence (KP696750) was 99% similarity to AB513852, while *EF1-α* and *RPB2* sequences (deposited as KP696749

and KP696751) were 99% similar to DQ247638 and EF469958, respectively. Alignment with known FSSC phylogenetic species confirmed S1 as FSSC 25. Pathogenicity was confirmed by placing 3 mm diam. PDA plugs of FSSC isolate S1, *Gm* isolate LM13GMN, both (split-plug), or sterile plugs into 10 cork borer-wounded 3-year-old *J. nigra* and *J. regia* saplings per treatment. Inoculated parafilmed trees were maintained in the greenhouse for 100 days. Inoculations with isolates *Gm* and S1 both individually and in combination on *J. nigra* resulted in cankers with similar mean areas of 4.8, 3.1, and 3.1 cm^2 and greater than ($P < 0.05$) the negative control (0.5 cm^2). For *J. regia*, cankers were 3× smaller compared with *J. nigra*, with similar mean areas of 1.5, 1.3, and 1.4 cm^2, respectively, and greater than ($P < 0.05$) the negative control (0.3 cm^2). Isolations from cankers and sequencing confirmed FSSC and/or *Gm*. These results support FSSC 25 as an early colonizer of WTB infested *J. nigra* and *J. regia*, and a contributing pathogen to early stages of TCD.

Plant Disease

Volume 99, Issue 8, Pages 1183

Identification of the arabidopsis RAM/MOR signalling network: Adding new regulatory players in plant stem cell maintenance and cell polarization

Zermiani, M.[a], *Begheldo, M.*[a], *Nonis, A.*[b], *Palme, K.*[cdef], *Mizzi, L.*[g], *Morandini, P.*[h], *Nonis, A.*[a], *Ruperti, B.*[a]

[a] Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università, 16, Legnaro (PD), Italy
[b] University Centre of Statistics for Biomedical Sciences, Università Vita-Salute San Raffaele, Via Olgettina 58, Milan, Italy
[c] Institute of Biology II/Molecular Plant Physiology, Faculty of Biology, Albert-Ludwigs-University of Freiburg, Schänzlestrasse 1, Freiburg, Germany
[d] Centre for Biological Systems Analysis, Albert-Ludwigs-University of Freiburg, Habsburgerstrasse 49, Freiburg, Germany
[e] Freiburg Institute for Advanced Sciences (FRIAS), Albert-Ludwigs-University of Freiburg, Albertstrasse 19, Freiburg, Germany
[f] BIOSS Centre for Biological Signalling Studies, Albert-Ludwigs-University of Freiburg, Albertstrasse 19, Freiburg, Germany
[g] Department of BioSciences, University of Milan, Via Celoria 26, Milan, Italy
[h] CNR Biophysics Institute (Milan Section), Via Celoria 26, Milan, Italy

Abstract

Background and Aims The RAM/MOR signalling network of eukaryotes is a conserved regulatory module involved in co-ordination of stem cell maintenance, cell differentiation and polarity establishment. To date, no such signalling network has been identified in plants.

Methods Genes encoding the bona fide core components of the RAM/MOR pathway were identified in *Arabidopsis thaliana* (arabidopsis) by sequence similarity searches conducted with the known components from other species. The transcriptional network(s) of the arabidopsis RAM/MOR signalling pathway were identified by running in-depth *in silico* analyses for genes co-regulated with the core components. *In situ* hybridization was used to confirm tissue-specific expression of selected RAM/MOR genes.

Key Results Co-expression data suggested that the arabidopsis RAM/MOR pathway may include genes involved in floral transition, by co-operating with chromatin remodelling and mRNA processing/post-transcriptional gene silencing factors, and genes involved in the regulation of pollen tube polar growth. The RAM/MOR pathway may act upstream of the ROP1 machinery, affecting pollen tube polar growth, based on the co-expression of its components with *ROP-GEFs*. *In silico* tissue-specific co-expression data and *in situ* hybridization experiments suggest that different components of the arabidopsis RAM/MOR are expressed in the shoot apical meristem and inflorescence meristem and may be involved in the fine-tuning of stem cell maintenance and cell differentiation.

Conclusions The arabidopsis RAM/MOR pathway may be part of the signalling cascade that converges in pollen tube polarized growth and in fine-tuning stem cell maintenance, differentiation and organ polarity.

Keywords: *Arabidopsis thaliana*; Cell polarity; Floral transition; *In situ* hybridization; RAM/MOR signalling network; Stem cell maintenance; Transcriptional

Annals of Botany

Volume 116, Issue 1, Pages 69-89

Metal partitioning in plant–substrate–water compartments under EDDS-assisted phytoextraction of pyrite waste with *Brassica carinata* A. Braun

Vamerali, T., Bandiera, M., Lucchini, P., Mosca, G.

Department of Agronomy, Food, Natural Resources, Animals and the Environment,
University of Padova, Viale dell'Università16, Legnaro, Padua, Italy

Abstract

Soil amendment with chelating agents can increase metal uptake and translocation in biomass species through increased metal bioavailability together with possible increases in metal leaching. In this study, we assessed the efficiency and environmental risk of the fast-degradable [*S,S*]-EDDS. Cu, Pb and Zn uptake in pot-cultivated *Brassica carinata* A. Braun, residual substrate metal bioavailability and leaching were investigated after one cycle of EDDS-assisted phytoextraction in mixed metal-contaminated pyrite waste, which is characterised by high Fe content. The chelator was supplied at doses of 2.5 and 5 mmol EDDS kg$^{(-1)}$ waste 1 week before harvest and 1 mmol EDDS kg$^{(-1)}$ waste repeated five times at 5- and 10-day intervals during the growing cycle. Here we demonstrate that EDDS generally increases shoot metal concentrations-especially of Cu-but only seldom improves removals because of markedly impaired growth. Considerable phytotoxicity and Cu leaching occurred under repeated EDDS treatments, although environmental risks may also arise from the single, close-to-harvest applications as Cu bioavailability in waste at plant harvest still remained very high (up to +67 % at 5 mmol EDDS kg$^{(-1)}$ vs. untreated controls). The residual bioavailability of Zn and Pb was instead generally reduced, perhaps due to shifts in cation exchange, whereas Fe mobility was not apparently affected. The amount of metals removed by plants represented a small fraction of the bioavailable pool (<1 %), and mobilised metals quickly reached deep layers in the substrate. We conclude that EDDS assistance can provide only some limited opportunities for improving phytoremediation of pyrite waste, major benefits being achieved by low doses to be traditionally applied shortly before harvest, with due attention to limiting groundwater pollution.

Keywords: Assisted phytoremediation; EDDS; Ethiopian mustard; Metal leaching; Pyrite waste

Environmental Science and Pollution Research

Volume 22, Issue 4, Pages 2434-2446

Nitrate sensing by the maize root apex transition zone: A merged transcriptomic and proteomic survey

Trevisan, S.[a], *Manoli, A.*[a], *Ravazzolo, L.*[a], *Botton, A.*[a], *Pivato, M.*[b], *Masi, A.*[a], *Quaggiotti, S.*[a]

[a] Department of Agriculture, Food, Natural Resources, Animals and Environment, University of Padova, Agripolis, Viale dell'Università, 16, Legnaro (PD), Italy
[b] Proteomics Centre of Padova University, VIMM, Padova University Hospital, Via Giuseppe Orus, 2, Padova, Italy

Abstract

Nitrate is an essential nutrient for plants, and crops depend on its availability for growth and development, but its presence in agricultural soils is far from stable. In order to overcome nitrate fluctuations in soil, plants have developed adaptive mechanisms allowing them to grow despite changes in external nitrate availability. Nitrate can act as both nutrient and signal, regulating global gene expression in plants, and the root tip has been proposed as the sensory organ. A set of genome-wide studies has demonstrated several nitrate-regulated genes in the roots of many plants, although only a few studies have been carried out on distinct root zones. To unravel new details of the transcriptomic and proteomic responses to nitrate availability in a major food crop, a double untargeted approach was conducted on a transition zone-enriched root portion of maize seedlings subjected to differing nitrate supplies. The results highlighted a complex transcriptomic and proteomic reprogramming that occurs in response to nitrate, emphasizing the role of this root zone in sensing and transducing nitrate signal. Our findings indicated a relationship of nitrate with biosynthesis and signalling of several phytohormones, such as auxin, strigolactones, and brassinosteroids. Moreover, the already hypothesized involvement of nitric oxide in the early response to nitrate was confirmed with the use of nitric oxide inhibitors. Our results also suggested that cytoskeleton activation and cell wall modification occurred in response to nitrate provision in the transition zone.

Keywords: iTRAQ; Nitrate; Nitric oxide; RNA-Seq;Rroot transition zone; *Zea mays* L.

Journal of Experimental Botany

Volume 66, Issue 13, Pages 3699-3715

Protocol: An improved and universal procedure for whole-mount immunolocalization in plants

Pasternak, T.[a], Tietz, O.[a], Rapp, K.[a], Begheldo, M.[d], Nitschke, R.[b], Ruperti, B.[d], Palme, K.[abce]

[a] University of Freiburg, Faculty of Biology, Institute of Biology II/Molecular Plant Physiology, Freiburg, Germany
[b] University of Freiburg, BIOSS Centre for Biological Signaling Studies, Freiburg, Germany
[c] University of Freiburg, Freiburg Institute for Advanced Studies (FRIAS), Freiburg, Germany
[d] University of Padova, Agripolis, Department of Agronomy, Food, Natural Resources, Animals and Environment, DAFNAE, Viale dell'Università, Legnaro, Padova, Italy
[e] University of Freiburg, Center for Biological Systems Analysis, Freiburg, Germany

Abstract

Rapid advances in microscopy have boosted research on cell biology. However sample preparation enabling excellent reproducible tissue preservation and cell labeling for in depth microscopic analysis of inner cell layers, tissues and organs still represents a major challenge for immunolocalization studies. Here we describe a protocol for whole-mount immunolocalization of proteins which is applicable to a wide range of plant species. The protocol is improved and robust for optimal sample fixation, tissue clearing and multi-protein staining procedures and can be used in combination with simultaneous detection of specific sequences of nucleic acids. In addition, cell wall and nucleus labelling can be implemented in the protocol, thereby allowing a detailed analysis of morphology and gene expression patterns with single-cell resolution. Besides enabling accurate, high resolution and reproducible protein detection in expression and localization studies, the procedure takes a single working day to complete without the need for robotic equipment.

Keywords: 3D reconstruction; Immunolocalization; Protein-protein interaction; Tissue multi-protein expression; Whole-mount

Plant Methods

Volume 11, Article number 50, Pages 1-10

Roles of ethylene production and ethylene receptor expression in regulating apple fruitlet abscission

Eccher, G.[a], Begheldo, M.[a], Boschetti, A.[b], Ruperti, B.[a], Botton, A.[a]

[a] Department of Agronomy, Food, Natural Resources, Animals, and Environment, University of Padova Agripolis, Legnaro, Italy
[b] Nanoscience Research Unit, Bruno Kessler Foundation, National Research Council-Institute of Materials for Electronics and Magnetism, Trento, Italy

Abstract

Apple (*Malus x domestica*) is increasingly being considered an interesting moadel species for studying early fruit development, during which an extremely relevant phenomenon, fruitlet abscission, may occur as a response to both endogenous and/ or exogenous cues. Several studies were carried out shedding light on the main physiological and molecular events leading to the selective release of lateral fruitlets within a corymb, either occurring naturally or as a result of a thinning treatment. Several studies pointed out a clear association between a rise of ethylene biosynthetic levels in the fruitlet and its tendency to abscise. A direct mechanistic link, however, has not yet been established between this gaseous hormone and the generation of the abscission signal within the fruit. In this work, the role of ethylene during the very early stages of abscission induction was investigated in fruitlet populations with different abscission potentials due either to the natural correlative inhibitions determining the so-called physiological fruit drop or to a well-tested thinning treatment performed with the cytokinin benzyladenine. A crucial role was ascribed to the ratio between the ethylene produced by the cortex and the expression of ethylene receptor genes in the seed. This ratio would determine the final probability to abscise. A working model has been proposed consistent with the differential distribution of four receptor transcripts within the seed, which resembles a spatially progressive cell-specific immune-like mechanism evolved by apple to protect the embryo from harmful ethylene.

Plant Physiology

Volume 169, Issue 1, Pages 125-137

Sulfadiazine uptake and effects in common hazel (*Corylus avellana* L.)

Michelini, L.[a], *Meggio, F.*[a], *Reichel, R.*[b], *Thiele-Bruhn, S.*[b], *Pitacco, A.*[a], *Scattolin, L.*[c], *Montecchio, L.*[c], *Alberghini, S.*[a], *Squartini, A.*[a], *Ghisi, R.*[a]

[a] Department of Agronomy, Food, Natural Resources, Animals, Environment (DAFNAE), University of Padova, Agripolis, viale dell'Università 16, Legnaro, Padova, Italy
[b] Soil Science Department, Faculty of Regional and Environmental Sciences, University of Trier, Behringstrasse 21, Trier, Germany
[c] Department of Land, Environment, Agriculture and Forestry (TESAF), University of Padova, Agripolis, viale dell'Università 16, Legnaro, Padova, Italy

Abstract

Soil contamination by antibiotics is a possible consequence of animal husbandry waste, sewage sludge, and reclaimed water spreading in agriculture. In this study, 1-year-old hazel plants (*Corylus avellana* L.) were grown in pots for 64 days in soil spiked with sulfadiazine (SDZ) in the range 0.01-100 mg kg($^{-1}$) soil. Leaf gas exchanges, fluorescence parameters and plant growth were measured regularly during the experiment, whereas plant biomass, sulfonamide concentrations in soil and plant tissues, and the quantitative variation of culturable bacterial endophytes in leaf petiole were analyzed at the end of the trial. During the experiment, photosynthesis and leaf transpiration as well as fluorescence parameters were progressively reduced by the antibiotic. Effects were more evident for leaf transpiration and for the highest SDZ spiking concentrations, whereas growth analyses did not reveal negative effects of the antibiotic. At the end of the trial, a high number of culturable endophytic bacteria in the leaf petiole of plants treated with 0.1 and 0.01 mg kg($^{-1}$) were observed, and SDZ was extractable from soil and plant roots for spiking concentrations ≥1 mg kg($^{-1}$). Inside plants, the antibiotic was mainly stored at the root level with bioconcentration factors increasing with the spiking dose, and the hydroxylated derivate 4-OH-SDZ was the only metabolite detected. Overall results show that 1-year-old hazel plants can contribute to the reduction of sulfonamide concentrations in the environment, however, sensitive reactions to SDZ can be expected at the highest contamination levels.

Keywords: Antibiotics; Endophytes; Photosynthesis; Phytoremediation; Riparian buffer strips; Sulfonamides

Environmental Science and Pollution Research

Volume 22, Issue 17, Pages 13362-13371

The dynamic regulation of microRNAs circuits in plant adaptation to abiotic stresses: A survey on molecular, physiological and methodological aspects

Begheldo, M.[a], Nonis, A.[b] , Trevisan, S.[a], Ruperti, B.[a], Quaggiotti, S.[a]

[a] Department of Agriculture, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padua, Agripolis, Viale dell'Università, 16, Legnaro, PD, Italy;
[b] Viticultural Research Center (CRA-VIT), Agricultural Research Council (CRA), Via XXVIII Aprile 26, Conegliano, TV, Italy

Abstract

Being sessile organisms, plants continuously face a complex array of environmental stresses and have naturally evolved multifaceted but well-coordinated adaptive responses to cope with them. Recent studies in various plant species and varieties have established microRNAs (miRNAs) as key elements in the post-transcriptional regulation of response to abiotic stresses. However, despite their critical role has been widely recognized, the joint action of miRNAs with the main pathways of response is still far from being completely clarified. Moreover, little is still known on the control of the biogenesis and maturation of stress responsive miRNAs.Here we give an overview on the involvement of microRNAs in plant response to nitrogen and phosphorous deprivation and on their less characterized role in the plant response to hypoxia and cold. Nitrogen and phosphorous are the most limiting nutrients for agricultural production and the plant responsiveness to their deficiency is significantly regulated at the transcriptional and post-transcriptional level. We reported the different miRNAs families involved, which sometimes are also common to different species and other nutrient or abiotic stresses. Indeed we reported that some miRNAs families involved in N and P deprivation are also acting in hypoxia and cold stresses. The results of the few existing studies on the miRNAs involvement on low oxygen and cold conditions are also described.Furthermore, we reviewed recent progress on methods for microRNAs localization/quantification and de novo identification, by exploring the main tools available and used.

Keywords: Cold tolerance; Hypoxia; *In situ* hybridization (*ISH*); MiRNA; NGS (next-generation sequencing); Nitrogen and phosphorus signalling

Environmental and Experimental Botany

Volume 114, Pages 65-79

The *KNOTTED*-like genes of peach (*Prunus persica* L. Batsch) are differentially expressed during drupe growth and the class 1 *KNOPE1* contributes to mesocarp development

Testone, G.[a]*, Condello, E.*[b]*, Di Giacomo, E.*[a]*, Nicolodi, C.*[a]*, Caboni, E.*[b]*,*
Rasori, A.[c]*, Bonghi, C.*[c]*, Bruno, L.*[d]*, Bitonti, M.B.*[d]*, Giannino, D.*[a]

[a] Institute of Agricultural Biology and Biotechnology, National Research Council of Italy (CNR), via Salaria km 29, 300, Monterotondo Scalo, Rome, Italy
[b] Fruit Tree Research Centre, Agriculture Research Council (CRA), Via di Fioranello 52, Rome,Italy
[c] Department of Agronomy, Food, Natural Resources, Animal and Environment (DAFNAE), University of Padova, Viale dell'Università, 16, Legnaro, Padova, Italy
[d] Department of Ecology, University of Calabria, Ponte Bucci, Arcavacata di Rende, Cosenza, Italy

Abstract

The KNOTTED-like transcription factors (KNOX) contribute to plant organ development. The expression patterns of peach *KNOX* genes showed that the class 1 members act precociously (S1-S2 stages) and differentially during drupe growth. Specifically, the transcription of *KNOPE1* and *6* decreased from early (cell division) to late (cell expansion) S1 sub-stages, whilst that of *STMlike1*, *2*, *KNOPE2*, *2.1* ceased at early S1. The *KNOPE1* role in mesocarp was further addressed by studying the mRNA localization in the pulp cells and vascular net at early and late S1. The message signal was first diffuse in parenchymatous cells and then confined to hypodermal cell layers, showing that the gene down-tuning accompanied cell expansion. As for bundles, the mRNA mainly featured in the procambium/phloem of collateral open types and subsequently in the phloem side of complex structures (converging bundles, ducts). The *KNOPE1* overexpression in Arabidopsis caused fruit shortening, decrease of mesocarp cell size, diminution of vascular lignification together with the repression of the major gibberellin synthesis genes *AtGA20ox1* and *AtGA3ox1*. Negative correlation between the expression of *KNOPE1* and *PpGA3ox1* was observed in four cultivars at S1, suggesting that the *KNOPE1* repression of *PpGA3ox1* may regulate mesocarp differentiation by acting on gibberellin homeostasis.

Keywords: Class 1 *KNOPE1*; Drupe development; Gibberellin synthesis genes; KNOX transcription factors; Mesocarp development; Peach

Plant Science

Volume 237, Issue , Pages 69-79

Time course of biochemical, physiological, and molecular responses to field-mimicked conditions of drought, salinity, and recovery in two maize lines

Morari, F., Meggio, F., Lunardon, A., Scudiero, E., Forestan, C., Farinati, S., Varotto, S.

Department of Agronomy, Animals, Food, Natural Resources and Environment,
University of Padova Agripolis Viale dell'Università, Padova, Italy

Abstract

Drought and salinity stresses will have a high impact on future crop productivity, due to climate change and the increased competition for land, water, and energy. The response to drought (WS), salinity (SS), and the combined stresses (WS+SS) was monitored in two maize lines: the inbred B73 and an F1 commercial stress-tolerant hybrid. A protocol mimicking field progressive stress conditions was developed and its effect on plant growth analyzed at different time points. The results indicated that the stresses limited growth in the hybrid and arrested it in the inbred line. In SS, the two genotypes had different ion accumulation and translocation capacity, particularly for Na^+ and Cl. Moreover, the hybrid perceived the stress, reduced all the analyzed physiological parameters, and kept them reduced until the recovery. B73 decreased all physiological parameters more gradually, being affected mainly by SS. Both lines recovered better from WS than the other stresses. Molecular analysis revealed a diverse modulation of some stress markers in the two genotypes, reflecting their different response to stresses. Combining biochemical and physiological data with expression analyses yielded insight into the mechanisms regulating the different stress tolerance of the two lines.

Keywords: Abiotic stress; Drought; Maize; Salinity; Stress marker genes; Stress response; Stress tolerance

Frontiers in Plant Science

Volume 6, Issue MAY, Pages 1-15

Beneficial bacteria isolated from grapevine inner tissues shape *Arabidopsis Thaliana* roots

Baldan, E.[a]*, Nigris, S.*[a]*, Romualdi, C.*[a]*, D'Alessandro, S.*[a]*, Clocchiatti, A.*[a]*,*
Zottini, M.[a]*, Stevanato, P.*[b]*, Squartini, A.*[b]*, Baldan, B.*[a]

[a] Dipartimento di Biologia, Universita Degli Studi di Padova, Padova, Italy
[b] Dipartimento DAFNAE, Department of Agronomy Food Natural Resources Animals and Environment, Legnaro (PD), Italy

Abstract

We investigated the potential plant growth-promoting traits of 377 culturable endophytic bacteria, isolated from *Vitis vinifera* cv. Glera, as good biofertilizer candidates in vineyard management. Endophyte ability in promoting plant growth was assessed in vitro by testing ammonia production, phosphate solubilization, indole-3-acetic acid (IAA) and IAA-like molecule biosynthesis, siderophore and lytic enzyme secretion. Many of the isolates were able to mobilize phosphate (33%), release ammonium (39%), secrete siderophores (38%) and a limited part of them synthetized IAA and IAA-like molecules (5%). Effects of each of the 377 grapevine beneficial bacteria on Arabidopsis thaliana root development were also analyzed to discern plant growth-promoting abilities (PGP) of the different strains, that often exhibit more than one PGP trait. A supervised model-based clustering analysis highlighted six different classes of PGP effects on root architecture. *A. thaliana* DR5:GUS plantlets, inoculated with IAA-producing endophytes, resulted in altered root growth and enhanced auxin response. Overall, the results indicate that the Glera PGP endospheric culturable microbiome could contribute, by structural root changes, to obtain water and nutrients increasing plant adaptation and survival. From the complete cultivable collection, twelve promising endophytes mainly belonging to the *Bacillus* but also to *Micrococcus* and *Pantoea* genera, were selected for further investigations in the grapevine host plants towards future application in sustainable management of vineyards.

PLoS ONE

Volume 10, Issue 10, e0140252

Effect of sulfate availability on root traits and microRNA395 expression in sugar beet

Stevanato, P., Fedito, P., Trebbi, D., Cagnin, M., Saccomani, M., Cacco, G.

DAFNAE, Dipartimento di Agronomia Animali Alimenti Risorse Naturali e Ambiente,
Università degli Studi di Padova, Viale dell'Università 16, Legnaro, Italy

Abstract

Nutritional stress is one of the main limits to sugar beet yield. This study evaluated morphological and molecular responses of sugar beet to changes in sulfate availability. Morphological characteristics of the root system and the accumulation of microRNA395 (miR395) were examined in sulfate(S)-supplemented and S-deprived seedlings under hydroponic conditions. We also investigated the functional role of miR395 in regulating the expression of APS1 gene coding for ATP-sulfurylase in roots and leaves. The S-deprived seedlings showed a significant increase in the number of root tips, in the miR395 expression in leaves but not in roots, and in the expression of APS1 gene. Our results indicate that miR395 may be a useful biomarker for sulfate status in sugar beet.

Keywords: ATP-sulfurylase; Beta vulgaris; Root morphology

Biologia Plantarum

Volume 59, Issue 3, Pages 491-496

Effects of phosphate and thiosulphate on arsenic accumulation in the species *Brassica juncea*

Grifoni, M.[b]*, Schiavon, M.*[a]*, Pezzarossa, B.*[b]*, Petruzzelli, G.*[b]*, Malagoli, M.*[a]

[a] Department of Agronomy, Food, Natural resources, Animal and Environment,
University of Padova, Agripolis—Viale dell'Università, Legnaro, PD, Italy
[b] CNR, Institute of Ecosystem Study—Via Moruzzi, Pisa, Italy

Abstract

Arsenic (As) is recognized as a toxic pollutant in soils of many countries. Since phosphorus (P) and sulphur (S) can influence arsenic mobility and bioavailability, as well as the plant tolerance to As, phytoremediation techniques employed to clean-up As-contaminated areas should consider the interaction between As and these two nutrients. In this study, the bioavailability and accumulation of arsenate in the species *Brassica juncea* were compared between soil system and hydroponics in relation to P and S concentration of the growth substrate. In one case, plants were grown in pots filled with soil containing 878 mg As kg^{-1}. The addition of P to soil resulted in increased As desorption and significantly higher As accumulation in plants, with no effect on growth. The absence of toxic effects on plants was likely due to high S in soil, which could efficiently mitigate metal toxicity. In the hydroponic experiment, plants were grown with different combinations of As (0 or 100 μM) and P (56 or 112 μM). S at 400 μM was also added to the nutrient solution of control (As) and As-treated plants, either individually or in combination with P. The addition of P reduced As uptake by plants, while high S resulted in higher As accumulation and lower P content. These results suggest that S can influence the interaction between P and As for the uptake by plants. The combined increase of P and S in the nutrient solution did not lead to higher accumulation of As, but enhanced As translocation from the root to the shoot. This aspect is of relevance for the phytoremediation of As-contaminated sites.

Keywords: Arsenic; Hydroponics; Phosphorus; Phytoextraction; Pot experiment; Sulfur

Environmental Science and Pollution Research

Volume 22, Issue 4, Pages 2423-2433

Effects of selenium biofortification on crop nutritional quality

Malagoli, M.[a], Schiavon, M.[a], Dall'Acqua, S.[b], Pilon-Smits, E.A.H.[c]

[a] Department of Agronomy, Food, Natural Resources, Animals and the Environment, University of Padova, Padova, Italy
[b] Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Padova, Italy
[c] Department of Biology, Colorado State University, Fort Collins, CO, United States

Abstract

Selenium (Se) at very low doses has crucial functions in humans and animals. Since plants represent the main dietary source of this element, Se-containing crops may be used as a means to deliver Se to consumers (biofortification). Several strategies have been exploited to increase plant Se content. Selenium assimilation in plants affects both sulfur (S) and nitrogen (N) metabolic pathways, which is why recent research has also focused on the effect of Se fertilization on the production of S- and N- secondary metabolites with putative health benefits. In this review we discuss the function of Se in plant and human nutrition and the progress in the genetic engineering of Se metabolism to increase the levels and bioavailability of this element in food crops. Particular attention is paid to Se biofortification and the synthesis of compounds with beneficial effects on health.

Keywords: Food; Nutritional quality; Plant biofortification; Secondary metabolites; Selenium

Frontiers in Plant Science

Volume 6, Issue APR, Pages 1-5

Exploring the importance of sulfate transporters and ATP sulphurylases for selenium hyperaccumulation — a comparison of *Stanleys pinnata* and *Brassica juncea* (*Brassicaceae*)

Schiavon, M.[a], Pilon, M.[b], Malagoli, M.[a], Pilon-Smits, E.A.H.[b]

[a] Department of Agronomy, Food, Natural Resources, Animals and the Environment, University of Padova, Legnaro, Padova, Italy
[b] Department of Biology, Colorado State University, Fort Collins, CO, United States

Abstract

Selenium (Se) hyperaccumulation, the capacity of some species to concentrate Se to levels upwards of 0.1% of dry weight, is an intriguing phenomenon that is only partially understood. Questions that remain to be answered are: do hyperaccumulators have one or more Se-specific transporters? How are these regulated by Se and sulfur (S)? In this study, hyperaccumulator *Stanleys pinnata* was compared with related non-hyperaccumulator *Brassica juncea* with respect to S-dependent selenate uptake and translocation, as well as for the expression levels of three sulfate/selenate transporters (*Sultr*) and three ATP sulphurylases (*APS*). Selenium accumulation went down ~10-fold with increasing sulfate supply in *B. juncea*, while *S. pinnata* only had a 2-3-fold difference in Se uptake between the highest (5mM) and lowest sulfate (0mM) treatments. The Se/S ratio was generally higher in the hyperaccumulator than the non-hyperaccumulator, and while tissue Se/S ratio in *B. juncea* largely reflected the ratio in the growth medium, S. pinnata enriched itself up to 5-fold with Se relative to S. The transcript levels of *Sultr1;2* and *2;1* and *APS1*, *2*, and *4* were generally much higher in *S. pinnata* than *B. juncea*, and the species showed differential transcript responses to S and Se supply. These results indicate that *S. pinnata* has at least one transporter with significant selenate specificity over sulfate. Also, the hyperaccumulator has elevated expression levels of several sulfate/selenate transporters and APS enzymes, which likely contribute to the Se hyperaccumulation and hypertolerance phenotype.

Keywords: ATP-sulphurylase; *Brassica juncea*; Gene expression; Selenium; *Stanleya pinnata*; Sulfur; Uptake

Frontiers in Plant Science

Volume 6, Issue JAN, Pages 1-13

IBAF - CNR

On-farm evaluation of integrated weed management tools for maize production in three different agro-environments in Europe: Agronomic efficacy, herbicide use reduction, and economic sustainability

Vasileiadis, V.P.[a], Otto, S.[a], van Dijk, W.[b], Urek, G.[c], Leskovšek, R.[c],

Verschwele, A.[d], Furlan, L.[e], Sattin, M.[a]
[a] National Research Council (CNR), Institute of Agro-Environmental and Forest Biology, Legnaro, PD, Italy
[b] Applied Plant Research, Wageningen University, Lelystad, Netherlands
[c] Agricultural Institute of Slovenia, Ljubljana, Slovenia
[d] Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Braunschweig, Germany
[e] Veneto Agricoltura, Legnaro, PD, Italy

Abstract

The development and implementation of integrated weed management (IWM) strategies that provide good weed control while reducing dependence on herbicides, and preferably without having side effects on the overall system economic performance, is still a challenge that has to be met. In 2011 and 2012, nine on-farm experiments (i.e., real field conditions on commercial farms, with natural weed flora) were conducted in three important European maize producing regions-countries, which represent the range of climatic and edaphic conditions in Europe, to evaluate the efficacy of different locally selected IWM tools for direct weed control in maize vs. the conventional approach (CON) followed by the farms. The IWM tools tested were: (1) early post-emergence herbicide band application combined with hoeing followed by a second hoeing in Southern Germany, (2) early post-emergence herbicide broadcast application when indicated by a predictive model of weed emergence after performing one scouting in the field to supply data for the model, followed by hoeing in Northern Italy, and (3) tine harrowing at 2-3rd leaf stage of maize and low dose of post-emergence herbicide in Slovenia. Results showed that the IWM tools tested in the different countries: (1) provided sufficient weed control without any significant differences in yields, (2) greatly reduced maize reliance on herbicides, and (3) IWM implementation was economically sustainable as no significant differences in gross margin were observed in any country compared to CON.

Keywords: Corn; Economic sustainability; Integrated pest management; Pesticide risk reduction

European Journal of Agronomy

Volume 63, Pages 71-78

Protocols for robust herbicide resistance testing in different weed species

Panozzo, S., Scarabel, L., Collavo, A., Sattin, M.

Institute of Agro-environmental and Forest Biology (IBAF), National Research Council (CNR), Italy

Abstract

Robust protocols to test putative herbicide resistant weed populations at whole plant level are essential to confirm the resistance status. The presented protocols, based on whole-plant bioassays performed in a greenhouse, can be readily adapted to a wide range of weed species and herbicides through appropriate variants. Seed samples from plants that survived a field herbicide treatment are collected and stored dry at low temperature until used. Germination methods differ according to weed species and seed dormancy type. Seedlings at similar growth stage are transplanted and maintained in the greenhouse under appropriate conditions until plants have reached the right growth stage for herbicide treatment. Accuracy is required to prepare the herbicide solution to avoid unverifiable mistakes. Other critical steps such as the application volume and spray speed are also evaluated. The advantages of this protocol, compared to others based on whole plant bioassays using one herbicide dose, are related to the higher reliability and the possibility of inferring the resistance level. Quicker and less expensive in vivo or in vitro diagnostic screening tests have been proposed (Petri dish bioassays, spectrophotometric tests), but they provide only qualitative information and their widespread use is hindered by the laborious set-up that some species may require. For routine resistance testing, the proposed whole plant bioassay can be applied at only one herbicide dose, so reducing the costs.

Keywords: Environmental sciences; Herbicide efficacy; Herbicide treatment; Monitoring; Resistance level; Resistant biotypes; Seed germination; Weed control; Weed science

Journal of Visualized Experiments

Volume 2015, Issue 101, Pages 1-10

IMAR: An interactive web-based application for mapping herbicide resistant weeds

Panozzo, S.[a], Colauzzi, M.[b], Scarabel, L.[a], Collavo, A.[a], Rosan, V.[a], Sattin, M.[a]

[a] National Research Council (CNR), Institute of Agro-environmental and Forest Biology (IBAF), Legnaro (PD), Italy
[b] Free-lance webmaster, Padova, Italy

Abstract

Herbicides are the major weed control tool in most cropping systems worldwide. However, the high reliance on herbicides has led to environmental issues as well as to the evolution of herbicide-resistant biotypes. Resistance is a major concern in modern agriculture and early detection of resistant biotypes is therefore crucial for its management and prevention. In this context, a timely update of resistance biotypes distribution is fundamental to devise and implement efficient resistance management strategies. Here we present an innovative webbased application called iMAR (interactive MApping of Resistance) for the mapping of herbicide resistant biotypes. It is based on open source software tools and translates into maps the data reported in the GIRE (Italian herbicide resistance working group) database of herbicide resistance at national level. iMAR allows an automatic, easy and cost-effective updating of the maps a nd provides two different systems, "static" and "dynamic". In the first one, the user choices are guided by a hierarchical tree menu, whereas the latter is more flexible and includes a multiple choice criteria (type of resistance, weed species, region, cropping systems) that permits customized maps to be created. The generated information can be useful to various stakeholders who are involved in weed resistance management: farmers, advisors, national and local decision makers as well as the agrochemical industry. iMAR is freely available, and the system has the potential to handle large datasets and to be used for other purposes with geographical implications, such as the mapping of invasive plants or pests.

Keywords: Biotype; Computer interface; Computer program; Cost effectiveness analysis; Data base; Geographic mapping; Herbicide resistance; Information dissemination; Internet; Nonhuman; Weed

PLoS ONE

Volume 10, Issue 8, Article number e0135328

Eight principles of integrated pest management

Barzman, M.[a], Bàrberi, P.[b], Birch, A.N.E.[c], Boonekamp, P.[d], Dachbrodt-Saaydeh, S.[e], Graf, B.[f], Hommel, B.[g], Jensen, J.E.[h], Kiss, J.[i], Kudsk, P.[j], Lamichhane, J.R.[a], Messéan, A.[a], Moonen, A.C.[b], Ratnadass, A.[k], Ricci, P.[l], Sarah, J.L.[m], Sattin, M.[n]

[a] INRA, UAR1240 ECO-INNOV, Avenue Lucien Brétignières, Thiverval Grignon, France
[b] Scuola Superiore Sant'Anna, Institute of Life Sciences, Land Lab, Via Santa Cecilia 3, Pisa, Italy
[c] The James Hutton Institute, Invergowrie, Dundee, Scotland, United Kingdom
[d] Plant Research International, Wageningen University, Postbus 69, Wageningen, Netherlands
[e] Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Stahnsdorfer Damm 81, Kleinmachnow, Germany
[f] Agroscope Changins-Wädenswil ACW, Plant Protection and Fruit and Vegetable Extension, Schloss 1, Wädenswil, Switzerland
[g] Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Institute for Ecological Chemistry, Königin-Luise-Str. 19, Berlin, Germany
[h] SEGES, 15 Agro Food Park, Skejby, Aarhus N, Denmark
[i] Plant Protection Institute, Szent Istvan University, Pater K. street 1, Gödöllö, Hungary
[j] Department of Agro-ecology, Aarhus University, Forsoegsvej 1, Flakkebjerg, Slagelse, Denmark
[k] CIRAD, Unité de recherche HortSys, Campus international de Baillarguet, Montpellier Cedex 5, France
[l] INRA, UMR1355 ISA, Sophia-Antipolis, France
[m] CIRAD, Direction Générale Déléguée à la Recherche et à la Stratégie, TA 179/04 Avenue Agropolis, Montpellier Cedex 5, France
[n] National Research Council, Institute of agro-environmental and forest biology, Agripolis, Legnaro (Padova), Italy

Abstract

The use of pesticides made it possible to increase yields, simplify cropping systems, and forego more complicated crop protection strategies. Over-reliance on chemical control, however, is associated with contamination of ecosystems and undesirable health effects. The future of crop production is now also threatened by emergence of pest resistance and declining availability of active substances. There is therefore a need to design cropping systems less dependent on synthetic pesticides. Consequently, the European Union requires the application of eight principles (P) of Integrated Pest Management that fit within sustainable farm management. Here, we propose to farmers, advisors, and researchers a dynamic and flexible approach that accounts for the diversity of farming situations and the complexities of agroecosystems and that can improve the resilience of cropping systems and our capacity to adapt crop protection to local realities. For each principle (P), we suggest that (P1) the design of inherently robust cropping systems using a combination of agronomic levers is key to prevention. (P2) Local availability of monitoring, warning, and forecasting systems is a reality to contend with. (P3) The decision-making process can integrate cropping system factors to develop longer-term strategies. (P4) The combination of non-chemical methods that may be individually less efficient than pesticides can generate valuable synergies. (P5) Development of new biological agents and products and the use of existing databases offer options for the selection of products minimizing impact on health, the environment, and biological regulation of pests. (P6) Reduced pesticide use can be effectively combined with other tactics. (P7) Addressing the root causes of pesticide resistance is the best way to find sustainable crop protection solutions. And (P8) integration of multi-season effects and trade-offs in evaluation criteria will help develop sustainable solutions.

Keywords: Alternatives; Europe; Integrated pest management; Pesticides; Resilient cropping system; Sustainable agriculture; Systems approach

Agronomy for Sustainable Development

Volume 35, Issue 4, Pages 1199-1215

Combining a weed traits database with a population dynamics model predicts shifts in weed communities

Storkey, J.[a], Holst, N.[b], Bøjer, O.Q.[b], Bigongiali, F.[c], Bocci, G.[c], Colbach, N.[d], Dorner, Z.[e], Riemens, M.M.[f], Sartorato, I.[g], Sønderskov, M.[b], Verschwele, A.[h]

[a] Agroecology Department, Rothamsted Research, Harpenden, United Kingdom
[b] Department of Agroecology, Aarhus University, Slagelse, Denmark
[c] Istituto di Scienze della Vita, Scuola Superiore Sant'Anna - Piazza Martiri della Libertà, Pisa, Italy
[d] INRA, UMR1347 Agroécologie, Dijon, France
[e] Institute of Plant Protection, Faculty of Agriculture and Environmental Science, Szent Istvan University, Godollo, Hungary
[f] Wageningen University and Research Centre, Agrosystems Research, Wageningen, Netherlands
[g] Istituto di Biologia Agroambientale and Forestale, CNR, Padua, Italy
[h] Federal Research Centre for Cultivated Plants, Julius Kuhn Institute, Braunschweig, Germany

Abstract

A functional approach to predicting shifts in weed floras in response to management or environmental change requires the combination of data on weed traits with analytical frameworks that capture the filtering effect of selection pressures on traits. A weed traits database (WTDB) was designed, populated and analysed, initially using data for 19 common European weeds, to begin to consolidate trait data in a single repository. The initial choice of traits was driven by the requirements of empirical models of weed population dynamics to identify correlations between traits and model parameters. These relationships were used to build a generic model, operating at the level of functional traits, to simulate the impact of increasing herbicide and fertiliser use on virtual weeds along gradients of seed weight and maximum height. The model generated 'fitness contours' (defined as population growth rates) within this trait space in different scenarios, onto which two sets of weed species, defined as common or declining in the UK, were mapped. The effect of increasing inputs on the weed flora was successfully simulated; 77% of common species were predicted to have stable or increasing populations under high fertiliser and herbicide use, in contrast with only 29% of the species that have declined. Future development of the WTDB will aim to increase the number of species covered, incorporate a wider range of traits and analyse intraspecific variability under contrasting management and environments.

Keywords: Agricultural intensification; Community assembly theory; Demographic model; Functional ecology; Weed management

Weed Research

Volume 55, Issue 2, Pages 206-218

Occurrence, genetic control and evolution of non-target-site based resistance to herbicides inhibiting acetolactate synthase (ALS) in the dicot weed *Papaver rhoeas*

Scarabel, L.[a], *Pernin, F.*[b], *Délye, C.*[b]

[a] Institute of Agro-environmental and Forest Biology (IBAF), CNR, AGRIPOLIS, Viale dell'Università 16, Legnaro (PD), Italy
[b] INRA, UMR1347 Agroécologie, 17 rue de Sully, Dijon, France

Abstract

Non-target-site resistance (NTSR) to herbicides is a major issue for the chemical control of weeds. Whilst predominant in grass weeds, NTSR remains largely uninvestigated in dicot weeds. We investigated the occurrence, inheritance and genetic control of NTSR to acetolactate synthase (ALS) inhibitors in *Papaver rhoeas* (corn poppy) using progenies from plants with potential NTSR to the imidazolinone herbicide imazamox. NTSR to imazamox was inherited from parents over two successive generations. NTSR to tritosulfuron (a sulfonylurea) was observed in F1 generations and inherited in F2 generations. NTSR to florasulam (a triazolopyrimidine) emerged in F2 generations. Our findings suggest NTSR was polygenic and gradually built-up by accumulation over generations of loci with moderate individual effects in single plants. We also demonstrated that ALS alleles conferring herbicide resistance can co-exist with NTSR loci in *P. rhoeas* plants. Previous research focussed on TSR in *P. rhoeas*, which most likely caused underestimation of NTSR significance in this species. This may also apply to other dicot species. From our data, resistance to ALS inhibitors in *P. rhoeas* appears complex, and involves well-known mutant ALS alleles and a set of unknown NTSR loci that confer resistance to ALS inhibitors from different chemical families.

Keywords: Acetolactate synthase (ALS); Corn poppy (*P. rhoeas*); Evolution; Herbicide; Non-target-site-based resistance (NTSR); Polygenic control

Plant Science

Volume 238, Pages 158-169

DAFNAE

Department of Agronomy Food
Natural resources Animals Environment

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This scientific report gives an overview of the research activity carried on during the fourth year of DAFNAE history, and of the wide and qualified collaboration with important research centers around the world in sectors as agronomy, food, natural resources, animals and environment.
DAFNAE was inaugurated on 1st January 2012, following the re-organization of the Italian University system, in accordance with Italian law 240/2010.

This third research yearbook of DAFNAE collects the abstracts of the papers published during the year 2015, on the ISI and Scopus databases by grouping them in the seven following different thematic areas:
Agronomy and soil science
Animal science
Ecology and environmental science
Entomology
Food science and technology
Genetics and genomics
Microbiology
Plant science
It offers an overview of DAFNAE research activity, that is mainly focused on the production and dissemination of basic and applied knowledge, to promote the quality of human life, the competitiveness of the agri-food sector, and the sustainable use of biotic and abiotic natural resources.

Prof. Maurizio Borin, head of DAFNAE, Department of Agronomy, Food, Natural resources, Animals and Environment, promoted the idea of publishing the DAFNAE annual yearbook as a tool to show and disseminate the level of the research activities of the Department.
All the researchers of DAFNAE contributed to this third edition.

ISBN 978-88-6938-074-7

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€ 15,00