

This volume of the Topics in Canadian Weed Science series is the result of a symposium that was held during the 2010 Annual Meeting of the Canadian Weed Science Society - Société canadienne de malherbologie in Regina, Saskatchewan, on November 16, 2010.

New crops such as camelina [*Camelina sativa* (L.) Crantz] and Ethiopian mustard (*Brassica carinata* A. Braun) are being considered for new uses, for example, industrial or biorefinery platforms. Other crops are being considered across North America for lignocellulose-based energy/biofuel feedstocks, such as switchgrass (*Panicum virgatum* L.), a C₄ native rhizomatous perennial grass, or intermediate wheatgrass [*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey], a C₃ nonnative rhizomatous perennial grass.

In addition to new crops, crops with second-generation traits (CNTs) – traits such as enhanced nutrient quality, bioproducts and pharmaceuticals, biomass/biofuels, altered plant morphology/physiology, biotic stress tolerance, and abiotic stress tolerance – are currently being tested in confined field trials around the world.

Some intentionally-introduced species became invasive in Canada. While most crops become volunteer weeds in subsequent crops, some have escaped and established in ruderal areas, and a few have invaded and transformed native landscapes. While invasive crops are rare, they are economically and environmentally costly. Dispersal for large-scale planting, agronomic nurturing, and seed dispersal (within fields at harvest and widely by the seed transport system) may shorten the lag period for invasion and reduce the opportunity for irradiation of weedy species. It is incumbent on the weed science community to engage with the crops industry and regulators to test new crops and second-generation CNTs under field conditions prior to release and to develop effective methods to monitor and mitigate following release.



Canadian Weed Science Society
Société canadienne de malherbologie
P.O. Box 674
Pinawa, Manitoba R0E 1L0
Canada



9 780968 897096

Topics in Canadian Weed Science

Volume 9

New Crops and Crops with Second-Generation Traits: Weed Management Challenges



Edited by
 Hugh J. Beckie and Linda M. Hall

Canadian Weed Science Society
 Société canadienne de malherbologie



New Crops and Crops with Second-Generation Traits: Weed Management Challenges

Edited by

Hugh J. Beckie¹ and Linda M. Hall²

¹ *Agriculture and Agri-Food Canada, Saskatoon Research Centre,
107 Science Place, Saskatoon, Saskatchewan*

² *Department of Agricultural, Food and Nutritional Science,
4-10 Agriculture/Forestry Center, University of Alberta, Edmonton,
Alberta*

Canadian Weed Science Society – Société canadienne de malherbologie
Pinawa, Manitoba, Canada

Disclaimer

All rights reserved. No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopy, recording, or any other information storage or retrieval system, without the written permission of the publisher.

This publication is designed to provide accurate and authoritative information. It is sold or distributed with the understanding that the publisher is not engaged in rendering professional services. If advice or expert assistance is required, the services of a competent professional person should be sought. Neither the publisher nor authors are responsible for errors or omissions. It remains the responsibility of the readers to follow product information contained on the product label. While every effort has been made to ensure accuracy, CWSS-SCM, its executive, committee members and contractors do not accept responsibility for any publication errors or any consequences resulting from the use of this publication.

To order copies of this publication, please contact our office or visit our web site at: www.cwss-scm.ca

© Copyright 2011
(ISBN 978-0-9688970-9-6)
Canadian Weed Science Society
Société canadienne de malherbologie
(CWSS-SCM)
P.O. Box 674
Pinawa (Manitoba) R0E 1L0

Telephone: + 1 204 753-2915
E-mail: assistant@cwss-scm.ca

Fax: + 1 204 753-2363

Citation for Volume:

Beckie, H. J. and Hall, L. M. eds. 2011. New Crops and Crops with Second-Generation Traits: Weed Management Challenges. Topics in Canadian Weed Science, Volume 9. Pinawa, Manitoba: Canadian Weed Science Society – Société canadienne de malherbologie. 101 pp.

Citation for Chapter:

Author(s). 2011. *Title*. Pages xx-xx in H. J. Beckie and L. M. Hall, eds. New Crops and Crops with Second-Generation Traits: Weed Management Challenges. Topics in Canadian Weed Science, Volume 9. Sainte Anne de Bellevue, Québec: Canadian Weed Science Society – Société canadienne de malherbologie.

Printed in Canada

Foreword

First-generation genetically-modified crops with novel agronomic traits have been grown in many countries since the mid-1990s. Weed scientists have contributed to resolving some of the challenges encountered with the adoption of these crops. Since that time, second-generation crops with novel traits are being evaluated in confined field trials around the world. These new traits range from enhanced nutrient quality, abiotic stress tolerances (e.g., nitrogen-use efficiency, drought, cold, salt, heat, flood), biotic stress resistances (e.g., fungal, bacterial, nematode, viral), plant morphology/physiology (e.g., altered flowering or maturity, yield increase), to bioproduct and pharmaceutical crops and biomass/biofuels. New industrial crops or bio-refinery platforms are also being developed by utilizing plants such as camelina and Ethiopian mustard. Great strides have been made towards the development of these second-generation crops with novel traits; however, the agronomic performance has yet to be evaluated to determine their success for adoption. There are several environmental and regulatory requirements and/or restrictions that need to be addressed, while other challenges also relevant to weed science include potential invasiveness of feral plants in adjacent non-cropped disturbed or natural areas and possible outcrossing to related crops or wild/weedy plant species. These have serious implications for identity preservation issues in crops and for the development of hybrid offspring that may become more weedy or invasive. The potential nontarget effects on flora and fauna diversity also need to be assessed. The Plenary Session and the resulting publications presented in this issue of *Topics in Canadian Weed Science* will provide an understanding of the role weed science must play in resolving some of the challenges facing the implementation of this technology. These include effective weed management tools and best agronomic practices that enhance crop competitiveness with weeds in various crop production systems. For second-generation crops to be successfully integrated into agriculture, collaborative multi-disciplinary research efforts are necessary for their potential to be realized in the marketplace.

Sue Boyetchko
President, 2010
CWSS-SCM

Preface

The Canadian Weed Science Society – Société canadienne de malherbologie (CWSS-SCM) is pleased to present “New crops/crops with second-generation traits: weed management challenges”, the 9th volume of Topics in Canadian Weed Science. This volume is a compilation of peer-reviewed papers that were presented during the plenary session at the 2010 CWSS-SCM annual meeting held in Regina, Saskatchewan.

Topics in Canadian Weed Science is intended to advance the knowledge of weed science and increase awareness of the consequences of weeds in agroecosystems, forestry, and natural habitats. The volumes cover a wide range of topics and provide a diverse source of information for weed science professionals and the general public.

The plenary session topics at the CWSS-SCM annual meeting are of both national and international interest, and we invite weed science professionals to attend our annual meetings. The annual meeting is usually held in late November, with locations alternating between Eastern and Western Canada. Meeting details are available on the CWSS-SCM website (www.weedscience.ca).

The CWSS-SCM Board of Directors expresses their gratitude to H. Beckie and L. Hall (editors), the Regina Local Arrangements Committee, the contributing authors, and the reviewers who have made this publication possible. Other volumes of Topics in Canadian Weed Science include:

Vol. 1: Field boundary habitats: Implications for seed, insect, and disease management.

Vol. 2: Weed management in transition;

Vol. 3: Soil residual herbicides: Science and management;

Vol. 4: The first decade of herbicide-resistant crops in Canada;

Vol. 5: Invasive plants: Inventories, strategies, and action.

Vol. 6: Physical weed control: progress and challenges.

Vol. 7: The politics of weeds.

Vol. 8: Climate change and the Canadian agricultural environment.

Most of these volumes are available for purchase and can be ordered through the CWSS-SCM website (www.weedscience.ca).

Stephen Darbyshire
Publications Director
CWSS-SCM

Acknowledgements

Cover

Top Photograph: Fall-seeded camelina in southern Saskatchewan, mid-June, 2010 (very wet spring that resulted in many unseeded or late-seeded fields) by Linda M. Hall

Bottom Photograph: Camelina large rosettes (and henbit) by Linda M. Hall

Cover Design for the Series: Ralph Underwood, Agriculture and Agri-Food Canada, Saskatoon Research Centre, Saskatoon, SK.

Reviewers

The editors would like to thank the following people for their assistance in reviewing the papers contained in this volume:

Eric Johnson, AAFC, Scott, SK
Kevin Falk, AAFC, Saskatoon, SK
Lisa Rew, Montana State University,
Bozeman, MT

Stephen Darbyshire, AAFC, Ottawa, ON
Yantai Gan, AAFC, Swift Current, SK
Christian Willenborg, University of
Saskatchewan, Saskatoon, SK

Karen Bailey, AAFC, Saskatoon, SK
Debra McLaren, AAFC, Brandon, MB
Cecil Vera, AAFC, Melfort, SK

Byron Irvine, AAFC, Brandon, MB
William May, AAFC, Indian Head, SK
Steven Shirliffe, University of
Saskatchewan, Saskatoon, SK

This volume was prepared for publication by the following persons:

Copy Editors: **Hugh J. Beckie**, Agriculture and Agri-Food Canada,
Saskatoon Research Centre, 107 Science Place, Saskatoon
and Agri-Food Canada, Saskatoon, Saskatchewan, Canada
and
Linda M. Hall, Department of Agricultural, Food and
Nutritional Science, 4-10 Agriculture/Forestry Center,
University of Alberta, Edmonton, Alberta, Canada

Production Editor: **Anita Drabyk**, Executive Assistant, Box 674, Pinawa,
Manitoba, Canada R0E 1L0

Contents

Introduction <i>Hugh J. Beckie and Linda M. Hall</i>	1
Camelina – a new industrial crop <i>Jack Grushcow</i>	5
Research perspective: a promising way forward in determining weediness and invasiveness of newly-introduced species <i>Jean H. Burns</i>	13
Agronomic challenges and opportunities for second-generation crops with novel traits and new crops <i>Eric N. Johnson, Stewart A. Brandt, Linda M. Hall and Elwin G. Smith</i>	31
Disease implications of canola-intensive crop rotations <i>H. Randy Kutcher, Christina Eynck, Bruce D. Gossen, T. Kelly Turkington, Stewart A. Brandt and Kequan Xi</i>	49
The development of new crops: case studies of niger and camelina <i>William E. May, Eric N. Johnson, Guy P. Lafond and Christopher B. Holzapfel</i>	67
Volunteer and feral crop plants and latent seed populations play an important role in novel trait containment <i>Rene C. Van Acker and Muthukumar V. Bagavathiannan</i>	83
Index	99

SYMPOSIUM

New crops and crops with second-generation traits: weed management challenges

Hugh J. Beckie¹ and Linda M. Hall²

¹*Agriculture and Agri-Food Canada, Saskatoon Research Centre, 107 Science Place, Saskatoon, SK S7N 0X2*

²*Department of Agricultural, Food and Nutritional Science, 4-10 Agriculture/Forestry Center, University of Alberta, Edmonton, AB T6G 2P5*
Email: hugh.beckie@agr.gc.ca

Introduction

New crops such as camelina [*Camelina sativa* (L.) Crantz] and Ethiopian mustard (*Brassica carinata* A. Braun) are being considered for new uses, for example, industrial or biorefinery platforms. Camelina is being examined as a source of biodegradable industrial oils and bioplastics. Ethiopian mustard is being considered as a source of oil and sinigrin for industrial applications. For the latter crop, high glucosinolate lines from a mutagenized breeding population are being examined for the development of biopesticides (e.g., nematode control) or fish feed.

Some desired characteristics of crops considered as industrial or biorefinery platforms include early maturity, disease resistance, seed shatter resistance, high seed yield, novel seed oil profiles, high glucosinolate meal (sinigrin or allyl glucosinolate), or other desirable properties for various value-added products (biopesticides, phytoremediation, biolubricants, novel feeds, bioplastics, etc.).

Other crops are being considered across North America for lignocellulose-based energy/biofuel feedstocks, such as switchgrass (*Panicum virgatum* L.), a C₄ native rhizomatous perennial grass, or intermediate wheatgrass [*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey], a C₃ non-native rhizomatous perennial grass. Desired characteristics include high harvestable biomass yields under low input conditions, e.g., low productivity/marginal lands. However, a prime potential environmental concern of these crops, including future genetically-enhanced cultivars, is

their invasiveness in ruderal (non-cropped disturbed) and natural areas, particularly if grown on millions of hectares annually.

In addition to new crops, crops with second-generation traits (CNTs) – traits such as enhanced nutrient quality, bioproducts and pharmaceuticals, biomass/biofuels, altered plant morphology/physiology, biotic stress tolerance, and abiotic stress tolerance – are currently being tested in confined field trials around the world (Warwick et al. 2009). Abiotic stress-tolerance traits include increased nitrogen-use efficiency, and cold, drought, salt, heat, flood, or general stress tolerance. All of the major world crops are candidates for one or more of these traits (Warwick et al. 2009). Similar to lignocellulose-based energy feedstocks, potentially fitness-enhancing abiotic stress-tolerance traits may impact weediness or invasiveness of some crops or cultivars.

There are weed management and other challenges posed by new crops or CNTs. Concerns that may be raised include the following: (1) weedy or weedier (e.g., competitiveness, persistence) crop volunteers in cultivated areas or potentially invasive in adjacent ruderal or natural areas; (2) potential for outcrossing to related crops (identity preservation) or related wild/weedy species whose hybrid offspring may become more weedy or more invasive; (3) potential impact on flora and fauna biodiversity; (4) availability of tools to effectively manage weeds in the new crop; and (5) best agronomic practices to enhance crop competitiveness against weeds.

Accurately predicting CNT invasiveness *a priori* is problematic, especially for traits that may enhance plant fitness and invasiveness. Beckie et al. (2010) have advocated post-release monitoring (PRM) of second-generation CNTs. As first-generation (agronomic or input traits) genetically modified/transgenic CNTs have been grown commercially in a number of countries since the mid-1990s, second-generation CNTs will follow in the near future. PRM of abiotic stress-tolerant and other second-generation CNTs will strengthen pre-release environmental risk assessments, for which protocols are currently being developed. In the review by Beckie et al. (2010), a comprehensive framework and protocol for case-specific PRM of such CNTs in Canada are outlined, using drought-tolerant canola (*Brassica napus* L.) as a model CNT. The primary potential environmental risk associated with cultivation of drought-tolerant canola is increased invasiveness of volunteers or feral plants (self-perpetuating populations outside of cultivated areas) and weedy relative-crop hybrids or backcrossed progeny in ruderal and natural areas adjacent to CNT cultivation, resulting in loss of abundance or biodiversity of native plant species. Thus, PRM can

effectively address the greater uncertainties in the environmental risk assessment of these second-generation vs. first-generation CNTs and thereby enhance environmental protection and security of the food supply.

In the opening Plenary Session, “New crops and crops with second-generation traits: weed management challenges”, the five speakers and presentation titles were the following:

1. Jack Grushcow* (Linnaeus Plant Sciences Inc.) – “*Industry perspective on new crops: the reward side of the risk reward equation*”
2. Gini Ardiel-Hill (Canadian Food Inspection Agency) – “*A regulatory perspective on new crops*”
3. Jean Burns* (Case Western Reserve University) – “*Research perspective: a promising way forward in determining weediness and invasiveness*”
4. Eric Johnson* (Agriculture and Agri-Food Canada) – “*Developing agronomic packages for new crops and 2nd-generation crops with novel traits: constraints and opportunities*”
5. Hugh Beckie[§] (Agriculture and Agri-Food Canada) – “*A role for post-release monitoring?*”

The Plenary Session was supplemented with a professional development workshop chaired by Steve Shirtliffe (Dept. Plant Sciences, University of Saskatchewan) entitled: “Weediness and agronomy of new crops”. The four speakers and presentation titles were the following:

1. Randy Kutcher* (Agriculture and Agri-Food Canada) – “*Implications of canola-intensive crop rotations*”
2. Bill May* (Agriculture and Agri-Food Canada) – “*Agronomy and weediness of camelina and niger*”
3. Nicholas Boersma (Iowa State University) – “*Agronomy and weediness of *Miscanthus**”
4. Rene Van Acker* (University of Guelph) – “*Risks associated with transgenic crops*”

Some intentionally-introduced species became invasive in Canada. While most crops become volunteer weeds in subsequent crops, some have escaped and established in ruderal areas, and a few have invaded and transformed native landscapes. While invasive crops are rare, they are economically and environmentally costly. Dispersal for large-scale planting, agronomic nurturing, and seed dispersal (within fields at harvest and widely by the seed transport system) may shorten the lag period for invasion and

reduce the opportunity for irradiation of weedy species. It is incumbent on the weed science community to engage with the crops industry and regulators to test new crops and second-generation CNTs under field conditions prior to release and to develop effective methods to monitor and mitigate following release.

By supporting this forum, the Canadian Weed Science Society is proactively exploring weed management and other issues related to new crops and CNTs. Six* of the nine speakers have summarized their presentations in this volume. The content for the presentation “*A role for post-release monitoring?*”[§] was previously detailed in a review article (Beckie et al 2010). We thank the authors and external reviewers for their contributions to the session, workshop, or this monograph.

Literature cited

- Beckie, H.J., L.M. Hall, M.-J. Simard, J.Y. Leeson and C.J. Willenborg. 2010. A framework for postrelease environmental monitoring of second-generation crops with novel traits. *Crop Sci.* 50:1587-1604.
- Warwick, S.I., H.J. Beckie and L.M. Hall. 2009. Gene flow, invasiveness and ecological impact of genetically modified crops. *The Year in Evolutionary Biology 2009: Ann. N.Y. Acad. Sci.* 1168:72-99.

Additional keywords: camelina, Ethiopian mustard, invasiveness, post-release monitoring, weediness

Camelina – a new industrial crop

Jack Grushcow

Linnaeus Plant Sciences Inc., Suite 244, 4438 West 10th Ave., Vancouver, BC V6R 4R8

Email: jack.gr@linnaeus.net

Camelina [*Camelina sativa* (L.) Crantz] is being developed as a new crop to replace castor bean (*Ricinus communis* L.) for the production of ricinoleic acid, a hydroxy fatty acid with a range of current uses ranging from lubricants to moulded and formed polyamide resins. Camelina is currently being grown for low-value biodiesel production, and is suited to dry and warm environments in areas where canola (*Brassica napus* L.) does not thrive. However, it has not been the focus of breeding or agronomic research efforts and remains a crop ‘in the rough’. To develop a new crop, it takes much more than a good idea and some novel genes. Industrial-value chains are deeply entrenched and difficult to change. There is a large bank of expertise required to shift a product into an existing market, especially one as entrenched as the petroleum industry.

Additional keywords: bioindustrial, bioplastics, castor bean, materials other than grains (MOG), ricinolein

Introduction

The petrochemical industry converts major raw materials – oil and natural gas – into thousands of industrial and consumer products, including plastics, paints, rubber, fertilizers, detergents, dyes, textiles, solvents, and lubricants. In so doing, it uses products made in plants by photosynthesis millions of year ago. The industry modifies the basic building blocks of carbon, oxygen, and hydrogen to form valuable feedstocks for other industries. Linnaeus Plant Sciences wants to use industrial crops to change the way we do chemistry – our mission: *we want to do chemistry in oilseeds* (Linnaeus Plant Sciences Inc. 2011).

Modern philosopher’s stone

Just as the ancient philosopher’s stone was said to be capable of turning base metals, especially lead, into gold, we want to use light and water

to make valuable industrial feedstocks. Specifically, we want to use non-food oilseeds as high-value non-fuel petroleum substitutes. Right now, many oilseed crops, including soybean [*Glycine max* (L.) Merr.], corn (*Zea mays* L.) and canola oil, can be converted to biodiesel. Canola oil for biodiesel is usually made from green seed and does not provide sufficient value to Canadian farmers, except as a market for substandard seed. We want to increase oilseed value for farmers beyond fuel. Rather than \$0.50 per litre, we want to produce a product that sells for \$5.00 per litre such as lubricants, hydraulic fluids, and greases. In so doing, we believe we can reduce our carbon footprint and make Canadian agriculture a key player in climate-change dialogue.

Oilseed crops are very effective at synthesis and concentration of fatty acids in the seed. A fatty acid is a carboxylic acid with a long unbranched aliphatic tail (chain), which is either saturated or unsaturated. Triglycerides (TAG) are formed from three fatty acids and glycerol. The location and position of saturated bonds in fatty acids and triglycerides forms a multitude of potential chemical compounds that vary widely in properties, such as lubricity, oxidative stability, drying ability, and nutritional content.

The tropics contain great biodiversity, and have long been a source of our most valued medicines. They are also a source of plant oil diversity. Tropical plants contain thousands of valuable industrial oils, but the crops lack agronomic traits and climatic adaptation to Canada. Castor oil, extracted from the seed of the castor bean (*Ricinus communis* L.), is one of these valuable oils. Castor bean is a rhizomatous shrub in the spurge family. The seed contains between 40 and 60% oil that is rich in triglycerides, of which 90% is ricinolein. The plant also produces a high level of highly-allergenic surface albumens, and seeds contains large amounts of the poison ricin, a water-soluble protein. Consequently, this makes hand-harvest of seeds a major human health risk.

One of the many uses of ricinolein is to manufacture Rilsan® Polyamide (PA) 11 resin (Fig. 1). Supplied in powder or pellet form, Rilsan PA resin can be processed by injection moulding, extrusion, blown film extrusion, or extrusion blow moulding, and are available in rigid, semi-flexible, and flexible grades. These resins have a wide range of applications, from automotive to sport equipment (Arkema 2011). Arkema has spent 20 yr trying to increase worldwide castor bean production through the development of germplasm, but with little success. The world supply of castor bean is limited, and the demand is increasing. Auto manufacturing represents a major new demand for green products. China currently is the

second largest importer of castor bean and demand is continually increasing. Arkema, the world's largest user, requires 50,000 t, almost 30% of total global annual exports in some years. India currently produces most of the world's supply of castor bean, at 830,000 t annually. Brazil, the second largest exporter, is increasingly subsidizing the use of castor bean for biodiesel, further limiting the supply of ricinoleic acid. A stable supply of castor oil would find a ready market.

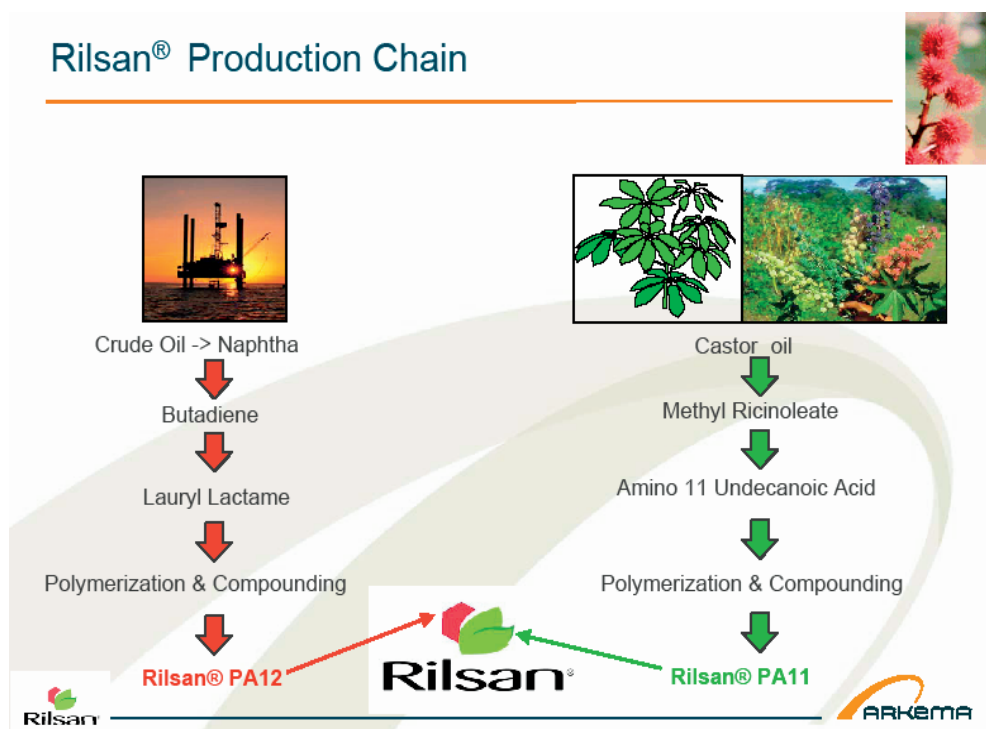


Figure 1. The use of castor oil and crude oil to manufacture Rilsan.(Arkema 2011).

Plants can produce large volumes of specialized oils at low cost. The replacement of castor bean with a North American broad-acre crop, without the worker safety concerns, would provide a stable and secure source of ricinolein. Linnaeus Plant Sciences has chosen camelina [*Camelina sativa* (L.) Crantz] as the crop to produce ricinolein. The company is the exclusive licensee of the Carnegie Institution of Washington's rights to castor bean oil synthesis genes, promoters, related technologies, and protocols. The critical

hydroxylase gene (Broun and Somerville 1997) was discovered by Dr. Chris Somerville, head of the Carnegie Institute's Department of Plant Biology at Stanford University and one of the world's top experts in plant biotechnology. These technologies allow Linnaeus Plant Sciences to create lines of agronomically-adapted oilseed crops that produce hydroxylated fatty acids, including ricinolein, as part of their seed oil profile. Camelina is amenable to transformation (Lu and Kang 2008). Genetically modified camelina oil will serve as an alternative source of castor oil. If 20% hydroxy fatty acid could be obtained in camelina seed, about 200,000 ha of production could satisfy the current United States (U.S.) market.

Camelina advantages

Camelina is an old world oilseed crop in the Brassica family, with small yellow flowers and globular seed pods (Francis and Warwick 2009). Interest in camelina as a potential oilseed crop for northern regions began to increase in Canada after trials were conducted in the late 1950s. Recently, camelina has received renewed interest in Europe, North America, and Australia, mostly due to its nutritional value. Camelina's fatty acid composition is largely unsaturated (greater than 90%), with significant amounts (30 to 40%) of linolenic acid, an important omega-3 fatty acid (Zubr 2003). This profile is similar to that of flax (*Linum usitatissimum* L.), making camelina oil a perfect fit for the nutraceutical market, where foods contain added health benefits (Peiretti and Meineri 2007).

Camelina is related to several weedy species: *C. alyssum*, *C. microcarpa*, and *C. rumelica* that were introduced to Canada as crop contaminants (Francis and Warwick 2009). There is no evidence from weed survey data (Leeson et al. 2005) or initial studies of demographics (Davis 2010) that any of these species are invasive or spreading within agronomic systems. It seems unlikely that camelina will be a persistent volunteer.

Camelina's small seed size, limited agronomic information (Gugel and Falk 2006; Urbaniak et al. 2008), lack of adequate weed control options, and current low value of the product are significant constraints to production. However, camelina is not currently being used as a food or export crop in North America, which reduces the concern for adventitious presence in food or export products. Camelina has not benefited from germplasm enhancement through breeding and selection of lines suited to Canada. Therefore, significant gains could still be achieved in seed size, yield, and abiotic and biotic stress tolerance. Because camelina is now 'coming of age',

it may benefit from rapid and less expensive sequencing, marker-assisted selection, and mutagenesis to reduce the time to reach breeding objectives.

Crop transformation, breeding, and production are just part of the expertise required to bring a green product to market (Fig. 2). Whole-crop processing and market development for products are also required. The least valuable component of the oil may be biofuel (Bernardo et al. 2003; Frohlich and Rice 2005; Moser and Vaughn 2010), but this product has an established market. Camelina meal has been accepted for feed use in the U.S., and has additional non-food uses such as mucilage applications; tocots, tocopherols, and omega fatty acids could be nutritionally-promising. Material other than grain (MOG) – pellets, composites using straw and seed hull – may find a market as well.

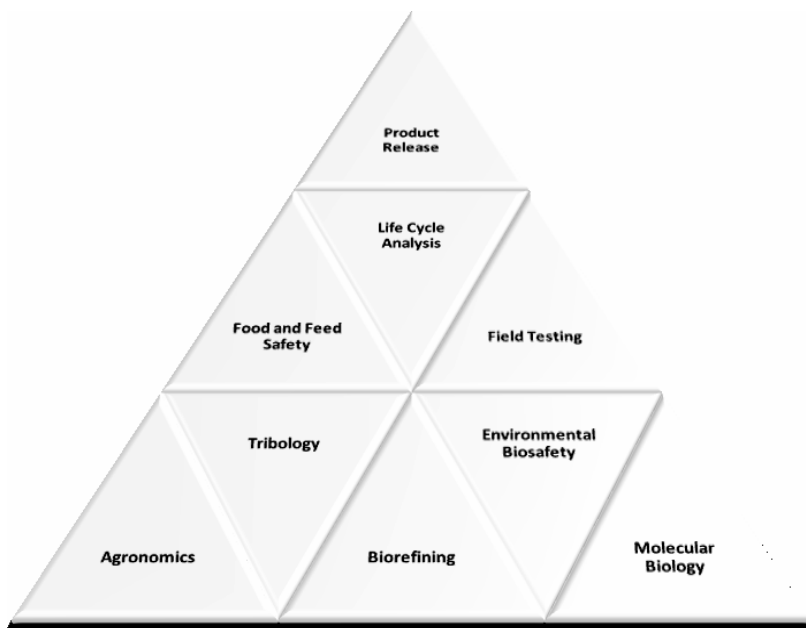


Figure 2. Some of the expertise required to develop a new crop and product stream.

Availability drives applications – the chicken and egg problem. If there was a market, then there would be production. If there was production, then there

would be a market. Which comes first? We are simultaneously working with camelina breeders and growers to develop the germplasm for conventional production, while enhancing the grower's agronomic skills and knowledge. This effort is occurring in parallel with product development, testing of meal for animal feed, and assessing physical and chemical attributes of bioproducts. Research will provide a stronger platform upon which to develop the higher value, modified fatty acid camelina production system.

Relevance to industry

Linnaeus Plant Sciences is part of a responsive biotechnology industry. We are cloning and engineering new genes, and working with public partners to further crop and product development. Because patents will control new materials, we are working with other industry partners such as DuPont. On September 13, 2010, Linnaeus Plant Sciences and DuPont announced a licensing agreement to develop an industrial oilseed platform and commercially exploit Linnaeus Plant Sciences/DuPont hydroxylated fatty acid intellectual property. It is our intention to obtain proof-of-concept for several DuPont industrial oil traits in camelina, and assess possible applications for DuPont oil profiles and related chemistries.

Relevance to government

Policy and regulations need to evolve to adapt to new products and crops. Linnaeus Plant Sciences is also working with the Canadian Food Inspection Agency to develop a biology document for camelina.

Relevance to agriculture

Linnaeus Plant Sciences is playing an active role in making agriculture part of the climate-change solution. There is a direct environmental advantage to replacing petroleum products with plant-made oils. Sustainable production practices can optimize this advantage.

It is also our responsibility to keep our growers competitive, by delivering a valuable new rotation crop. Offering more crop rotation options may break the commodity price cycle and drive added value at the farm gate. In 2010, over 6 million ha of canola were planted in Canada, providing a

significant economic return to growers and the Canadian economy. Camelina may provide an alternative crop to canola, particularly in areas too warm and dry for successful canola production. Thus, camelina could offer greater crop diversity and an additional high-value crop.

Acknowledgments

I want to acknowledge the contribution of the group of visionary scientists who work with Linnaeus Plant Sciences. These include Kevin Falk, Eric Johnson (Agriculture and Agri-Food Canada), Mark Smith (National Research Council), Linda Hall (University of Alberta), Tony Slabas (University of Durham), and Chris Somerville (Carnegie Institution of Washington and Stanford University). We continue to look for collaborators with creative and novel ideas and skill sets.

Literature cited

- Arkema. 2011. Arkema Inc., Philadelphia, PA. [Online] Available: <http://www.arkema-inc.com/index.cfm>.
- Bernardo, A., R. Howard-Hildige, A. O'Connell, R. Nichol, J. Ryan, B. Rice, E. Roche and J.J. Leahy. 2003. Camelina oil as a fuel for diesel transport engines. *Ind. Crop Prod.* 17:191-197.
- Broun, P. and C. Somerville. 1997. Accumulation of ricinoleic, lesquerolic, and densipolic acids in seeds of transgenic *Arabidopsis* plants that express a fatty acyl hydroxylase cDNA from castor bean. *Plant Physiol.* 113:933-942.
- Davis, P.B. 2010. The Invasion Potential and Competitive Ability of *Camelina Sativa* (L.) Crantz (Camelina) in Rangeland Ecosystems. M.Sc. dissertation. Bozeman, Montana: Montana State University.
- Francis, A. and S.I. Warwick. 2009. The biology of Canadian weeds. 142. *Camelina alyssum* (Mill.) Thell.; *C. microcarpa* Andr. ex DC.; *C. sativa* (L.) Crantz. *Can. J. Plant Sci.* 89:791-810.
- Frohlich, A. and B. Rice. 2005. Evaluation of *Camelina sativa* oil as a feedstock for biodiesel production. *Indust. Crops Prod.* 21:25-31.
- Gugel, R.K. and K.C. Falk. 2006. Agronomic and seed quality evaluation of *Camelina sativa* in western Canada. *Can. J. Plant Sci.* 86:1047-1058.
- Leeson, J.Y., A.G. Thomas, L.M. Hall, C.A. Brenzil, T. Andrews, K.R. Brown and R.C. Van Acker. 2005. Prairie Weed Surveys of Cereal,

- Oilseed and Pulse Crops from the 1970s to the 2000s. Weed Survey Series Publ. 05-1CD. Saskatoon, SK: Agriculture and Agri-Food Canada. 395 pp.
- Linnaeus Plant Sciences Inc. 2011. Linnaeus Plant Sciences Inc., Vancouver, BC. [Online] Available: <http://www.linnaeus.net>.
- Lu, C. and J. Kang. 2008. Generation of transgenic plants of a potential oilseed crop *Camelina sativa* by *Agrobacterium*-mediated transformation. Plant Cell Rep. 27:273-278.
- Moser, B.R. and S.F. Vaughn. 2010. Evaluation of alkyl esters from *Camelina sativa* oil as biodiesel and as blend components in ultra low-sulfur diesel fuel. Bioresour. Technol. 101:646-653.
- Peiretti, P.G. and G. Meineri. 2007. Fatty acids, chemical composition and organic matter digestibility of seeds and vegetative parts of false flax (*Camelina sativa* L.) after different lengths of growth. Anim. Feed Sci. Technol. 133:341-350.
- Urbaniak, S.D., R. Lada, L. Luan, C.D. Caldwell and V.D. Zheljazkov. 2008. The effect of seeding rate, seeding date and seeder type on the performance of *Camelina sativa* L. in the maritime provinces of Canada. Can. J. Plant Sci. 88:501-508.
- Zubr, J. 2003. Dietary fatty acids and amino acids of *Camelina sativa* seed. J. Food Qual. 26:451-462.

Research perspective: a promising way forward in determining weediness and invasiveness of newly-introduced species

Jean H. Burns

Department of Biology, Case Western Reserve University, 2080 Adelbert Rd., 307 DeGrace Hall, Cleveland, OH 44106-7080

Email: jbm122@case.edu

Tools to predict and prevent biological invasions are greatly needed because controlling invaders after they have established is far more expensive than prevention. Existing tools to predict plant invasions include trait-based models, such as the Australian Weed Risk Assessment and modifications thereof, and pathways models, which target dispersal corridors for monitoring. An additional tool, demographic modeling, predicts whole-population level behaviours, such as projected population growth rate, and is thus closely related to the invasion process. Evidence in the literature is consistent with the prediction that invasive and weedy species have higher projected population growth rates than noninvasive and less weedy relatives. However, phylogenetically-broad experimental tests of this hypothesis that control for environmental variation are still needed. Demographic methods must carefully consider the environmental conditions, including both biotic and abiotic conditions, under which these models are parameterized, to most accurately predict potential invasiveness or weediness. The most powerful approach to predicting and preventing invasions of newly arrived, or potential introductions, on a country or regional scale, may be a multi-pronged approach that makes iterative use of the information in pathways models, weed risk assessment models, and demographic models to predict potential invasiveness.

Additional keywords: demography, modeling, population dynamics, prediction, prevention

Introduction

Invasive species are those that have been introduced and spread rapidly outside their native range, as defined by Richardson et al. (2000). Like invasive species, weedy species often spread rapidly, and may have

severe ecological or economic effects, but may be either native or introduced, and are more likely to be a problem in disturbed or agricultural settings than in natural areas (USDA 2010). In most cases, the costs of prediction and prevention of invasions would be far less than the costs of control (Finnoff et al. 2007); thus, developing useful prediction tools is an important goal for invasion biology.

A combination of approaches for predicting and preventing biological invasions is most likely to succeed in the face of this highly complex regulatory problem (Fig. 1). Tools to predict biological invasions include pathways models (Hulme et al. 2008), which use introduction pathways to predict invasion corridors, trait-based prediction models, such as the Australian Weed Risk Assessment (WRA) models and their progeny (e.g., Daehler et al. 2004; McClay et al. 2010; Pheloung et al. 1999) and demographic models, which use population parameters, such as survival and fecundity, to predict future population growth (Caswell 2001) (e.g., Burns 2008; Ramula et al. 2008). Here, I briefly summarize the literature on these prediction tools and suggest an integrative approach that might enhance prediction and prevention by making use of the strengths of multiple tools (Fig. 1).

Pathways models

Regulators might propose prevention strategies based on pathways of introduction (Fig. 1). Pathways models use modes of introduction, such as shipping routes, to predict likely sources of invasions, allowing managers to focus their limited time on key locations or sources (Hulme et al. 2008). Hulme et al. (2008) identify six major pathways of introduction, ranging from fully-intentional introductions, to fully-unintentional introductions, or ‘unaided’ dispersal. Intentional introductions include commodity introductions that are deliberately released – their ‘release’ category, such as plants for forage or erosion control. Unaided dispersal may occur for many introduced species after an initial introduction, such as for *Sargassum* invasion in Europe, a very aggressive macroalga (Hulme et al. 2008). Terrestrial plants are most often originally ‘escaped’ commodities, which were intentionally introduced, such as for horticulture or agriculture, but became naturalized unintentionally (Hulme et al. 2008).

By classifying pathways of introduction, regulations could be standardized by pathway type. For example, when movement via seed

contaminants is likely, vehicles could be regularly inspected and cleaned to prevent invasions (Hulme et al. 2008). Evidence suggests that the proportion of invasions that result from unintentional introduction is increasing, especially among invertebrates and microorganisms, suggesting that monitoring and control of these pathways will be especially critical in the future (Hulme et al. 2008). This approach is particularly useful for accidental introductions, where the traits of species cannot be used to filter potentially weedy or invasive species *a priori* (Fig. 1).

Trait-based models

When species are introduced intentionally, as is often the case for terrestrial plants (Hulme et al. 2008), trait-based models may provide a mechanism to predict potential invasive species (Fig. 1). Trait-based models, or WRAs, use information about species' traits, such as fecundity, vegetative reproduction, and self-compatibility, to predict the probability of invasiveness or weediness (e.g., Daehler et al. 2004; Daehler and Virtue 2010; Dawson et al. 2009; Gasso et al. 2010; Gordon et al. 2008a; Krivanek and Pyšek 2006; McClay et al. 2010; Pheloung et al. 1999; Skarpaas and Okland 2009; Weber et al. 2009). Trait-based models also often use a number of traits that are not necessarily morphological, but could be historical factors that correlate with invasion success, including propagule pressure, timing of introduction, and perhaps the best predictor, 'invasive elsewhere' (Pheloung et al. 1999). While these traits provide useful information predicting potential invasiveness, they are not always available; for example, if a species has not been introduced beyond its native range, then whether it is invasive elsewhere cannot be evaluated. This may be especially true for new crop or biofuel crop introductions, which by nature of their recent development, will lack information such as whether they are invasive elsewhere. For such species, a second level of evaluation is most likely necessary (Fig. 1).

Trait-based models are typically accurate at predicting invasiveness at a regional scale, correctly classifying invasive species as invasive in cross-validation analyses 86 to 100% of the time (e.g., Daehler and Virtue 2010; Gordon et al. 2008b; Pheloung et al. 1999). However, trait-based models are often less good at accurately predicting noninvasive species, in some cases only correctly predicting 44 to 50% of noninvasive species (McClay et al. 2010, but see for example Daehler et al. 2004). Further, while these models

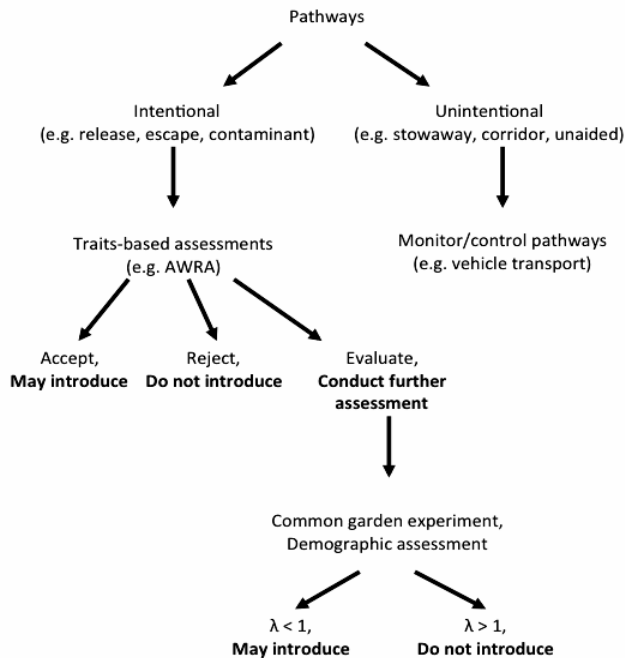


Figure 1. A proposed integrated approach to invasive and weedy species risk assessment, using an iterative model including pathways, trait-based assessments, and detailed demographic assessments when necessary. When populations are introduced unintentionally (pathways categories from Hulme et al. (2008); note that intention is continuous in Hulme's framework), it may be necessary to monitor high traffic pathways for potential invaders or implement risk-reduction strategies. If species or their vectors are introduced intentionally, trait-based approaches can be used to identify species of high risk of invasiveness or weediness and prevent their introduction. Trait-based models have been designed to yield recommendations for introduction: if high risk, then reject; if low risk, then accept; or evaluate further. Demographic models can be used to further evaluate potential invasiveness by making predictions about projected population growth rate, λ (lambda). By making predictions at the population level, these models provide predictions closely tied to the invasion process. At its simplest, if a population is predicted to have population growth, $\lambda > 1$, it might be considered a higher-risk introduction, and if a population is predicted to shrink, $\lambda < 1$, it might be considered a lower-risk introduction.

have worked well in some areas, such as Australia and Hawaii (e.g., Daehler et al. 2004; Pheloung et al. 1999; Weber et al. 2009), they seem to work less well in other regions, such as Canada (McClay et al. 2010). These models also usually recommend some proportion of species that fall into an ‘evaluate further’ category for additional analysis (e.g., 13%, Daehler et al. 2004). Complementary approaches are needed when trait-based models provide ambiguous results, or for newly-developed genotypes such as novel crops, which cannot be evaluated using standard WRA models (Fig. 1).

Demographic modeling

Demographic models are an empirical summary of all of the factors in a species' life cycle that influence population growth, including survival, growth, retrogression, and fecundity (Caswell 2001), and thus they are a method of summarizing all of that information into a measure of population performance. Demographic models can take either continuous form, as in integral projection models (Easterling and Ellner 2000) or discrete form, as in age- or stage-structured models (Caswell 2001). Stage-structured demographic matrix models are the most frequently used demographic modeling tool for plants (Buckley et al. 2010; Burns et al. 2010; Crone et al. 2011; Ramula et al. 2008). Stage-based demographic matrix models take the form of a projection matrix, where the elements of the matrix describe the probabilities of transitions from one stage class to another (equation 1; Fig. 2).

For example, a stage-based demographic matrix model for a hypothetical perennial plant with life-cycle diagram (Fig. 2) could take the form:

$$A = \begin{pmatrix} P_1 & F_2 & F_3 \\ G_1 & P_2 & 0 \\ 0 & G_2 & P_3 \end{pmatrix} \quad [1]$$

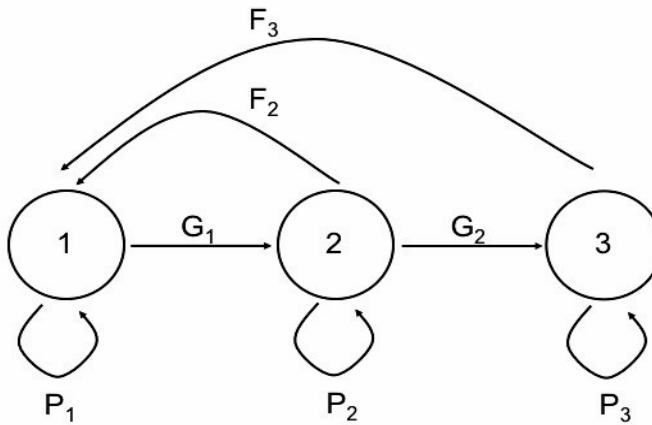


Figure 2. Hypothetical life cycle diagram for a perennial plant with nonreproductive stage class 1, and reproductive stage classes 2 and 3 (modified from Caswell (2001)), where circles indicate stage classes, and arrows represent transition probabilities. Individuals in this model can stay in a stage class (P_i), grow to the next stage class (G_i), or reproduce (F_i) over the course of 1 yr. The transition probabilities are a function of vital rates, such as fecundity, survival, and germination proportion (Caswell 2001).

where ‘A’ is the population projection matrix, ‘ P_i ’ is the probability of surviving and remaining in stage class ‘i’ over the projection interval – often 1 yr. ‘ G_i ’ is the probability of growing from stage class ‘i’ to the next stage class, and ‘ F_i ’ is the fecundity of stage class ‘i’ individuals, which, in this case, germinate and become nonreproductive, stage class 1 individuals over the course of 1 yr (equation 1). In this example, the projection interval is 1 yr, but this could be altered as appropriate for the life cycle. This example has no seed bank, but the model can easily be modified to include a seed bank stage. The values of the transition probabilities can be fixed (e.g., $P_1 = 0.1$) or can be functions, for example, of density (Caswell 2001).

The projected population growth rate, λ (lambda) describes the rate of increase of the population at equilibrium, assuming that conditions remain the same as when the vital rates were measured. Lambda can be easily calculated from demographic matrix projection models, and is equal to the

dominant eigenvalue of the projection matrix (Caswell 2001). Values of $\lambda > 1$ indicate a population that is projected to grow; values of $\lambda < 1$ indicate a population that is projected to shrink. Some empirical evidence suggests that demographic modeling may be a useful predictive tool for invasive and weedy species, where one might predict that invasive species would have greater λ than noninvasive species (Burns et al. 2008; Ramula et al. 2008).

Because demographic modeling can provide population-level predictions about potential invasiveness, demographic models can provide another tool for evaluation (Fig. 1). The unit of management interest for invasive and weedy species is typically the population, not individuals or species (Davis 2010; Davis et al. 2011; Rew et al. 2007). To the extent that invasiveness and weediness are emergent properties of populations, measuring performance at the level of the population is more likely to succeed than measuring individual traits (Davis et al. 2000; Davis 2010; Davis et al. 2011). For example, if density dependence regulates population growth (Halpern and Underwood 2006), fecundity might increase population growth only up to a point where density suppresses additional germination and seedling survival, resulting in a saturating relationship between population density and population growth (Fig. 3). In such cases, fecundity alone (a trait often used in WRA models, e.g., Pheloung et al. 1999), will not scale with population growth or invasiveness in a one-to-one fashion. Thus, measures of population growth may be more relevant for management purposes than individual traits (Rew et al. 2007).

Positive population growth is a necessary, though not sufficient requirement for a population to be considered invasive or weedy across the landscape (Richardson et al. 2000). Invasion, or spread in the landscape (Richardson et al. 2000), is a function of both demographic and dispersal processes (Neubert and Caswell 2000). A population could have high population growth at some sites, but still fail to invade, if dispersal limits expansion across the landscape (Neubert and Caswell 2000), or if environment-dependent demography results in lower population growth at some sites. Demography is, in principle, more closely tied to the invasion process than any individual trait, like fecundity, because it incorporates all of the stages of a species' life cycle into a single descriptor of population-level processes (Halpern and Underwood 2006). However, measuring demography is not identical with measuring the rate of spread across the landscape (Neubert and Caswell 2000), or even necessarily with future population behaviour (Crone et al. 2011).

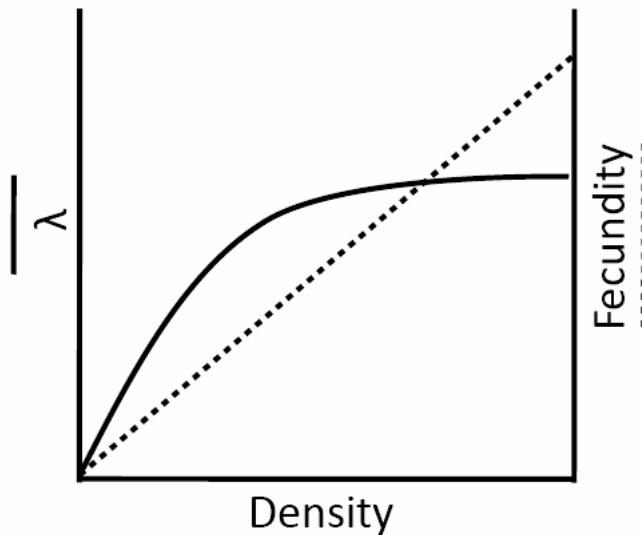


Figure 3. Density dependence could suppress population growth at high densities, via effects on other vital rates, even if fecundity is not suppressed at high densities.

Evidence that demographic modeling can predict weediness or invasiveness

Projected population growth rates are higher for invasive than for noninvasive species in the dayflower family (Commelinaceae)

To test the hypothesis that λ is associated with invasiveness, I conducted a factorial greenhouse experiment manipulating water and nutrients for four pairs of invasive and noninvasive congeners (Burns 2006). I focused on a single family for these experiments, the dayflower family (Commelinaceae), because there are multiple invasive species in the family across multiple genera, and because there are many species with known introduction histories that have failed to invade. For the purposes of the experiment, species were considered invasive if they were so classified by

published sources and authorities (e.g., USDA National Invasive Species Information Center database; Florida Exotic Pest Plant Council database).

The experiment manipulated water and nutrients in a factorial design with high and low water and nutrient availability (see Burns (2006) for experimental details), and demographic models were parameterized based on the experiment (see Burns (2008) for details). The invasive species had consistently and significantly higher λ than their noninvasive relatives under high nutrient conditions (Fig. 4). These results suggest that invasive species might have higher λ than their noninvasive relatives, under conditions relevant to invasion. However, the experiment was conducted in a greenhouse with a phylogenetically-limited sample of species, limiting the ability to generalize from the results. The observed λ values are probably also much higher than might be expected under field conditions, suggesting that field sampling is necessary. The meta-analysis described below assesses the degree of generality of this pattern, with respect to additional phylogenetic sampling and field conditions.

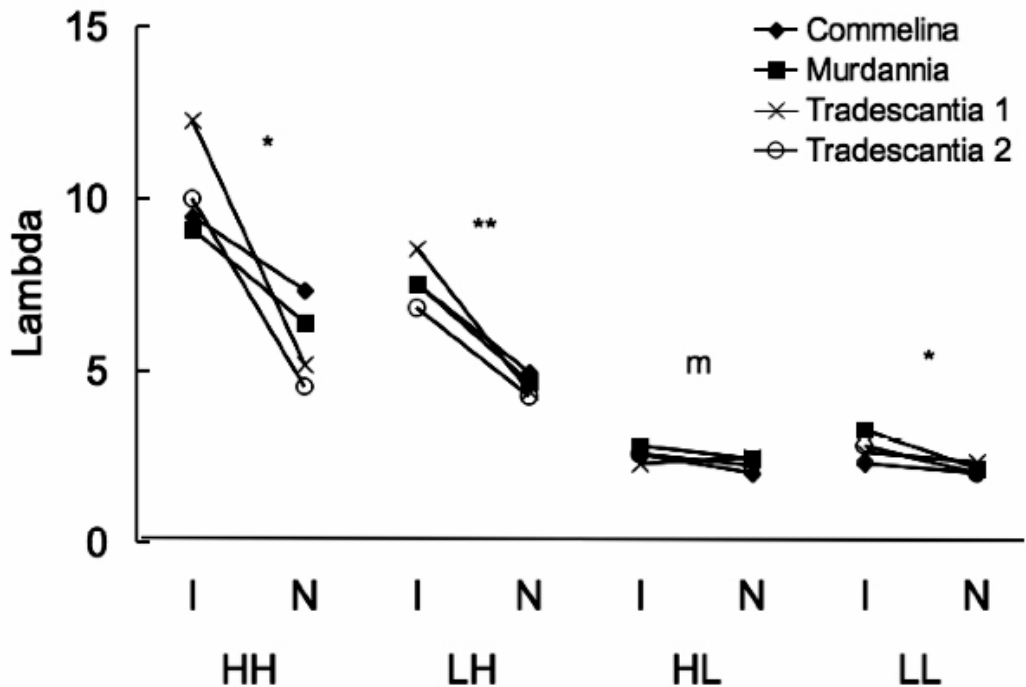


Figure 4. Population growth rate (λ) based on plants grown under a factorial experimental design, with high and low water and high and low nutrient availabilities (e.g., HL=high water, low nutrients, etc.). Response

variables are presented for individual species, with invasive (I) and noninvasive (N) species nested within related pairs. Lines connect pairs. Lambda was higher for invasive species than for their noninvasive congeners under high-nutrient conditions (m: $P < 0.10$; *: $P < 0.05$; **: $P < 0.01$). Reprinted with permission from Burns (2008).

Projected population growth rates are higher for invasive populations than for native populations in a global meta-analysis

To determine whether species in the invaded range, i.e., invasive populations, had higher λ than species in their native range, i.e., native populations, we conducted a meta-analysis of the plant demographic literature from 1975 to 2006, collected demographic matrix information including estimates of λ , and conducted a meta-analysis comparing the demography of invasive and native populations (details in Ramula et al. (2008)). The data set consisted of 21 invasive populations, where demography was measured in the invaded range and invasiveness was as defined by the study authors, and 179 populations in the species' native range. In this data set, there were no species that fell into both categories, i.e., were in the data set both in their introduced range and in their native range. We used an ANCOVA with introduction status as a factor (native, invasive), lifespan as a covariate, and λ log-transformed to ask whether invasive species differed in λ from native species. Note that not all native species are noninvasive, and demography of a given species might differ between its native and introduced range; however, this provides a first approximation of the signal of invasiveness on λ .

The invasive species had significantly higher λ than the native species (Fig. 5); unsurprisingly, native species had a λ very close to 1, suggesting that the populations are relatively stable, on average. The higher λ of invasive populations is consistent with the prediction that demographic models may be useful tools for predicting potential invasiveness (Fig. 1).

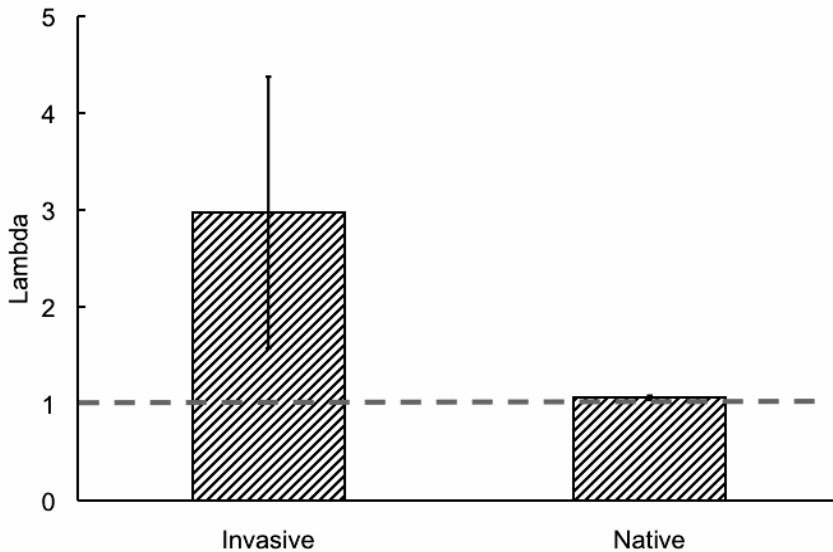


Figure 5. Invasive species had higher lambda (λ) than native species in a meta-analysis (modified from data in Ramula et al.(2008)). The dashed line indicates the point above which populations are projected to increase. Results were also significant with removal of the outlier, *Lespedeza cuneata*, which had an unusually high λ of 22.45 (least square mean = 1.47 and 1.05, for invasive and native species, respectively; $F_{1,19} = 10.04$ $P = 0.005$).

An example application

Projected population growth suggests that camelina (*Camelina sativa*) may be unlikely to be weedy

Davis and colleagues (Davis 2010; Davis et al. 2011) have conducted a demographic analysis of camelina (gold of pleasure, large-seeded false flax) [*Camelina sativa* (L.) Crantz] to predict its potential for weediness in two rangeland ecosystems, and compared the results of demographic simulations to predictions of invasiveness by the Australian WRA (Pheloung et al. 1999 and modifications thereof). Camelina is a proposed biofuel crop, which has potential to be weedy, especially as it has been bred for rapid growth. Population models under a wide range of scenarios predicted that populations of camelina in two Montana rangelands will decline ($\lambda < 1$),

suggesting that it is probably safe to introduce camelina to Montana, if rangeland invasions are the primary factor affecting introduction decisions (Davis 2010; Davis et al. 2011).

Predictions of a modified Australian WRA for camelina were inconsistent with the demographic predictions; under no scenario did the WRA evaluation result in an ‘accept’ recommendation (Davis 2010; Davis et al. 2011). Under some scenarios, the WRA recommendation would be ‘reject’, as when climate matching was assumed to be high or hybridization with weedy relatives was assumed to occur, and under others, it would be ‘evaluate further’, as when climate matching, dispersal, and hybridization were all assumed to be low (Davis 2010; Davis et al. 2011). It will be instructive to assess whether camelina invades Montana rangelands in the future, to assess whether WRA or demographic modeling predictions were more accurate.

Caveats and recommendations

Demographic models can only make accurate predictions about future population growth or decline if their parameters are measured under appropriate environmental conditions (Burns et al. 2008; Rew et al. 2007; see also Crone et al. 2011 for a discussion of model limitations). It is widely recognized that disturbed systems are more likely to be invaded than undisturbed systems (Davis et al. 2000), and that the outcome of introductions is dependent on the interaction between species traits and the non-native environment (e.g., Burns 2006). For example, fecundity is responsive to environmental quality, including nutrient availability in most plants (Burns 2006), and higher quality environments may result in higher fecundity and higher λ (Fig. 4; Burns 2008). Thus, fecundity measured under low nutrient conditions would not reflect the potential invasiveness of that population under high nutrient conditions. To accurately predict invasiveness using demographic models, it will be critical to measure population parameters under appropriate environmental conditions, that is, those environments potentially subject to invasion.

In addition to careful choice of the environments in which to parameterize demographic models, studies of the demography of potential introductions should also consider the sample sizes used to estimate model parameters (Fiske et al. 2008). The sample size necessary to minimize bias in estimates of transition rates will vary across populations and for different

transitions, but for a relatively typical perennial plant, *Heliconia acuminata*, across six plots and 6 yr of sampling, samples of ca. 100 individuals were adequate when the probability of survival was relatively high (> 0.80), and larger sample sizes were necessary when survival was low (Fiske et al. 2008). Studies that suffer from small sample sizes, for example, due to unforeseen mortality, might consider using a continuous demographic modeling approach such as integral projection modeling (Easterling and Ellner 2000), which may be more robust than the discrete matrix model approach when sample sizes are small.

The density-independent population dynamics may be of most interest in governing the early stages of invasion (Neubert and Caswell 2000), unless Allee effects play a role in early introduction success; however, density-dependent dynamics could be important at later stages of invasion and might have management implications (Halpern and Underwood 2006; Pardini et al. 2009; Parker 2000). Demographic models of potential invasiveness might benefit from considering the effects of density dependence on population growth. Models can incorporate density dependence either using standard density-dependent models, where density functions are incorporated into the model in lieu of point estimates for vital rates (Caswell 2001), or multiple population projection matrices could be fit at different population densities, following the density-structured modeling approach of Taylor and Hastings (2004). Incorporating density dependence in demographic models could shed light on the role of density dependence in structuring invasions and could enhance control efforts (Halpern and Underwood 2006; Pardini et al. 2009).

Population dynamics are known to vary greatly across years and populations (e.g., Buckley et al. 2010), and attempts to predict future population dynamics would be enhanced by considering the role of environmental stochasticity in determining population dynamics of proposed introductions (Caswell 2001). Samples across years or populations are necessary to parameterize stochastic demographic models, and several techniques are available for incorporating among-year or population variation, including sampling the environmental states from a fixed distribution, discrete-state Markov chains, or autoregressive-moving-average models (Caswell 2001). The stochastic population growth rate, λ_s , can be calculated using simulation or Tuljapurkar's approximation (Tuljapurkar 1989, 1990), and in theory could be used to make predictions about future population dynamics of introduced species.

While population growth ($\lambda > 1$) is necessary for invasion to occur, determining projected population growth may not be sufficient for making a management decision. Whether a species has a negative effect on local systems, native species, or ecosystem processes will be a function of population growth (Davis et al. 2000; Rew et al. 2007) and other factors such as functional group (Vitousek and Walker 1989). For example, species in the legume family (Fabaceae) have nitrogen-fixing rhizobia, and may have ecosystem effects disproportionate to their population size (Vitousek and Walker 1989). Any management decisions using demographic tools will have to be based on what projected population growth rates are deemed 'acceptable' – a value-based judgment. Demographic models, like other quantitative tools, cannot make these judgments, but can provide an objective scale on which to base introduction decisions. What λ value is the appropriate cut-off for assessing potential invasiveness or weediness, and what other criteria need to be considered in making such a decision remain a judgment that policy-makers adopting this tool would need to make.

Conclusions

Predicting whether species or genotypes will be weedy or invasive is a difficult problem, which is most likely to be solved using a combination of approaches, including understanding the pathways of introduction, using trait-based predictive models, and using demographic modeling to predict the potential for future population growth (Fig. 1). Demographic models must take special care to consider the appropriate environments for evaluation, as a population is only weedy or invasive in a particular context (e.g., Burns 2008). When parameterized in the environments subject to potential invasion, demographic models should provide useful information about the potential for population growth, and thus invasiveness (e.g., Davis 2010; Davis et al. 2011), though caution should be used in interpreting these models, as the extent to which demographic models accurately predict future population growth is a matter of debate (see discussion in Crone et al. (2011)). By taking a multi-step process to evaluate potential invasiveness, we may be able to create more accurate predictions even in the face of uncertainty, and demographic modeling could be an important component of such prediction efforts (Fig. 1).

Acknowledgments

I thank Hugh Beckie and Linda Hall for organizing this publication, and Tiffany Knight, Stephen Darbyshire, and an anonymous reviewer for constructive comments and ideas on the manuscript. Thanks also to Case Western Reserve University for funding.

Literature cited

- Buckley, Y.M., S. Ramula, S.P. Blomberg, J.H. Burns, E.E. Crone, J. Ehrlén, T.M. Knight, J.-B. Pichancourt, H. Quested and G.M. Wardle. 2010. Causes and consequences of variation in plant population growth rate: a synthesis of matrix population models in a phylogenetic context. *Ecol. Lett.* 13:1182-1197.
- Burns, J.H. 2006. Relatedness and environment affect traits associated with invasive and noninvasive introduced Commelinaceae. *Ecol. Appl.* 16:1367-1376.
- Burns, J.H. 2008. Demographic performance predicts invasiveness of species in the Commelinaceae under high-nutrient conditions. *Ecol. Appl.* 18:335-346.
- Burns, J.H., S.P. Blomberg, E.E. Crone, J. Ehrlén, T.M. Knight, J.B. Pichancourt, S. Ramula, G. M. Wardle and Y.M. Buckley. 2010. Empirical tests of life-history evolution theory using phylogenetic analysis of plant demography. *J. Ecol.* 98:334-344.
- Burns, J.H., P. Munguia, B.E. Nomann, S.J. Braun, C.P. Terhorst and T.E. Miller. 2008. Vegetative morphology and trait correlations in 54 species of Commelinaceae. *Bot. J. Linn. Soc.* 158:257-268.
- Caswell, H. 2001. *Matrix Population Models: Constructions, Analysis, and Interpretation* (second edition). Sunderland, Massachusetts: Sinauer Associates, Inc.
- Crone, E.E., E.S. Menges, M.M. Ellis, T. Bell, P. Bierzychudek, J. Ehrlén, T.N. Kaye, T.M. Knight, P. Lesica, W.F. Morris, G. Oostermeijer, P.F. Quintana-Ascencio, A. Stanley, T. Ticktin, T. Valverde and J.L. Williams. 2011. How do plant ecologists use matrix population models? *Ecol. Lett.* 14:1-8.
- Daehler, C.C., J.S. Denslow, S. Ansari and H.C. Kuo. 2004. A risk-assessment system for screening out invasive pest plants from Hawaii and other Pacific Islands. *Conser. Biol.* 18:360-368.
- Daehler, C. and J.G. Virtue. 2010. Likelihood and consequences: reframing the Australian weed risk assessment to reflect a standard model of risk. *Plant Prot. Quart.* 25:52-55.
- Davis, M.A., J.P. Grime and K. Thompson. 2000. Fluctuating resources in plant communities: a general theory of invasibility. *J. Ecol.* 88:528-534.

- Davis, P.B. 2010. The Invasions Potential and Competitive ability of *Camelina sativa* (L.) Crantz (Camelina) in Rangeland Ecosystems. MS. dissertation. Bozeman, MT: Montana State University.
- Davis, P.B., F.D. Menalled, R.K.D. Peterson and B.D. Maxwell. 2011. Refinement of weed risk assessments for biofuels using *Camelina sativa* as a model species. J. Appl. Ecol. doi: 10.1111/j.1365-2664.2011.01991.x.
- Dawson, W., D. Burslem and P.E. Hulme. 2009. The suitability of weed risk assessment as a conservation tool to identify invasive plant threats in East African rainforests. Biol. Conser. 142:1018-1024.
- Easterling, M.R. and S.P. Ellner. 2000. Dormancy strategies in a random environment: comparing structured and unstructured models. Evol. Ecol. Res. 2:387-407.
- Finnoff, D., J.F. Shogren, B. Leung and D. Lodge. 2007. Take a risk: preferring prevention over control of biological invaders. Ecol. Econ. 62:216-222.
- Fiske, I.J., E.M. Bruna and B.M. Bolker. 2008. Effects of Sample Size on Estimates of Population Growth Rates Calculated with Matrix Models. Plos One 3:e3080.
- Gasso, N., C. Basnou and M. Vila. 2010. Predicting plant invaders in the Mediterranean through a weed risk assessment system. Biol. Inv. 12:463-476.
- Gordon, D.R., C. Gantz and D.A. Onderdonk. 2008a. Accuracy and impact of the Australian weed risk assessment modified for the U.S. Hortscience 43:1051.
- Gordon, D.R., D.A. Onderdonk, A.M. Fox and R.K. Stocker. 2008b. Consistent accuracy of the Australian weed risk assessment system across varied geographies. Divers. Distrib. 14:234-242.
- Halpern, S.L. and N. Underwood. 2006. Approaches for testing herbivore effects on plant population dynamics. J. Appl. Ecol. 43:922-929.
- Hulme, P.E., S. Bacher, M. Kenis, S. Klotz, I. Kuhn, D. Minchin, W. Nentwig, S. Olenin, V. Panov, J. Pergl, P. Pysek, A. Roques, D. Sol, W. Solarz and M. Vila. 2008. Grasping at the routes of biological invasions: a framework for integrating pathways into policy. J. Appl. Ecol. 45:403-414.
- Krivaneck, M. and P. Pyšek. 2006. Predicting invasions by woody species in a temperate zone: a test of three risk assessment schemes in the Czech Republic (Central Europe). Divers. Distrib. 12:319-327.
- McClay, A., A. Sissons, C. Wilson and S. Davis. 2010. Evaluation of the Australian weed risk assessment system for the prediction of plant invasiveness in Canada. Biol. Inv. 12:4085-4098.
- Neubert, M.G. and H. Caswell. 2000. Demography and dispersal: calculation and sensitivity analysis of invasion speed for structured populations. Ecol. 81:1613-1628.
- Pardini, E.A., J.M. Drake, J.M. Chase and T.M. Knight. 2009. Complex population dynamics and control of the invasive biennial *Alliaria petiolata* (garlic mustard). Ecol. Appl. 19:387-397.
- Parker, I.M. 2000. Invasion dynamics of *Cytisus scoparius*: a matrix model approach. Ecol. Appl. 10:726-743.

- Pheloung, P.C., P.A. Williams and S.R. Halloy. 1999. A weed risk assessment model for use as a biosecurity tool evaluating plant introductions. *J. Environ. Manage.* 57:239-251.
- Ramula, S., T.M. Knight, J.H. Burns and Y.M. Buckley. 2008. General guidelines for invasive plant management based on comparative demography of invasive and native plant populations. *J. Appl. Ecol.* 45:1124-1133.
- Rew, L.J., E.A. Lehnhoff and B.D. Maxwell. 2007. Non-indigenous species management using a population prioritization framework. *Can. J. Plant Sci.* 87:1029-1036.
- Richardson, D.M., P. Pysek, M. Rejmánek, M.G. Barbour, F.D. Panetta and C.J. West. 2000. Naturalization and invasion of alien plants: concepts and definitions. *Divers. Distrib.* 6:93-107.
- Skarpaas, O. and B. Okland. 2009. Timber import and the risk of forest pest introductions. *J. Appl. Ecol.* 46:55-63.
- Taylor, C.M. and A. Hastings. 2004. Finding optimal control strategies for invasive species: a density-structured model for *Spartina alterniflora*. *J. Appl. Ecol.* 41:1049-1057.
- Tuljapurkar, S. 1989. An uncertain life – demography in random-environments. *Theor. Pop. Biol.* 35:227-294.
- Tuljapurkar, S. 1990. *Population Dynamics in Variable Environments*. New York, NY: Springer-Verlag.
- [USDA] United States Department of Agriculture. 2010. USDA Plants Database. [Online] Available: <http://plants.usda.gov>.
- Vitousek, P.M. and L.R. Walker. 1989. Biological invasion by *Myrica faya* in Hawaii – plant demography, nitrogen-fixation, ecosystem effects. *Ecol. Monogr.* 59:247-265.
- Weber, J., F.D. Panetta, J. Virtue and P. Pheloung. 2009. An analysis of assessment outcomes from eight years' operation of the Australian border weed risk assessment system. *J. Environ. Manage.* 90:798-807.

Agronomic challenges and opportunities for second-generation crops with novel traits and new crops

**Eric N. Johnson¹, Stewart A. Brandt¹, Linda M. Hall²
and Elwin G. Smith³**

¹*Agriculture and Agri-Food Canada, P.O. Box 10, Scott, SK S0K 4A0*

²*Department of Agricultural, Food and Nutritional Science, 4-10 Agriculture/Forestry
Center, University of Alberta, Edmonton, AB T6G 2P5*

³*Agriculture and Agri-Food Canada, P.O. Box 3000, Lethbridge, AB T1J 4B1
Email: eric.johnson@agr.gc.ca*

Crops with novel traits have been rapidly adopted in Canada and worldwide. To date, most of the traits have been monogenic herbicide- or insect-resistance traits. A number of second-generation input and output traits are currently under development. Many of the traits under development are fitness traits, and it is difficult to predict the impact of these fitness traits on invasiveness. Improved vigour and competitiveness, stress tolerance, and nitrogen-use efficiency have already been observed in hybrid canola developed through traditional plant breeding techniques. This improvement in crop fitness has occurred without a corresponding increase in invasiveness in managed or natural ecosystems. Second-generation traits have been inserted into canola to improve drought tolerance and nitrogen-use efficiency. In both cases, improvements in drought tolerance and nitrogen-use efficiency are reported; however, the field evaluation studies to assess their performance are quite limited. Agronomists, plant physiologists, and soil scientists will need to collaborate with molecular biologists early in the evaluation phase in order that second-generation traits can be adequately evaluated in the field. New crops provide both economic and environmental benefits to growers; however, there are many agronomic and commercial challenges to be overcome for them to be widely adopted and successfully commercialized. The development of *Saponaria vaccaria* L. from a weed (cow cockle) to a potential crop is presented as a case study to outline the challenges of new crop development.

Additional keywords: abiotic stress, crop diversification, fitness traits, nutrient-use efficiency, transgenic crops

Introduction

Genetically-modified (GM) crops and crops with novel traits (CNT) have been rapidly adopted worldwide. The area devoted to transgenic crops has grown by about 10 million ha annually since their introduction in 1996; in 2009, this area was estimated to be 134 million ha worldwide (Marshall 2010). Crops with monogenic herbicide- and insect-resistance traits accounted for 74 and 16% of the total area, respectively. Since 2003, crops with stacked (two or more) traits have risen globally from 5.8 to 28.7 million ha in 2009, and make up 20% of the total area devoted to transgenic crops. In addition to herbicide and insect resistance, there are also traits for viral resistance, male sterility, and oil quality (Que et al. 2010). The major GM crops include soybean [*Glycine max* (L.) Merr.], corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), and canola (*Brassica napus* L.). In 2009, 8.2 million ha of GM crops were seeded in Canada, the fifth largest area seeded in the world (James 2009). Canola is the primary GM crop at 6.2 million ha, followed by soybean (1.4 million ha), corn (1.2 million ha), and sugarbeet (*Beta vulgaris* L.) (15,000 ha). Acetolactate synthase (ALS)-resistant canola, developed by mutagenesis, is considered a CNT and occupies about 6% of the total canola hectares; therefore, the area seeded to CNT canola is slightly higher than the 6.2 million ha (James 2009).

Second-generation crops with novel traits (SG-CNTs) provides both challenges and opportunities for weed scientists and agronomists. Traits being developed include tolerance to drought, cold, and salinity; nutrient-use efficiency; value-enhanced crops (crops with output characteristics modified to add end-user value); and those developed for molecular farming or bioproduct production (Jefferson-Moore and Traxler 2005; Warwick et al. 2009). Contrary to first-generation traits such as herbicide resistance, some of the traits in SG-CNTs are fitness traits and their environmental risk for invasiveness is difficult to predict (Beckie et al. 2010). On the other hand, it may be argued that traditional plant breeding has improved stress tolerance and improved nutrient-use efficiency in a crop such as canola without impacting its invasiveness. Agronomic research to support this argument will be provided herein.

The need for crop diversification in western Canada has been recognized since the late 1960s when cropping was dominated by spring wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and oat (*Avena sativa* L.) (Carlyle 2002). Declining spring wheat prices and changes to grain transportation policies have resulted in declining spring wheat hectares, and

compensatory increases in crops such as canola and pulses. The Prairie provinces are now the leading exporter of lentil (*Lens culinaris* L.), field pea (*Pisum sativum* L.), condiment mustard (*Brassica juncea* L. and *Sinapis alba* L.), and canaryseed (*Phalaris canariensis* L.) in the world (Johnson et al. 2010). In addition, the Prairies have thriving industries with a number of other non-traditional crops (Blade and Slinkard 2002).

Some 'new' crops under development have weedy characteristics and some have been or are currently listed as noxious weeds in the Canadian Weeds Seeds Order or in Provincial Noxious Weeds Acts. These include hemp (*Cannabis sativa* L.), camelina [*Camelina sativa* (L.) Crantz], cow cockle (*Saponaria vaccaria* L.), and field pennycress/stinkweed (*Thlapsi arvense* L.) (Minister of Justice 2005; The Queens Printer 1999; The Queens Printer for the Province of Manitoba 1987). Issues have been raised on the potential invasiveness of cellulosic biofuel crops, particularly non-native perennial species such as giant reed (*Arundo donax* L.) and the hybrid grass *Miscanthus x giganteus* (Barney and DiTomaso 2008; Raghu et al. 2006); however, there is limited development in biomass-derived energy currently underway in western Canada.

SG-CNTs and new crops provide agronomic, environmental, and economic opportunities and challenges for weed scientists, agronomists, plant breeders, producers, and regulators. This review will focus on agronomic opportunities and challenges; in addition, barriers to development and adoption of new crops will be discussed. Environmental issues surrounding SG-CNTs and new crops are a prime concern, particularly for regulators. Some of the issues include transgene movement to wild and weedy relatives, and potential for increased weediness and invasiveness of the crop. These issues will not be addressed, as they have been comprehensively addressed by Warwick et al. (2009).

Second-generation CNTs

Second-generation traits such as abiotic and biotic stress tolerance, nitrogen-use efficiency (NUE), and fungal resistance are currently being field tested in crops such as canola, wheat, barley, corn, and soybean in Canada (Warwick et al. 2009). Since canola is the primary CNT grown in western Canada, the discussion on second-generation traits will be limited to this crop. It is difficult to know all the second-generation traits being evaluated in canola due to proprietary information; however, Warwick et al.

(2009) list NUE and stress tolerance as traits currently being evaluated in canola.

Challenges – monoculture and loss of crop diversification

One primary argument against genetic modification is that it leads to transgenic monocultures (Altieri 2001). The widespread adoption of glyphosate-resistant soybean and corn has led to gene monoculture in many U.S. states, and an alarming rise in the number of glyphosate-resistant weeds (Powles 2008). Stacked traits will allow for tank-mixes with different modes of action, which is an effective means of managing herbicide resistance (Beckie and Reboud 2009); however, the impact of the corn-soybean rotation on soil resources, water quality, energy use, and biodiversity remains a concern (Karlen et al. 2006; Liebman et al. 2008). Will the introduction of second-generation traits in corn and soybean continue to encourage corn-soybean rotations and discourage crop diversification? Wheat growers in the United States (U.S.) have brought forward the argument for GM wheat development in that they are losing ground to corn and soybean (Anonymous 2009). The area seeded to corn and soybean has risen since the 1990s, while acreage of wheat, barley, and other crops has declined (Schnitkey 2010). From a western Canadian perspective, will the introduction of second-generation traits into canola lead to further canola intensification and erode the production of small acreage crops that are important to the Prairie economy?

GM canola was introduced in Canada in 1996. The areas seeded to canola fluctuated after its introduction, although there has been a steady increase since 2002 (Gan et al. 2010). The increase has come from more intensive cropping of canola in the black soil zone and more production in the non-traditional canola-growing areas of the southern Prairies. While canola hectares have increased, so has the hectareage of field pea and lentil (Gan et al. 2010). The increased hectareage of canola and pulse crops has come at the expense of spring wheat and summerfallow hectares. The area seeded to flax (*Linum usitatissimum* L.) and condiment mustard has seen a downward trend since 1999 (Fig. 1), and their respective commodity groups feel that they cannot compete with the improved weed control and higher seed yields of canola. In response, both groups have identified herbicide resistance as a high development priority, but markets will only allow CNTs produced through seed mutagenesis. The Flax Council of Canada and the

Government of Canada are making a \$5.5 million investment in site-directed mutagenesis with the hope of attaining glyphosate resistance (Dietz 2010).

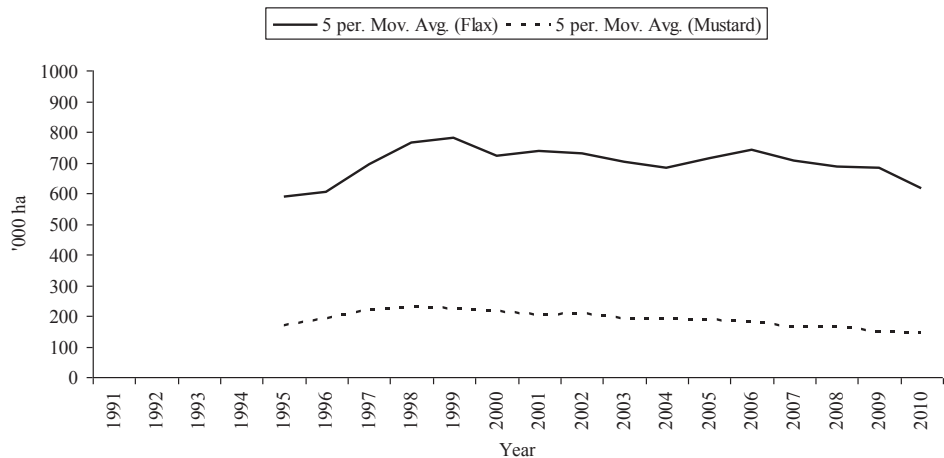


Figure 1. Five-year moving average of flax and mustard hectareage in the Canadian Prairies, 1991 to 2010 (source: Statistics Canada 2010).

Drought tolerance is an important trait that would allow canola expansion into southwest Saskatchewan and southern Alberta, areas where it is currently produced on less than 10% of the cropped area. Drought tolerance is a needed trait; in addition, the ability of canola to tolerate high temperatures particularly during the flowering phase would be desirable (Young et al. 2004). If oilseed production can be expanded in the southern Prairies, then one could argue that the introduction of a fitness trait may actually reduce monoculture in this area by diversifying the crop mix available to growers. It is possible that SG-CNTs may encourage growers in the traditional canola-growing areas to grow canola more frequently in the rotation; however, there are a number of reasons that it will not necessarily follow the same intensification as U.S. corn and soybean. Canola is not well suited to the introduction of industrial or pharmaceutical output traits due to issues with commingling and outcrossing (Smyth et al. 2004). Other crops that become bioplatfroms for industrial and output traits not allowed in canola may provide alternatives for growers. Pulse crops will remain an important crop in a producer's cropping system due to their nitrogen-fixing potential. Genetic modification of corn and soybean has certainly contributed to their popularity; however, one cannot underestimate the influence of U.S.

Farm Policy and the changes to the Freedom to Farm Act in the 1990s (Schnitkey 2010). Canadian producers tend to make their cropping decisions based on markets and less so on government programs. Still, it is prudent for governments to invest in new crop development and improvement of alternative crops to reduce the potential for canola monoculture.

Opportunities

Increasing grain yield, achieving maximum economic yield, yield stability, and improved grain quality are the goals of agronomists. In canola, the development of GM traits is striving to achieve these goals through improved moisture stress, drought stress, and improved NUE. Canola is very sensitive to moisture and drought stress, particularly during the flowering and seed-filling stage. Drought and heat avoidance strategies such as early seeding can reduce the impact of environmental stress at flowering (Kirkland and Johnson 2000); however, this is not always an option in many parts of the Prairies. Increasing the sensitivity of canola to abscisic acid through genetic transformation resulted in less stomatal conductance, and reduced leaf transpiration under drought conditions (Wang et al. 2005). Under moderate drought stress in both the laboratory and the field, the transformed plants were more tolerant to flower and seed abortion under conditions of moderate drought stress compared to untransformed parental plants. Under conditions of adequate water, transgenic canola plants produced the same amount of seed as the parental control.

It is difficult to predict the effect that a drought-tolerance trait will impart upon the weediness and invasiveness of a plant since few weeds exhibit high levels of drought tolerance (Warwick et al. 2009). Many weeds waste water since their stomata are less sensitive to declining soil water potential than domestic crops; however, C₄ weeds such as kochia [*Kochia scoparia* (L.) Schrad.] and Russian thistle (*Salsola tragus* L.) are more competitive with crops under dry soil conditions (Patterson 1995). Luxury consumption of water allows weeds to exhaust the water supply available to a competing crop.

One question that needs to be addressed with increased drought tolerance is, "Could this result in volunteers that are more difficult to control with herbicides?" Beckie et al (2003) reported that genetic transformation for herbicide resistance in canola did not cause pleiotropic effects, and that canola with herbicide-resistance genes exhibited similar tolerance to

alternative herbicides as did nontransgenic lines. It has been observed that many weeds are more difficult to control with herbicides when the plants are under drought stress conditions (Kudsk 2002). This may be due to a number of factors such as smaller leaves and thicker cuticles, and a higher wax deposit, which reduces herbicide retention and uptake. Furthermore, drought-stressed plants close their stomata; therefore, if the drought-tolerance trait causes earlier stomatal closure, could this impact herbicidal efficacy? The role of stomata in herbicide penetration is inconsistent and may play a minimal role (Devine et al. 1993); however, closure in stomata results in reduced photosynthesis and phloem translocation of assimilates, which may impact herbicide efficacy (Kudsk 2002). Control of drought-tolerant volunteers will need to be investigated prior to their unconfined release into the environment.

Nitrogen is one of the most important nutrients required by crop plants. Despite improvements in efficiency from better application methods, crop plants are generally able to extract and convert only 20 to 50% of the applied nitrogen, with the remainder bound to organic matrices within the soil or utilized by soil microbes and lost to the environment (Andrews et al. 2009; Strange et al. 2008). Estimates of efficiency of applied phosphorus and potassium fertilizer are about 10% and 40%, respectively (Baligar et al. 2001). Improving the efficiency by which plants take up, translocate, and assimilate applied fertilizer nutrients would provide economic and environmental benefits.

Nitrogen is one of the most expensive nutrients to supply (Good et al. 2004). Improvements in NUE of crops such as canola could reduce production costs, provide energy savings, and reduce environmental impact (Strange et al. 2008). There are a number of definitions and formulae used to describe NUE, but we will limit our definition to:

$$\text{NUE} = \text{Gw} / \text{Ns} \quad [1]$$

where Gw = grain weight (kg/ha) and Ns = nitrogen supply (kg/ha).

Plants can improve NUE in two ways: either extract nitrogen more efficiently (increased uptake efficiency) or assimilate the nitrogen they extract more efficiently (increased utilization efficiency) (Garnett et al. 2009). NUE in plants is a complex process that is controlled by a large number of genes acting individually or in combination (Hirel et al. 2001).

There are a number of genes that have been overexpressed in plants in an attempt to improve NUE (Good et al. 2004). GM canola plants that

overexpress alanine aminotransferase (*AlaAT*) have been engineered by introducing a gene from barley with a stress promoter (Good et al. 2007). Canola plant biomass and seed yield were higher in the transformed plants compared to a wild-type canola under low nitrogen conditions in both laboratory and greenhouse studies. Yields were similar between the transformed and wild-type under high nitrogen conditions. In a limited number of field trials, a 40% decrease in the amount of applied nitrogen was required for the transgenic plants to yield the same as wild-type canola plants.

Nitrogen-use efficiency may affect competitiveness of a plant; however, it is unknown how it might affect invasiveness (Warwick et al. 2009). The NUE of canola has improved dramatically with traditional plant breeding, the introduction of canola hybrids, and improved agronomic practices. The NUE of hybrids ranges from 10 to 35% higher than open-pollinated cultivars (Fig. 2). This increase in NUE is similar to the improvement in NUE that Good et al. (2007) reported in genetic transformation of an older open-pollinated canola cultivar. Therefore, it is unknown whether the genetic transformation is just improving the NUE of the OP cultivar to the level of current high-yielding hybrids or whether the transgene would provide similar benefits to high-yielding hybrids. An understanding of the reasons for improved NUE with hybrid canola is required to further improve the NUE of canola.

While genetic modification for herbicide resistance has had a major impact on controlling weeds in canola (Harker et al. 2007), significant canola cultivar improvements such as disease resistance, vigour and weed competitiveness, uniform maturity, NUE, and seed yield have also occurred from traditional plant breeding and hybridization (Beckie et al. 2008a, 2008b; Brandt et al. 2007; Smith et al. 2010). Have these improvements increased the invasiveness of canola? Should one be concerned with invasiveness of a transgene that improves the abiotic stress tolerance or NUE of canola when there is some indication that traditional plant breeding has provided similar improvements? There does not appear to be any evidence that traditional plant breeding has increased the invasiveness of canola; therefore, regulators should take this into account when conducting environmental assessments of SG-CNTs, particularly those that deal with abiotic stresses and improvements in NUE.

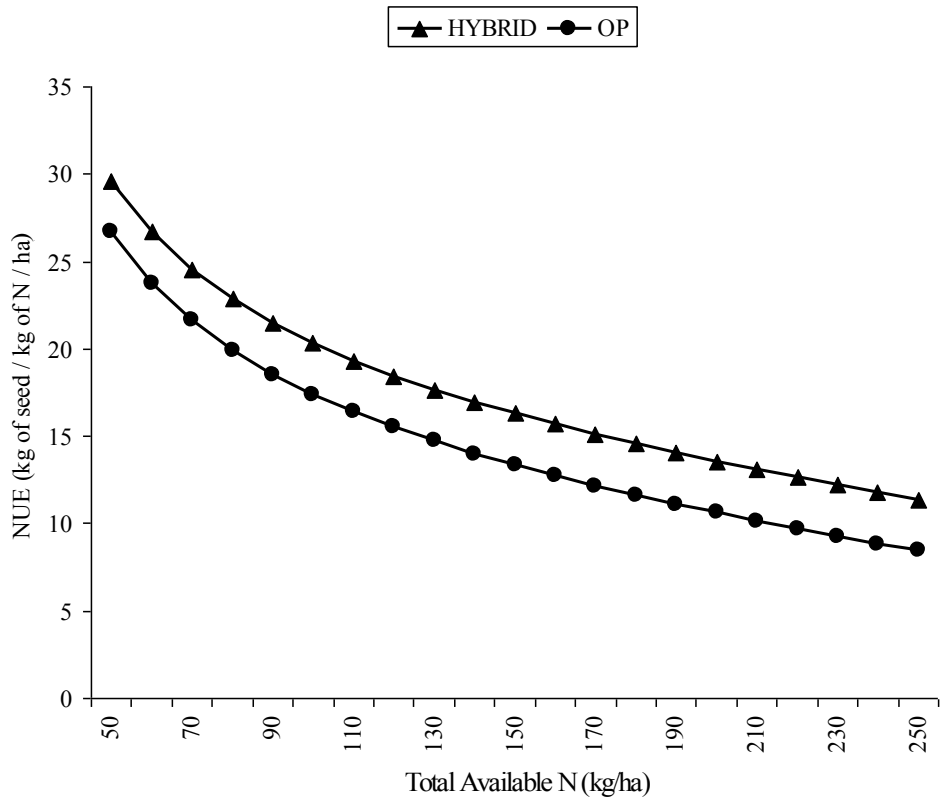


Figure 2. Nitrogen-use efficiency of hybrid vs. open-pollinated (OP) canola (adapted from Smith et al. (2010)).

In reviewing the literature on traits that impart drought tolerance and NUE in canola, it became apparent that the inclusion of plant physiologists and agronomists in the preliminary evaluation and field testing of these traits is desperately needed. Many of the transformations are conducted in inferior germplasm, which may be necessary from a molecular point of view; however, superior germplasm needs to be included as checks. The methodology of field testing drought tolerance is somewhat questionable in Wang et al. (2005) since irrigation treatments were not replicated, although trends were consistent over years. A properly designed field facility to test abiotic stress tolerance is urgently needed in western Canada. A second option is to evaluate the transformed and untransformed cultivars over a number of diverse environments to adequately evaluate the genotype by environment interactions. Both are costly, particularly during the confined-release stage of field evaluation; however, proper evaluation is critical to

ensure that the transformed plants deliver what they are advertised to do and are not just an artefact of the experimental process.

Nitrogen-use efficiency is not that well understood by growers, and its delivery may be over-promised at this time. In order to fully exploit NUE in canola, an understanding of existing variability of NUE in non-transformed canola germplasm is required. This has been done with corn by using a quantitative genetic approach that associates metabolic functions and agronomic traits to DNA markers (Hirel et al. 2001). Observed differences in NUE between genotypes can be dependent on whether plants are grown in soils of high or low nitrogen concentration (Garnett et al. 2009). Genetic variation in corn NUE at high nitrogen levels is related to differences in nitrogen uptake efficiency, while at low nitrogen, it was related to differences in nitrogen-utilization efficiency. As with drought and abiotic stress, more collaboration between soil chemists, plant physiologists, agronomists, and molecular biologists is required. It may be desirable to provide agronomists with molecular training (or vice versa) and develop a field of 'molecular agronomy' in order to properly evaluate the potential of SG-CNTs.

New crops – opportunities and challenges

Inclusion of alternative crops such as legumes in cereal-based cropping systems improves water-use efficiency, reduces production risk, and enhances economic sustainability (Johnson et al. 2010). Crop diversification has led to a reduction in summerfallow area in western Canada, which minimizes the risk of soil degradation (Gan et al. 2010). Diversification into canola and pulse crops has also resulted in local processing compared to the predominately export spring wheat economy of the 1960s (Carlyle 2002). Local processing provides employment and other economic spin-offs, particularly for rural areas.

According to Blade and Slinkard (2002), the success of crop diversification in western Canada is due to four major influences: (1) the need to diversify crop production due to large global surpluses in some of our primary crops; (2) a large agricultural land area available with a diversity of climatic conditions; (3) motivated, knowledgeable growers who are willing to adopt new technologies and embrace new opportunities; and (4) a strong research community, and well-organized industry and commodity groups that support new crop research and market development.

To meet the growing world oilseed demand, there is a need to expand the oilseed production area from the cooler, wetter black soil zone to the warmer, drier brown soils of the southern Prairies. Plant breeding programs and agronomic studies are underway to adapt camelina and Ethiopian mustard (*Brassica carinata* A. Braun) to western Canada (Gugel and Falk 2006; Taylor et al. 2010). Camelina has been shown to be well adapted to the brown and dark brown soil zones providing similar yields to a high-yielding canola hybrid in six of nine site-years (Blackshaw et al. 2011). Agronomic evaluations and opportunities for camelina are discussed elsewhere in this monograph (see Grushcow (2011) and May et al. (2011) this volume), and have also been recently reviewed by Johnson et al. (2010). Taylor et al (2010) provide a comprehensive review of the breeding advances and potential uses of Ethiopian mustard. The crop requires a long growing season due to its late maturity; however, seeding rates that result in plant densities of 80 to 170 plants/m² has been shown to reduce days to physiological maturity by 5 to 7 d (Johnson, unpublished data). Blackshaw et al. (2011) reported that oils of both camelina and Ethiopian mustard were easily converted to biodiesel, and quality analyses indicated they would be suitable for biodiesel feedstock.

The emerging Canadian and global plant-based bioeconomy will require the identification of new crops and modification of existing crops to provide the feedstock demand (Chapotin and Wolt 2007). CNTs will be imperative to the success of biorefineries (Gressel 2008); however, regulatory approvals, market adoption, and public acceptance will present major challenges to their introduction (Chapotin and Wolt 2007). In anticipation of the need for CNTs to fulfill the feedstock demand of the emerging bio-based economy, a number of domesticated crops have undergone tiered environmental biosafety evaluations in western Canada, including flax (Jhala et al. 2009, 2010), triticale (*X Triticosecale* Wittmack) (Hills et al. 2007), and safflower (*Carthamus tinctorius* L.) (McPherson et al. 2004, 2008, 2009). Similar studies are underway for camelina (L. Hall, unpublished data). These biosafety evaluations provide a description of the biology of the crop, which is required prior to modification in order to provide comparative data with the CNT. The following are the five key criteria of environmental safety assessments: altered weediness potential; potential for outcrossing; altered plant pest potential; impact on non-target organisms, and other impacts on biodiversity (Canadian Food Inspection Agency 2010). Whether these crops will be chosen as platforms for novel industrial and/or pharmaceutical traits remains to be seen.

Many new crops under development have limited weed control options. Both camelina and Ethiopian mustard will require broadleaf weed control options for producers to consider growing them on a large scale. To date, screening of potential broadleaf herbicides has been relatively unsuccessful; therefore, genetic transformation or mutagenesis may be required to provide herbicide resistance. This will add to the expense of developing these crops due to the regulatory costs involved.

Another challenge for developing new crops is finding research dollars for breeding and agronomic studies. The Province of Saskatchewan has been very supportive in providing research funding for the development of both camelina and Ethiopian mustard. Most new crops are being commercialized by small companies with limited financial resources. As an agronomist or plant breeder, one must look at the opportunity cost of conducting research on new crops. An improvement of 1% in the performance of canola has a huge economic benefit for an industry that generates \$14 billion dollars annually. It will take many years of research and development for a new crop to provide that level of economic impact. Additionally, simple reductionist studies like seeding rate, fertilizer response, and pesticide screening are required in the early development of new crops. This basic knowledge is required before multi-factor system trials can be conducted; therefore, publication of results in journals with high impact factors is unlikely in the developmental stage. An excellent review on the opportunities and challenges for new crop development is provided by Blade and Slinkard (2002). Other challenges listed by these authors include: lack of market development, a 'get-rich quick mentality' and false promotion of new crop attributes, a lack of investment capital, and lack of government-support programs such as crop insurance.

***Saponaria vaccaria* – a case study in new crop development**

Ralph Waldo Emerson described a weed as a "plant whose virtues have not been discovered". *Saponaria vaccaria*, commonly known as cow cackle, has the potential to fit this definition. It was first investigated as an oilseed crop, but the seed has very low oil content and was found to contain a high concentration of starch (64%) with some unique properties (Goering et al. 1966). Mazza et al. (1992) reported that the starch granules were densely packed, fine and round, making it desirable for the cosmetic industry. In addition to its unique starch characteristics, *Saponaria vaccaria* seed contains unique saponins as well as other potential medicinal compounds

such as cyclic peptides (Balsevich et al. 2006). A landrace of cow cockle was inadvertently developed at the Scott Research Farm. Wild cow cockle seed was collected from a farm near Regina in the 1970s and was multiplied for weed control trials. Wild mustard (*Sinapis arvensis* L.) could not be controlled in the cow cockle multiplication plots; therefore, the wild mustard seed was removed by hand-screening. This cleaning also removed small cow cockle seeds. The cleaned, large seed was multiplied and large seed was inadvertently selected for over time. This resulted in a 'Scott' landrace with seed that has an average thousand kernel weight of 6.9 g, compared to 3.9 g for wild cow cockle seed (Johnson, unpublished data). In addition to its large seed size, desirable agronomic characteristics of the Scott landrace included: relatively uniform emergence and seed vigour; days to physiological seed maturity similar to spring wheat; and good resistance to seed shattering. It also exhibited good yield potential, with yields ranging from 1,500 to 2,500 kg/ha (Johnson, unpublished data). In addition to these desirable traits, the wild cow cockle species declined in relative abundance and distribution in weed surveys conducted on the Prairies from the 1970s to the 2000s (Thomas and Leeson 2007). A number of herbicides are also available to control volunteer plants (Saskatchewan Ministry of Agriculture 2011). Based on its desirable seed composition and agronomic traits, a small company based out of Saskatoon attempted to commercialize the plant under the trademark name "Prairie Carnation".

Interestingly, one of the main agronomic challenges of *Saponaria vacarria* was broadleaf weed control, although some potential solutions were identified in screening trials (Johnson, unpublished studies). Isoxaflutole was one herbicide that the crop tolerated and provided efficacious weed control; however, its potential for carryover and injury to rotational crops limited its use on Prairie soils and it was not supported for registration. Other agronomic issues that need further addressing are seed dormancy and seed bank persistence, and its susceptibility to *Alternaria* leaf diseases when grown in monoculture. Unfortunately, the Saskatoon-based company ran into financial trouble in 2009; thus, the commercialization of this species is currently on hold.

Conclusions

Second generation CNTs provide new cropping opportunities for western Canadian producers. In order to fully capitalize on the potential and

to address the challenges of SG-CNTs, agronomists need to become an important collaborator with molecular biologists much earlier in the evaluation stage. Significant efforts are required to develop research protocols that will adequately evaluate CNTs that have been genetically modified to tolerate abiotic stress or to be more nutrient-use efficient. Ecologists and regulators should look at the impact that traditional plant breeding has had on invasiveness of a crop when trying to evaluate the impact of some second-generation traits. Crop diversification has been critical for the survival of agriculture on the Canadian Prairies, and will continue to be in the future. For a plant breeder or agronomist, it is challenging to determine the potential of a new crop in its infancy. Typically, there are more failures than successes; therefore, scientists must accept the high risk/reward associated with new crop development. It remains to be seen whether the promise of SG-CNTs or new crops such as camelina and Ethiopian mustard will be fully realized in western Canada.

Literature cited

- Altieri, M.A. 2001. The ecological impacts of agricultural biotechnology. [Online] Available: <http://www.actionbioscience.org/biotech/altieri.html>.
- Andrews, M., P.J. Lea, J.A. Raven and R.A. Azevedo. 2009. Nitrogen use efficiency. 3. Nitrogen fixation, genes and costs. *Ann. Appl. Biol.* 155:1-13.
- Anonymous. 2009. The case for biotech wheat. Industry paper prepared by the National Association of Wheat Growers, U.S. Wheat Associates, North American Millers' Association, Independent Bakers Association and the Wheat Foods Council. [Online] Available: <http://www.smallgrains.org/PGConf08/Coppock1.pdf>.
- Baligar, V.C., N.K. Fageria and Z.L. Hue. 2001. Nutrient use efficiency in plants. *Commun. Soil Sci. Plant Anal.* 32:921-950.
- Balsevich, J.J., G.G. Bishop and I. Ramirez-Erosa. 2006. Analysis of bisdesmosidic saponins in *Saponaria vaccaria* L. by HPLC-PAD-MS: identification of new quillaic acid and gypsogenin 3-O-trisaccharides. *Phytochem. Anal.* 17:414-423.
- Barney, J.N. and J.M. DiTomaso. 2008. Nonnative species and bioenergy: are we cultivating the next invader? *Bioscience* 58:64-70.
- Beckie, H.J., L.M. Hall, M.-J. Simard, J.Y. Leeson and C.J. Willenborg. 2010. A framework for post-release environmental monitoring of second-generation crops with novel traits. *Crop Sci.* 50:1587-1604.
- Beckie, H.J., E.N. Johnson, R.E. Blackshaw and Y.T. Gan. 2008a. Productivity and quality of canola and mustard cultivars under weed competition. *Can. J. Plant Sci.* 88:367-372.

- Beckie, H.J., E.N. Johnson, R.E. Blackshaw and Y.T. Gan. 2008b. Weed suppression by canola and mustard cultivars. *Weed Technol.* 22:182-185.
- Beckie, H.J. and X. Reboud. 2009. Selecting for weed resistance: herbicide rotation and mixture. *Weed Technol.* 23:363-370.
- Beckie, H.J., G. Séguin-Swartz, H. Nair, S.I. Warwick and E. Johnson. 2003. Multiple herbicide-resistant canola (*Brassica napus*) can be controlled by alternative herbicides. *Weed Sci.* 52:152-157.
- Blackshaw, R.E., E.N. Johnson, Y. Gan, W.E. May, D.W. McAndrew, V. Barthet, T. McDonald and D. Wispinsk. 2011. Alternative oilseed crops for biodiesel feedstock on the Canadian Prairies. *Can. J. Plant Sci.* 91:889-895.
- Blade, S.F. and A.E. Slinkard. 2002. New crop development: the Canadian experience. Pages 62-75 in J. Janick and A. Whipkey, eds. *Trends in New Crops and New Uses*. Alexandria, VA: ASHS Press.
- Brandt, S.A., S.S. Malhi, D. Ulrich, G.P. Lafond, H.R. Kutcher and A.M. Johnston. 2007. Seeding rate, fertilizer level and disease management effects on hybrid versus open pollinated canola (*Brassica napus* L.). *Can. J. Plant Sci.* 87:255-266.
- Canadian Food Inspection Agency. 2010. Conducting Confined Research Field Trials of Plants with Novel Traits in Canada. Canadian Food Inspection Agency Regulatory Directive 2000-07. [Online] Available: <http://www.inspection.gc.ca/english/plaveg/bio/dir/dir0007e.shtml>.
- Carlyle, W.J. 2002. Cropping patterns in the Canadian Prairies: thirty years of change. *Geogr. J.* 168:97-115.
- Chapotin, S.M. and J.D. Wolt. 2007. Genetically modified crops for the bioeconomy: meeting public and regulatory expectations. *Transgenic Res.* 16:657-688.
- Devine, M.D., S.O. Duke and C. Fedtke. 1993. *Physiology of Herbicide Action*. Englewood Cliffs, NJ: Prentice-Hall. 441 pp.
- Dietz, J. 2010. Non-GMO path opens for flax and canola. *Top Crop Manager*. [Online] Available: <http://www.topcropmanager.com/content/view/5293/59/>.
- Gan, Y., H.R. Kutcher, F. Menalled, G.P. Lafond and S.A. Brandt, 2010. Crop diversification and intensification with broadleaf crops in cereal-based cropping systems in the Northern Great Plains of North America. Pages 277-301 in S.S. Malhi, Y. Gan, J.J. Schoenau, R.L. Lemke and M.A. Liebig, eds. *Recent Trends in Soil Science and Agronomy Research in the Northern Great Plains of North America*, Trivandrum, Kerala, India: Research Signpost.
- Garnett, T., V. Conn and B.A. Kaiser. 2009. Root based approaches to improving nitrogen use efficiency in plants. *Plant Cell Environ.* 32:1272-1283.
- Goering K.J., R.F. Eslick, C.A. Watson and J. Keng. 1966. Utilization and agronomic studies of cow cockle (*Saponaria vaccaria*). *Econ. Bot.* 20:429-433.
- Good, A.G., S.J. Johnson, M. de Pauw, R.T. Carroll, N. Savidov, J. Vidmar, Z. Lu, G. Taylor and V. Stroehrer. 2007. Engineering nitrogen use efficiency with alanine aminotransferase. *Can. J. Bot.* 85:252-262.

- Good, A.G., A.K. Shrawat and D.G. Muench. 2004. Can less yield more? Is reducing nutrient input into the environment compatible with maintaining crop production? *Trends Plant Sci.* 9:597-605.
- Gressel, J. 2008. Transgenics are imperative for biofuel crops. *Plant Sci.* 181:246-263.
- Gugel, R.K. and K.C. Falk. 2006. Agronomic and seed quality evaluation of *Camelina sativa* in western Canada. *Can. J. Plant Sci.*, 86:1047-1058.
- Harker, K.N., G.C. Clayton and H.J. Beckie. 2007. Weed management with herbicide resistant crops in western Canada. Pages 15-31 in R.H. Gulden and C.J. Swanton, eds. *The First Decade of Herbicide-Resistant Crops in Canada. Topics in Canadian Weed Science, Volume 4.* Sainte Anne de Bellevue, Québec: Canadian Weed Science Society.
- Hills, M.J., L.M. Hall, D.F. Messenger, R.J. Graf and B.L. Beres. 2007. Evaluation of crossability between triticale (X *Triticosecale* Wittmack) and common wheat, durum wheat, and rye. *Environ. Biosafety Res.* 6:249-257.
- Hirel, B., P. Bertin, I. Quillerè, W. Bourdoncle, C. Attagnant, C. Dellay, A. Gouy, S. Cadiou, C. Retalliau, M. Falque and A. Gallais. 2001. Towards a better understanding of the genetic and physiological basis for nitrogen use efficiency in maize. *Plant Physiol.* 125: 1258-1270.
- James, C. 2009. Global Status of Commercialized Biotech/GM Crops: 2009. ISAAA Briefs No. 41. Ithaca, NY: ISAAA. [Online] Available: <http://www.isaaa.org/resources/publications/briefs/41/executivesummary/default.asp>.
- Jefferson-Moore, K.Y. and G. Traxler. 2005. Second-generation GMOs: where to go from here? *AgBioForum* 8:143-150.
- Jhala, A., H. Bhatt, K. Topinka and L.M. Hall. 2010. Pollen mediated gene flow in flax (*Linum usitatissimum* L.): can genetically engineered and organic flax coexist? *Heredity* 105:1-10.
- Jhala, A., R.J. Weselake and L.M. Hall. 2009. Genetically engineered flax: potential benefits, risks, regulations, and mitigation of transgene movement. *Crop Sci.* 49:1943-1954.
- Johnson, E.N., Y. Gan, W.E. May, P. Balasubramanian, C. Vera, K. Friesen and A. Hermann 2010. Selected special crops on the Canadian Great Plains. Pages 245-275 in S.S. Malhi, Y. Gan, J.J. Schoenau, R.L. Lemke and M.A. Liebig, eds. *Recent Trends in Soil Science and Agronomy Research in the Northern Great Plains of North America.* Trivandrum, Kerala, India: Research Signpost.
- Karlen, D.L., E.G. Hurley, S.S. Andrews, C.A. Cambardella, D.W. Meek, M.D. Duffy and A.P. Mallarino. 2006. Crop rotation effects on soil quality at three northern corn/soybean belt locations. *Agron. J.* 98:484-495.
- Kirkland, K.J. and E.N. Johnson. 2000. Alternative seeding dates (fall and April) affect *Brassica napus* canola yield and quality. *Can. J. Plant Sci.* 80:713-719.

- Kudsk, P. 2002. Optimising herbicide performance. Pages 323-353 in R.E.L. Naylor, ed. *Weed Management Handbook* (ninth edition). Oxford, UK: The British Crop Protection Council and Blackwell Science Ltd.
- Liebman, M., L.R. Gibson, D.N. Sundberg, A.H. Heggenstaller, P.R. Westerman, C.A. Chase, R.G. Hartzler, F.D. Menalled, A.S. Davis and P.M. Dixon. 2008. Agronomic and economic performance characteristics of conventional and low-external-input cropping systems in the central corn belt. *Agron. J.* 100:600-610.
- Marshall, A. 2010. 2nd-generation GM traits progress. *Nature Biotech.* 28:306.
- Mazza, G., C.G. Biliaderis, R. Przybylski and B.D. Oomah. 1992. Compositional and morphological characteristics of cow cockle (*Saponaria vaccaria*) seed, a potential alternative crop. *J. Agric. Food Chem.* 40:1520-1523.
- McPherson, M.A., A.G. Good, A.K.C. Topinka and L.M. Hall. 2004. Theoretical hybridization potential of transgenic safflower (*Carthamus tinctorius* L.) with weedy relatives in the New World. *Can. J. Plant Sci.* 84:923-934.
- McPherson, M.A., A.G. Good, A.K.C. Topinka, R. Yang, R.H. McKenzie, R.J. Cathcart, J. A. Christianson, C. Strobeck and L.M. Hall. 2009. Pollen mediated gene flow from transgenic safflower (*Carthamus tinctorius* L.) intended for plant molecular farming to conventional safflower. *Environ. Biosafety Res.* 8:19-32.
- McPherson, M.A., R. Yang, A.G. Good, R.L. Nelson and L.M. Hall. 2008. Potential for seed-mediated gene flow in agroecosystems from transgenic safflower (*Carthamus tinctorius* L.) intended for plant molecular farming. *Transgenic Res.* 17:281-299.
- Minister of Justice. 2005. Weed Seeds Order. SOR/2005-220. Ottawa, ON. 8 pp.
- Patterson, D.T. 1995. Effects of environmental stress on weed/crop interactions. *Weed Sci.* 43: 483-490.
- Powles, S.B. 2008. Evolved glyphosate-resistant weeds around the world: lessons to be learnt. *Pest Manag. Sci.* 64:360-365.
- Que, Q., M.M. Chilton, C.M. de Fontes, C. He, M. Nuccio, T. Zhu, Y. Wu, J.S. Chen and L. Shi. 2010. Trait stacking in transgenic crops. *GM Crops* 1:1-10.
- Raghu, S., R.C. Anderson, C.C. Daehler, A.S. Davis, R.N. Wiedenmann and D. Simberloff. 2006. Adding biofuels to the invasive species fire? *Science* 313:1742.
- Saskatchewan Ministry of Agriculture. 2011. Guide to Crop Protection. Regina, SK: Saskatchewan Ministry of Agriculture. 474 pp.
- Schnitkey, G. 2010. Changes in crop acres since Freedom to Farm. *Farm Economics: Facts and Opinions*. Dept. of Agricultural and Consumer Economics. Urbana-Champaign, IL. University of Illinois. 3 pp.
- Smith, E.G., B.M. Upadhyay, M.L. Favret and R.E. Karamanos. 2010. Fertilizer response for hybrid and open-pollinated canola and economic optimal nutrient levels. *Can. J. Plant Sci.* 90:305-310.

- Smyth, S., G.G. Khachatourians and P. Phillips. 2004. The liability from regulating gene flow in plant-made pharmaceuticals. *Biotechnol. Genet. Eng. Rev.* 21:277-297.
- Statistics Canada. 2010. Table 001-0010 – Estimated Areas, Yield, Production, and Average Farm Price of Principal Field Crops, in metric units, annual, CANSIM (database). [Online] Available: <http://cansim2.statcan.gc.ca/cgi-win/cnsmcgi.exe?Lang=E&CNSM-Fi+CII/CII-1-eng.htm>.
- Strange, A., J. Park, R. Bennett and R. Phipps. 2008. The use of life-cycle assessment to evaluate the environmental impacts of growing genetically modified, nitrogen use-efficient canola. *Plant Biotech. J.* 6:337-345.
- Taylor, D.C., K.C. Falk, C.D. Palmer, J. Hammerlindl, V. Babic, E. Mietkiewska, A. Jadhav, E. Marilla, T. Francis, T. Hoffman, E. Giblin, V. Katavic and W. Keller. 2010. *Brassica carinata* – a new molecular farming platform for delivering bio-industrial oil feedstocks: case studies of genetic modifications to improve very long-chain fatty acid and oil content in seeds. *Biofuels Bioprod. Bioref.* 4:538-561.
- The Queens Printer. 1999. The noxious weeds designation regulations. Chapter N-9.1 Reg 2 of the Saskatchewan Noxious Weeds Act 1984. Regina, SK. 4 pp.
- The Queens Printer for the Province of Manitoba. 1987. The Noxious Weeds Act. C.C.S.M. c. N110. Winnipeg, MB. 36 pp.
- Thomas, A.G. and J.Y. Leeson. 2007. Tracking long-term changes in the arable weed flora of Canada. Pages 43-67 in D.R. Clements and S.J. Darbyshire, eds. *Invasive Plants: Inventories, Strategies, and Action. Topics in Canadian Weed Science, Volume 4.* Sainte Anne de Bellevue, Québec: Canadian Weed Science Society.
- Wang, Y., Y. Jifeng, M. Kuzma, M. Chalifoux, A. Sample, C. McArthur, T. Uchacz, C. Sarvas, J. Wan, D.T. Dennis, P. McCourt and Y. Huang. 2005. Molecular tailoring of farnesylation for plant drought tolerance and yield protection. *The Plant J.* 43:413-424.
- Warwick, S.I., H.J. Beckie and L.M. Hall. 2009. Gene flow, invasiveness and ecological impact of GM crops. *The Year in Evolutionary Biology 2009.* Ann. N.Y. Acad. Sci. 1168:72-99.
- Young, L.W., R.W. Wilen and P.C. Bonham-Smith. 2004. High temperature stress of *Brassica napus* during flowering reduces micro- and meagametophyte fertility, induces fruit abortion, and disrupts seed production. *J. Exp. Bot.* 55:485-495.

Disease implications of canola-intensive crop rotations

H. Randy Kutcher¹, Christina Eynck², Bruce D. Gossen², T. Kelly Turkington³, Stewart A. Brandt⁴ and Kequan Xi³

¹College of Agriculture and Bioresources, University of Saskatchewan, 3C02 Agriculture Building, 51 Campus Drive, Saskatoon, SK S7N 5A8; ²AAFC, 107 Science Place, Saskatoon, SK S7N 0X2; ³AAFC, 6000 C and E Trail, Lacombe, AB T4L 1W1; and ⁴AAFC, P.O. Box 10, Scott, SK S0K 4A0

Email: randy.kutcher@usask.ca

Recent technological improvements in canola (*Brassica napus* L.) have enhanced effective control of two difficult pests. Herbicide resistance has facilitated a high level of broad-spectrum weed control, and good blackleg [*Leptosphaeria maculans* (Desmaz.) Ces. & de Not.] resistance has significantly reduced the damage caused by this disease. Unfortunately, the benefit of these technologies may be their undoing as canola growers intensify production practices in response to market demand. Cultivation of a diverse mix of field crops in a rotation has been used for thousands of years as a strategy to mitigate plant diseases and to deal with other agronomic issues. Crop rotation is the foundation for successful crop production and the maintenance of host-plant resistance for many plant diseases, as well as the inhibition of fungicide insensitivity. Inclusion of non-host crops in the rotation effectively removes the selection pressure for these pathogen races within the particular field. However, the number of crop species grown in the rotation must be sufficient to allow residue breakdown of each crop that harbours the important pathogens before the crop is grown again. This paper discusses blackleg of canola to illustrate the beneficial effects of crop rotation in disease management and the potential pitfalls of intensive production. The principles discussed apply to other diseases of canola, as well as diseases of many other field crops.

Additional keywords: disease risk mitigation, blackleg, *Brassica napus*, canola, *Leptosphaeria maculans*

Intensification of canola rotations

Technological progress has facilitated the grower's ability to increase canola production from the initial development of the crop to the present. In the past, weed and disease management issues were major impediments to production of canola. Controlling weeds and mitigating blackleg disease [*Leptosphaeria maculans* (Desmaz.) Ces. & de Not.] were managed by following a 4-yr rotation (one canola crop no more than once every 4 years on a particular field). However, the development of herbicide-resistant canola cultivars has facilitated weed control and reduced the importance of crop rotation in weed management. Similarly, the development of cultivars with strong resistance to blackleg, and more recently to clubroot [*Plasmidiophora brassicae* Woronin] and sclerotinia stem rot [*Sclerotinia sclerotiorum* (Lib.) de Bary] is interpreted by many growers as removing the need for crop rotation to reduce canola diseases.

High prices for canola relative to other crops provide an incentive to grow canola as frequently as possible. For many growers in western Canada, canola is the most profitable crop in their rotation, and it has a positive impact on cash flow because it may be sold as a cash crop without the restrictions or delays imposed on other crops such as wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.). Promotion by the canola industry to reach the goal of 15 million tonne of production by 2015 (Canola Council of Canada 2008) complements this economic incentive. This increase in production is projected to be necessary to meet the expected increase in demand for increased consumption of healthy cooking oils such as canola, particularly in China, and for biodiesel produced from the energy-rich canola seed (Zegada-Lizarazu and Monti 2010).

The impact of crop rotation on disease

Crop rotation, defined as the cultivation of several crop species in a field over time, has been used for thousands of years in field crop production because of the yield benefits that arise (Bullock 1992; Karlen et al. 1994). Recent research supports these observations, demonstrating that a diverse selection of crops grown in rotation increases overall crop productivity (Bailey et al. 2000; Johnston et al. 2005). Crop rotation is a fundamental control strategy for many plant pests, especially diseases (Curl 1963). One important benefit of a diverse crop rotation is to reduce the population of major pathogens, in order that significant disease damage does not occur

during production of the subsequent crop. This reduction occurs because crop rotation affects the growth, survival, and reproduction of pathogens. Increasing the species diversity of the crops cultivated in a field over time reduces genetic uniformity, and thus improves disease management because disease proliferation and severity are limited by the population's genetic diversity, as well as the host's genetic makeup and the variation among genotypes (Zadoks and Schein 1979).

The effectiveness of crop rotation to control any particular plant disease will depend on the nature of the pathogen. Good candidates for control through crop rotation are pathogens that have a limited host range, such as those that cause disease on only one of the crop species in the rotation, and that do not survive as long-lived resting structures in the soil, such as *L. maculans*. Pathogens with a wide host range that can also survive a long time in an inactive state in the absence of host plants or residue, such as clubroot and sclerotinia, will be less well controlled by crop rotation.

Generally, the greater the differences among crops in a rotation sequence, the better the control of pests that can be expected (Francis and Clegg 1990). On the Canadian Prairies, production of annual field crops is restricted to one crop per year, so that over 3 to 5 yrs, three to five different annual field crop species might be grown. Growing cereals, pulse crops, and even other oilseeds in rotation with canola should mitigate many diseases of canola, compared to intensive canola rotations. Cereals have few diseases in common with canola, with the exception of some root and seedling diseases such as fusarium and rhizoctonia (Bailey et al. 2003). Pulse crops such as field pea (*Pisum sativum* L.) and other oilseeds such as flax (*Linum usitatissimum* L.) share only a few diseases with canola, the most common being sclerotinia stem rot.

Camelina [*Camelina sativa* (L.) Crantz] (also known as false flax) is a new oilseed crop that may have a place in a rotation with canola in the drier regions of western Canada. Although camelina has a number of diseases in common with canola, it is highly resistant to alternaria black spot (Kolte et al. 1991; Plümper and Sacristan 1991; Sharma et al. 2002) and blackleg (Li et al. 2005; Salisbury 1987). Isolates of *L. maculans* common to western Canada failed to infect the cotyledons of camelina accessions under controlled conditions (Séguin-Swartz and Etienne, unpublished data). Including camelina in a canola rotation would therefore contribute to the mitigation of the disease pressure caused by these two pathogens. Similar to flax or pulse crops, the disease likely to be of potentially greatest consequence to both camelina and canola is sclerotinia stem rot (Séguin-

Swartz et al. 2009), although clubroot could also be a problem in some areas of western Canada.

Crop rotation cannot be expected to completely eradicate a particular pathogen since many pathogens have more than one way to survive. Pathogens may infect susceptible volunteer plants and weed species, or they may survive for prolonged periods on crop residue or as resting structures in the soil. Additionally, some pathogens may also move relatively long distances from inoculum sources in nearby fields. The primary aim of crop rotation is to reduce and maintain the population of the pathogen at a sufficiently low level to limit damage to an acceptable level in subsequent susceptible crop species. Under continuous monoculture, conditions are conducive for pathogens to continue their life cycle with no break or interruption, resulting in rapid multiplication and increase in disease severity. By growing a number of crop species, pathogens with a relatively narrow host range and without the capacity for long survival or dispersal will not survive in the absence of a suitable host.

Effect of rotation on blackleg of canola

Blackleg of canola is an example of a disease that is effectively mitigated through host genetic resistance when combined with a sustainable crop rotation. Blackleg became a problem for western Canadian growers in the mid-1970s (McGee and Petrie 1978), and yield losses of as much as 50% were reported in individual fields by the 1980s (Gugel and Petrie 1992). Growing canola in a 4-yr rotation has been a recommended strategy to deal with blackleg (Rimmer et al. 2003). A rotation of 4 yrs or more allows much of the residue to decompose before canola is grown again. Adherence to a 4-yr rotation and the availability of blackleg-resistant cultivars in the early 1990s (Saskatchewan Agriculture and Food 1993) proved to be a reliable strategy to mitigate this disease for most canola growers in western Canada as indicated by recent disease surveys (Dokken-Bouchard et al. 2010). Yield and quality losses associated with the disease were dramatically reduced. Although rotation can reduce *L. maculans*, it is unlikely to eradicate the pathogen because it survives on crop residue and by infecting volunteer plants or weed species. Therefore, control of volunteer canola and weeds is an important management tool for blackleg that is compromised by short canola rotations, even with good herbicide rotation.

Seeding canola into canola stubble with no break between crops consistently results in lower yields than seeding canola into stubbles of other crop species (Cathcart et al. 2006; Christen and Sieling 1995; Johnston et al. 2005; Krupinsky et al. 2006). Blackleg severity was highest and yield reduced in canola seeded into canola stubble compared with canola seeded into the stubble of other crops (Johnston et al. 2005). Blackleg disease was also found to be more severe in canola-on-canola rotations in Manitoba (Guo et al. 2005). In Saskatchewan, herbicide-resistant canola cultivars with a high level of resistance to blackleg grown in rotations of 2 yrs with wheat produced canola yields similar to canola grown in 4-yr rotations (Kutcher and Brandt 2009; Kutcher et al. 2003). However, blackleg severity was elevated in the 2-yr rotation compared to the 4-yr rotation and was correlated with the amount of infested crop residue (Fig. 1). Over time, repeated use of short rotations results in increased residue from the previous canola crops, resulting in disease increase. The rate of residue breakdown depends on environmental conditions (Sosnowski et al. 2006). In Victoria and South Australia, rotations of 2 yrs and 2 to 3 yrs, respectively, were suggested to be sufficient to mitigate blackleg disease because the cooler and moister conditions in Victoria promote faster residue decomposition than the warmer and drier conditions in South Australia. However, a 4-yr rotation was recommended for Western Australia, because hot, dry summers preserve residues for longer periods. In the semi-arid environment of Saskatchewan, infected canola residue may produce spores for 5 to 7 yrs (Petrie 1995).

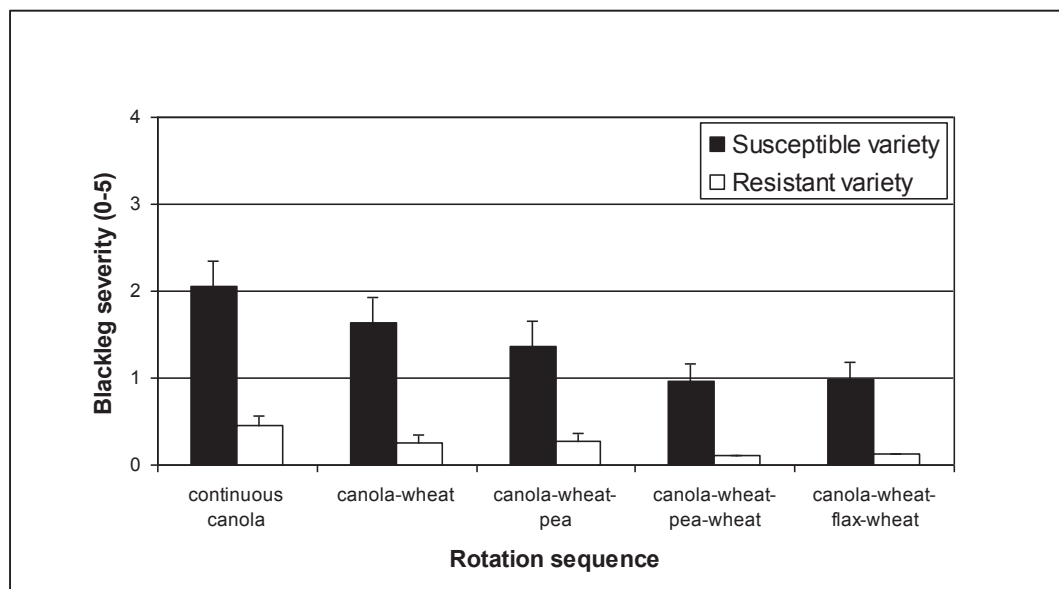


Figure 1. Severity of blackleg symptoms from rotation studies conducted at Melfort and Scott, SK from 2000 to 2006. Blackleg-resistant (Invigor hybrids) and -susceptible (cv. ‘Westar’) cultivars were grown continuously or every second, third and fourth year with wheat and field pea or flax (capped lines represent standard error, based on 13 site-years).

Resistance to blackleg

Resistance to *L. maculans* in *Brassica* spp. is reported to be of two types: qualitative and quantitative (Delourme et al. 2006). Qualitative resistance is usually effective at the site of infection on the cotyledons and leaves, and is controlled by specific resistance genes. Specific resistance genes in the host interact with corresponding avirulence genes in the pathogen in a gene-for-gene manner (Ansan-Melayah et al. 1998; Flor 1942). Quantitative resistance is the sum of small effects of many genes and is expressed at the adult plant stage as reduced severity of basal stem cankers compared with susceptible cultivars.

Isolates of *L. maculans* are classified into pathogenicity groups (PGs) using a system based on the *B. napus* cultivars ‘Quinta’ and ‘Glacier’. Isolates designated PG2 elicit a resistant reaction on both cultivars, PG3h – Quinta resistant, Glacier susceptible; PGT – Quinta susceptible, Glacier

resistant; and PG4 – both lines susceptible (Keri et al. 2001; Mengistu et al. 1991). Previous to the release of canola cultivars resistant to *L. maculans*, isolates from Saskatchewan and Manitoba collected in 1988 and 1989 (Kutcher et al. 1993) were all PG2 (Fig. 2). However, PGT and PG3 isolates were present in collections made between 1998 and 2000, shortly after the introduction of resistant cultivars (Keri et al. 2001; Kutcher et al. 2007); more recent collections of isolates have yielded all four PG types (Bradley et al. 2005; Chen and Fernando 2006).

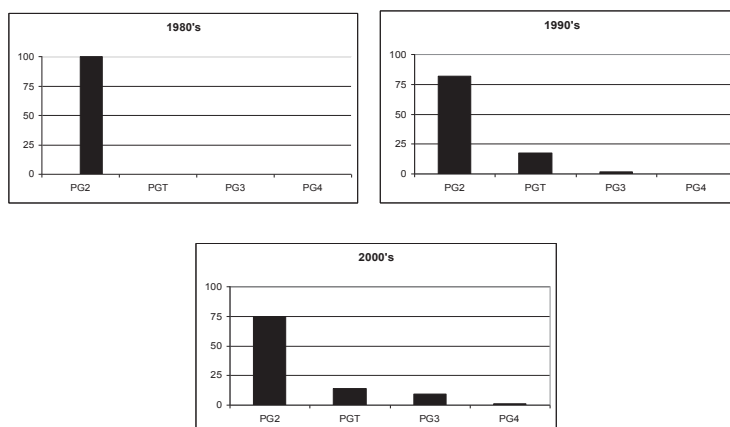


Figure 2. Percentage of isolates in each *Leptosphaeria maculans* pathogenicity group observed in the 1980s, 1990s and 2000s in western Canada (adapted from Chen and Fernando (2006); Kutcher et al. (1993, 2007)).

The PG classification system has been useful as a tool to recognize virulence changes in the population of *L. maculans* in western Canada since the late 1980s. However, it did not provide information on the underlying gene-for-gene interactions, as the resistance genes in cultivars Quinta and Glacier were not known until recently. Rimmer (2007) listed 14 resistance genes in various *Brassica* species that have been reported to condition resistance to *L. maculans*. If all 14 of these putative resistance genes are unique, there are theoretically 2^{14} or 16,384 races of the pathogen possible. An analysis of western Canadian isolates of *L. maculans* collected between 1997 and 2005 indicated that there was considerable variation for the avirulence genes corresponding to 10 of the resistance genes (Fig. 3), and identified 16 races among 96 isolates (Kutcher et al. 2010). Only the resistance genes *Rlm6* and *Rlm10*, corresponding to the avirulence genes

AvrLm6 and *AvrLm10*, condition resistance to all of the isolates tested, and isolates that overcame the eight other resistance genes were identified. This indicates that isolates that have the ability to cause disease on canola cultivars carrying one or more of these eight resistance genes are already present in western Canada. Growing cultivars that rely on any of these eight genes for resistance to *L. maculans* will select for the isolates that can overcome these resistance genes, and thus increase in the pathogen population. It is noteworthy that *Rlm6* has been used only experimentally, but isolates that overcame this resistance were obtained after only 3 yrs of continuous cultivation (monoculture) of a *B. napus* line carrying *Rlm6* in France (Brun et al. 2000). Similarly, *Rlm10* has only recently been detected and is not known to be in commercial use (A.-M. Chèvre, personal communication; Kutcher et al. 2010).

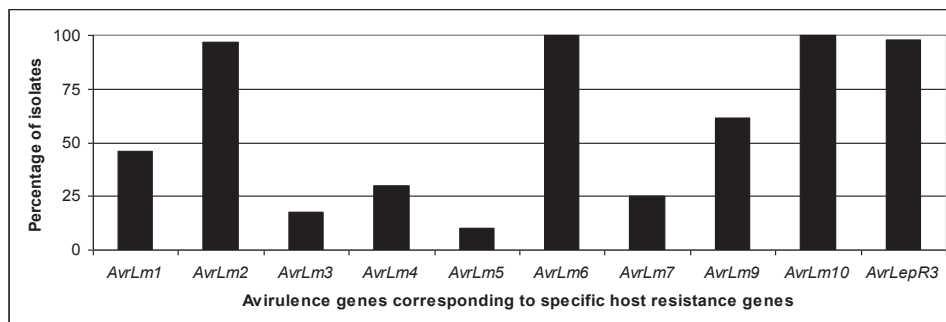


Figure 3. Percentage of *Leptosphaeria maculans* isolates carrying various avirulence genes in collections made in western Canada between 1997 and 2005 (adapted from Kutcher et al. (2010)).

In Canada, almost all of the commonly grown canola cultivars are presently rated as resistant or moderately resistant to *L. maculans* (Saskatchewan Ministry of Agriculture 2010a), although the type of resistance or the specific genes that each cultivar carries is not known (Rimmer 2006). The combination of genetic resistance to *L. maculans* and a 4-yr crop rotation has been a successful strategy against blackleg disease in western Canada. However, the virulence of pathogen populations in western Canada has changed over time, as shown by changes in the disease reaction of individual cultivars; several cultivars that were rated as resistant to blackleg when first released are now susceptible. For example, Q2 was a popular cultivar in the late 1990s (Saskatchewan Agriculture and Food 1999; Seed Manitoba 1999), and was used as a resistant check in variety

development trials, but is now susceptible in many regions of western Canada. Similarly in Europe and Australia, examples of failure of previously resistant cultivars demonstrate that exclusive reliance on genetic resistance is not sustainable (Brun et al. 2000; Li et al. 2003; Rouxel et al. 2003).

Integration of crop rotation and genetic resistance strategies

Maintaining genetic resistance in a host crop to a pathogen depends on effective integration of management practices. With few exceptions, such as resistance to flax rust (Rashid 2003) or loose smut in barley (Mathre 1997; Thomas and Menzies 1997), durable resistance has not been achieved using single resistance genes. Instead, breakdown of single-gene resistance is common, e.g., in cereals attacked by rust species (Mathre 1997; McCallum et al. 2007), as is the loss of blackleg resistance in canola cultivars dependent on single genes in Canada, Europe, and Australia. As previously discussed, the amount of blackleg-infested canola residue decreases as the length of the rotation increases. The greater the time available for the infected residue to decompose before the next canola crop, the greater the reduction in the pathogen population. Rotation to non-host crops and control of susceptible volunteers and weed species are perhaps the most important management strategies that can be used to prolong the lifespan of scarce genetic resources to combat blackleg disease of canola. Under continuous monoculture or short rotations where infected canola residue does not decompose, or where volunteer plants or susceptible weed species maintain infested residues, *L. maculans* can continue its life cycle without interruption. Growing a number of crop species in the rotation, such as wheat, barley, field pea, flax and forages (Johnston et al. 2005), and varying the cultivar of each crop, to utilize different sources of resistance (Turkington et al. 2005) interrupts the life cycle of the pathogen.

Sexual recombination of *L. maculans* on canola residue results in the production of ascospores that may carry novel combinations of the genes to overcome specific resistance genes in the host, and therefore represent new races of the pathogen. Ascospores are wind-borne and can be carried 10 km or more from the source. This means that these new races can travel from the field where they are produced to nearby canola crops, and thus move substantial distances over several years. Reducing the impact of ascospore movement between fields may be accomplished by maintaining an effective buffer distance of 50 to 100 m between future canola fields and fields

containing infected residue (Guo and Fernando 2005). These new races can also be transported over long distances on infected seed or in infested crop residue on equipment. In western Canada, most canola seed is treated with fungicides from the supplier, which is also a recommended practice to reduce the risk of disseminating new races.

Knowledge of the genes for resistance that are available in individual canola cultivars, combined with knowledge of the distribution of specific races, is necessary for the development and implementation of resistance management strategies. These strategies could be developed for use at various scales, from large regions of the prairies to individual farms. For example, this knowledge could be used to identify the canola cultivars that carry the best combinations of genes to manage the entire range of races that are present in a particular region and could also be used by individual producers to select a rotation of canola cultivars for use on their own farms. Similarly, this knowledge might provide a basis for development of a mixture of cultivars that differ in the specific resistance genes to *L. maculans*, or to develop a multiline (genotypes identical for all traits, but varying for specific resistance genes). Use of multilines or mixtures that carry different specific resistance genes may slow the evolution of the pathogen to virulence on any single resistance gene (Mundt 2002). A multiline would likely be more acceptable to the industry than a mixture because the harvested product and other plant characteristics would be uniform. However, development of a multiline takes much longer than a mixture and so this approach is less flexible and less responsive to changes in the pathogen population over time.

Knowledge of the specific resistance genes carried by canola cultivars and of the races in the population of *L. maculans* could also be used to develop strategic blackleg resistance breeding objectives. An effective specific resistance gene might be incorporated into more than one cultivar, but substituted for another when the pathogen population is observed to shift to a significant proportion of isolates that can overcome that resistance gene. Gene pyramiding, i.e., combining more than one specific resistance gene in a cultivar, also has the potential to be an effective management strategy against *L. maculans*, particularly when combined with knowledge of the pathogenic races present in the various regions of western Canada. The use of a specific resistance gene in combination with an effective source of quantitative resistance has been shown to prolong the durability of the specific resistance gene compared to reliance on the gene without a source of quantitative resistance (Brun et al. 2010). All of these resistance management strategies

using specific resistance, either alone or in a background of quantitative resistance, will require that the pathogen population structure be monitored at regular intervals to detect new pathogenic races.

Foliar fungicides: another tool that requires integrated management

In the 1980s the systemic fungicide flutriafol, applied at seeding as a coating on fertilizer granules, was shown to markedly reduce blackleg disease and increase yield in Australia (Ballinger et al. 1988). This fungicide and application method was evaluated in field trials in Saskatchewan and Manitoba, but showed marginal and variable disease control (Xi et al. 1991). The first foliar fungicide registered for control of blackleg of canola in Canada (propiconazole or Tilt[®]), was introduced in the early 1990s. It was not highly effective (Bailey et al. 2000), and was not widely adopted by canola growers. In the early 2000s, the strobilurin fungicide azoxystrobin (Quadris[®]) became available and showed beneficial effects on susceptible cultivars (Kutcher et al. 2003). It too was not widely adopted, in large part because it came to the market as cultivars that were highly resistant to blackleg became available and growers were still adhering to rotations with 3 and 4 yrs between canola crops. Subsequently, growers have tended to shorten rotations to maximize returns. This has led to anecdotal reports from many sources, of increased severity of blackleg in canola crops across the region. As a result, some canola growers are using or are considering the use of foliar fungicides as part of their blackleg management package. In 2010, a second strobilurin fungicide (pyraclostrobin, Headline[®]) was registered to control blackleg of canola.

One reason for the low level of adoption of foliar fungicides by growers may be that achieving effective disease reduction with a single application is a challenge due to the wide window of infectivity of the pathogen. Canola seedlings and plants are susceptible throughout the season, but the risk of stem canker becomes higher the earlier that infection occurs (Ghanbarnia et al. 2009). Achieving improved blackleg control may require multiple applications of foliar fungicides. Fungicide application may be considered by canola growers more frequently in the future, especially if genetic resistance should fail.

Frequent use of a limited number of fungicides to control plant diseases increases the risk that the pathogen may become insensitive to the

effect of the products (Brent and Hollomon 2007). Insensitivity to a fungicide, sometimes called “acquired resistance”, develops in the target pathogen population as a result of repeated exposure. The development of insensitivity in the pathogen population and the loss of genetic resistance to a disease in the host crop as a result of changes in the pathogen population are parallel situations in many respects. Both are heritable traits in the pathogen population. As is the case for a pathogen overcoming genetic resistance in the host, insensitivity to a fungicide is generally produced by a mutation in one or more genes, and may be present at low levels in a pathogen population even before the host resistance gene is deployed or the fungicide product applied. Fungicide-insensitive strains of the pathogen will increase in frequency in the pathogen population in response to repeated use of the same fungicide or mode of action, higher rates of applied product or dose, the presence of large pathogen populations, and short pathogen generation time (Brent and Hollomon 2007). This is similar to the situation of host genetic resistance breakdown as a result of short rotations or continuous production of the same crop species. Intensive canola production, such as 2-yr rotations of canola, increases the size of the pathogen population on which selection pressure is imposed and therefore increases the risk that races of the pathogen with the ability to overcome the resistance will occur. As with host resistance genes, the potential loss of efficacy is particularly high if a single class of fungicides is used over a large area or if exposure is prolonged (e.g., multiple applications per season).

Insensitivity to certain classes of fungicides has been observed to occur very rapidly and is characterized by a clear-cut difference in the reaction of sensitive and insensitive pathogen populations (Brent and Hollomon 2007). This has been referred to as ‘qualitative’, ‘single-step’ or ‘major gene’ resistance, examples of which were observed in Saskatchewan with the development of insensitivity to strobilurin fungicides in *Ascochyta rabiei* (Pass.) Labrousse, the cause of ascochyta blight of chickpea (*Cicer arietinum* L.) (Chang et al. 2007; Gossen and Anderson 2004; Thaher 2011). The common mutation to insensitivity to strobilurin fungicides is a simple point mutation that results in a single amino acid change in the target protein, but this change is responsible for a high level of insensitivity (Brent and Hollomon 2007). Other examples of pathogens that developed resistance to strobilurin fungicides in as little as 2 yr of commercial use were reported as early as 2000 (Heaney et al. 2000). Insensitivity to benomyl in *S. sclerotiorum* developed in a similar pattern on the Canadian Prairies. It had a large impact on the efficacy of the product, which resulted in rapid loss of

control of the disease (Gossen and Rimmer 2001). A decline in disease control that occurs gradually over time, and that may be partial and variable in degree, is referred to as ‘quantitative’, ‘multi-step’ or ‘polygenic’. This type of insensitivity is caused by the accumulation of mutations for a number of genes, each with a partial effect, and appears to be developing for several classes of fungicide in *A. rabiei* in Saskatchewan (Thaher 2011; Thaher et al. 2010).

Development of fungicide insensitivity is a serious risk to consider if foliar fungicides are to be used as a major strategy to control blackleg, especially under short rotation intervals between canola crops. While there are three products currently registered for blackleg management in canola, two (azoxystrobin and pyraclostrobin) are strobilurins that have the same mode of action (Group 11), and a high risk for the development of insensitivity (Saskatchewan Ministry of Agriculture 2010b). Propiconazole (Group 3) carries a moderate risk of insensitivity, but provides less consistent disease reduction. Each of these products is registered for use on many of the field crops on the prairies and therefore be used frequently each season. A fungicide management plan to promote judicious and efficacious use should be a high priority for growers and industry. For example, a fungicide rotation strategy, similar to rotation of herbicides, may be beneficial to producers to reduce the risk of selecting pathogen strains that are insensitive to these products.

Conclusions

Disease management strategies such as genetic resistance, crop rotation, and judicious use of foliar fungicide to control blackleg must be considered as a package, rather than independent strategies. The adoption of a 2-yr wheat-canola cropping system in many parts of the prairies is a concern because of the increased risk associated with the development of new pathogenic races and strains that are insensitive to fungicides. Typically, growers rely only on resistant cultivars in these short-rotation canola production systems. Management strategies to maintain genetic resistance to blackleg disease and to reduce the risk of fungicide insensitivity in the pathogen population must include a sustainable crop rotation to minimize the overall pathogen population and maximize the efficiency of disease control. Development of disease-resistant cultivars or new chemistries for disease

mitigation is a long and expensive process that can be undermined quickly if adequate rotation intervals and good agronomic practices are not followed.

Literature cited

- Ansan-Melayah, D., M.H. Balesdent, R. Delourme, M.L. Pilet, X. Tanguy, M. Renard and T. Rouxel. 1998. Genes for race-specific resistance against blackleg disease in *Brassica napus* L. Plant Breed. 117:373-378.
- Bailey, K.L., B.D. Gossen, R.K. Gugel and R.A.A. Morrall. 2003. Diseases of Field Crops in Canada (third edition). Saskatoon, SK: The Canadian Phytopathological Society.
- Bailey, K.L., A.M. Johnston, H.R. Kutcher, B.D. Gossen and R.A.A. Morrall. 2000. Managing crop losses from foliar diseases with fungicides, rotation, and tillage in the Saskatchewan Parkland. Can. J. Plant Sci. 80:169-175.
- Ballinger, D.J., P.A. Salisbury, J.I. Dennis, J.F. Kollmorgen and T. D. Potter. 1988. Evaluation of fungicides, applied at sowing, for control of blackleg in rapeseed. Austr. J. Exp. Agric. 28:511-515.
- Bradley, C.A., P.S. Parks, Y. Chen and W.G.D. Fernando. 2005. First report of pathogenicity groups 3 and 4 of *Leptosphaeria maculans* on canola in North Dakota. Plant Dis. 89:776.
- Brent, K.J. and D.W. Hollomon. 2007. Fungicide resistance in crop pathogens: how can it be managed? Fungicide Resistance Action Committee, Monograph No. 1 (revised edition). [Online] Available: <http://www.frac.info>.
- Brun, H., A.M. Chèvre, B.D.L. Fitt, S. Powers, A.-L. Besnard, M. Ermel, V. Huteau, B. Marquer, F. Eber, M. Renard and D. Andrivon. 2010. Quantitative resistance increases the durability of qualitative resistance to *Leptosphaeria maculans* in *Brassica napus*. New Phytologist 185:285-299.
- Brun H., S. Levivier, D. Ruer, I. Somda, A.M. Chèvre and M. Renard. 2000. A field method for evaluating the potential durability of new resistance sources: application to the *Leptosphaeria maculans*/*Brassica napus* pathosystem. Phytopathology 90:961-966.
- Bullock, D.G. 1992. Crop rotation. Crit. Rev. Plant Sci. 11:309-326.
- Canola Council of Canada. 2008. Our Great Journey. Annual Report 2008. P. 2.
- Cathcart, R.J., A.K. Topinka, P. Kharbanda, R. Lange, R.-C. Yang and L.M. Hall. 2006. Rotation length, canola variety and herbicide resistance system affect weed populations and yield. Weed Sci. 54:726-734.
- Chang, K.F., H.U. Ahmed, S.F. Hwang, B.D. Gossen, S.E. Strelkov, S.F. Blade and G.D. Turnbull. 2007. Sensitivity of field populations of *Ascochyta rabiei* to chlorothalonil, mancozeb and pyraclostrobin fungicides and effect of strobilurin fungicides on the progress of ascochyta blight of chickpea. Can. J. Plant Sci. 87:937-944.

- Chen, Y. and W.G.D. Fernando. 2006. Prevalence of pathogenicity groups of *Leptosphaeria maculans* in western Canada and North Dakota, USA. *Can. J. Plant Pathol.* 28:533-539.
- Christen, O. and K. Sieling. 1995. Effect of different preceding crops and crop rotations on yield of winter oil-seed rape (*Brassica napus* L.). *J. Agron. Crop Sci.* 174:265-271.
- Curl, E.A. 1963. Control of plant diseases by crop rotation. *Bot. Rev.* 29:413-479.
- Delourme, R., A.M. Chèvre, H. Brun, T. Rouxel, M.H. Balesdent, J.S. Dias, P. Salisbury, M. Renard and S.R. Rimmer. 2006. Major gene and polygenic resistance to *Leptosphaeria maculans* in oilseed rape (*Brassica napus*). *Eur. J. Plant Pathol.* 114:41-52.
- Dokken-Bouchard, F.L., K.A. Bassendowski, T. Boyle, L.E. Cowell, R.K. Gugel, J. Ippolito, C.L. Kirkham, H.R. Kutcher, Z. Lewchuk, S.G. Miller, R.A.A. Morrall, S. Phelps, I. Schemenauer, S. Sommerfeld and V. Vakulabharanam. 2010. Survey of canola diseases in Saskatchewan, 2009. *Can. Plant Dis. Surv.* 90:127-129.
- Flor, H.H. 1942. Inheritance of pathogenicity in *Melampsora lini*. *Phytopathology* 32:653-669.
- Francis, C.A. and M.D. Clegg. 1990. Crop rotations in sustainable production systems. Page 107 in C.A. Edwards, ed. *Sustainable Agricultural Systems*. Ankeny, IO: Soil and Water Conservation Society.
- Ghanbarnia, K., W.G.D. Fernando and G. Crow. 2009. Developing rainfall- and temperature-based models to describe infection of canola under field conditions caused by pycnidiospores of *Leptosphaeria maculans*. *Phytopathology* 99:879-886.
- Gossen, B.D. and K.L. Anderson. 2004. First report of resistance to strobilurin fungicides in *Didymella rabiei*. *Can. J. Plant Pathol.* 26:411.
- Gossen, B.D. and S.R. Rimmer. 2001. First report of resistance to benomyl fungicide in *Sclerotinia sclerotiorum*. *Plant Dis.* 85:1206.
- Gugel, R.K. and G.A. Petrie. 1992. History, occurrence, impact, and control of blackleg of rapeseed. *Can. J. Plant Pathol.* 14:36-45.
- Guo, X.W. and W.G.D. Fernando. 2005. Seasonal and diurnal patterns of spore dispersal by *Leptosphaeria maculans* from canola stubble in relation to environmental conditions. *Plant Dis.* 89:97-104.
- Guo, X.W., W.G.D. Fernando and M. Entz. 2005. Effects of crop rotation and tillage on blackleg disease of canola. *Can. J. Plant Pathol.* 27:53-57.
- Heaney, S.P., A.A. Hall, S.A. Davis and G. Olaya. 2000. Resistance to fungicides in the Qol-STAR cross-resistance group: current perspectives. Pages 755-762 in *Proc. Brighton Crop Protection Conference – Pests & Diseases*.
- Johnston, A.M., H.R. Kutcher and K.L. Bailey. 2005. Impact of crop sequence decisions in the Saskatchewan Parkland. *Can. J. Plant Sci.* 85:95-102.
- Karlen, D.L., G.E. Varvel, D.G. Bullock and R.M. Cruse. 1994. Crop rotations for the 21st century. *Adv. Agron.* 53:1-45.

- Keri, M., H.R. Kutcher and S.R. Rimmer. 2001. Virulence of isolates of *Leptosphaeria maculans* from western Canada on *Brassica napus* differentials. Can. J. Plant Pathol. 23:199.
- Kolte, S.J., D.K. Bordoloi and R.P. Awasthi. 1991. The search for resistance to major diseases of rapeseed and mustard in India. Pages 219-225 in D.I. McGregor, ed. Proc. GCIRC 8th International Rapeseed Congress, Vol. 1. Groupe Consultatif International de Recherche sur la Colza, Paris, France.
- Krupinsky, J.M., D.L. Tanaka, S.D. Merrill, M.A. Liebig and J.D. Hanson. 2006. Crop sequence effects on 10 crops in the northern Great Plains. Agric. Systems 88:227-254.
- Kutcher, H.R., M.H. Balesdent, S.R. Rimmer, T. Rouxel, A.M. Chevre, R. Delourme and H. Brun. 2010. Frequency of avirulence genes among isolates of *Leptosphaeria maculans* in western Canada. Can. J. Plant Pathol. 31:77-85.
- Kutcher, H.R. and S.A. Brandt. 2009. Increased frequency of canola in crop rotations associated with increased blackleg disease. Can. J. Plant Pathol. 31:123-124.
- Kutcher, H.R., M. Keri, D.L. McLaren and S.R. Rimmer. 2007. Pathogenicity of *Leptosphaeria maculans* in western Canada. Can. J. Plant Pathol. 29:388-393.
- Kutcher, H.R., D. Ulrich and S.A. Brandt. 2003. Plant disease implications of intensive crop rotations of canola and field pea in western Canada. Pages 273-276 in Proc. Dynamic Cropping Systems: Principles, Processes and Challenges, Bismarck, ND.
- Kutcher, H.R., C.G.J. van den Berg and S.R. Rimmer. 1993. Variation in pathogenicity of *Leptosphaeria maculans* on *Brassica* spp. based on cotyledon and stem reactions. Can. J. Plant Pathol. 15:253-258.
- Li, H., M.J. Barbetti and K. Sivasithamparam. 2005. Hazard from reliance of cruciferous hosts as sources of major gene-based resistance for managing blackleg (*Leptosphaeria maculans*) disease. Field Crops Res. 91:185-198.
- Li, H., K. Sivasithamparam and M.J. Barbetti. 2003. Breakdown of a *Brassica rapa* subsp. *sylvestris* single dominant blackleg resistance gene in *B. napus* rapeseed by *Leptosphaeria maculans* field isolates in Australia. Plant Dis. 87:752.
- Mathre, D.E. 1997. Compendium of Barley Diseases (second edition). St. Paul, MN: APS Press. 90 pp.
- McCallum, B.D., T. Fetch and J. Chong. 2007. Cereal rust control in Canada. Aust. J. Agr. Res. 58:639-647.
- McGee, D.C. and G.A. Petrie. 1978. Variability of *Leptosphaeria maculans* in relation to blackleg of rapeseed. Phytopathology 68:625-630.
- Mengistu, A., S.R. Rimmer, E. Koch and P.H. Williams. 1991. Pathogenicity grouping of isolates of *Leptosphaeria maculans* on *Brassica napus* cultivars and their disease reaction profiles on Rapid – Cycling Brassicas. Plant Dis. 75:1279-1282.
- Mundt, C.C. 2002. Use of multiline cultivars and cultivar mixtures for disease management. Annu. Rev. Phytopathol. 40:381-410.

- Petrie, G.A. 1995. Long-term survival and sporulation of *Leptosphaeria maculans* (blackleg) on naturally-infected rapeseed/canola stubble in Saskatchewan. Can. Plant Dis. Surv. 75:23-34.
- Plümper, B. and M.D. Sacristan. 1991. Resistenz gegen *Alternaria brassicicola* in der Familie *Cruciferae*. Vortr. Pflanzenzüchtung. 19:250-251.
- Rashid, K. 2003. Diseases of flax. Pages 147-154 in K.L. Bailey, B.D. Gossen, R.K. R.A.A. Morrall, eds. Diseases of Field Crops in Canada. Saskatoon, SK: Canadian Phytopathological Society.
- Rimmer, S.R. 2006. Resistance genes to *Leptosphaeria maculans* in *Brassica napus*. Can. J. Plant Pathol. 28:S288-S297.
- Rimmer, S.R. 2007. Chasing genes for resistance to blackleg and sclerotinia in *Brassica napus*. Proc. 12th International Rapeseed Congress, Wuhan, China.
- Rimmer, S.R., H.R. Kutcher and R.A.A. Morrall. 2003. Diseases of canola and mustard. Pages 29-146 in K.L. Bailey, B.D. Gossen, R.K. Gugel and R.A.A. Morrall. Diseases of Field Crops in Canada (third edition). Saskatoon, SK: The Canadian Phytopathological Society, University Extension Press, University of Saskatchewan.
- Rouxel, T., A. Penaud, X. Pinochet, H. Brun, L. Gout, R. Delourme, J. Schmit and M.H. Balesdent. 2003. A 10-year survey of populations of *Leptosphaeria maculans* in France indicates a rapid adaptation towards the *Rlm1* resistance gene of oilseed rape. Eur. J. Plant Pathol. 109:871-881.
- Salisbury, P.A. 1987. Blackleg resistance in weedy crucifers. Cruciferae Newslett. 12:90.
- Saskatchewan Agriculture and Food. 1993. Varieties of Grain Crops 1993. Regina, SK: Saskatchewan Agriculture and Food.
- Saskatchewan Agriculture and Food. 1999. Varieties of Grain Crops 1999. Regina, SK: Saskatchewan Agriculture and Food.
- Saskatchewan Ministry of Agriculture. 2010a. Varieties of Grain Crops 2010. Regina, SK: Saskatchewan Ministry of Agriculture.
- Saskatchewan Ministry of Agriculture. 2010b. 2010 Guide to Crop Protection: Weeds, Plant Diseases, Insects. Regina, SK: Saskatchewan Ministry of Agriculture. 442 pp.
- Seed Manitoba. 1999. Manitoba Agriculture Variety Recommendations 1999. Winnipeg, MB: Manitoba Agriculture, Food and Rural Initiatives.
- Séguin-Swartz, G., C. Eynck, R.K. Gugel, S.E. Strelkov, C.Y. Olivier, J.L. Li, H. Klein-Gebbinck, H. Borhan, C.D. Caldwell and K.C. Falk. 2009. Diseases of *Camelina sativa* (false flax). Can. J. Plant Pathol. 31:375-386.
- Sharma, G., V.D. Kumar, A. Haque, S.R. Bhat, S. Prakash and V.L. Chopra. 2002. Brassica coenospecies: a rich reservoir for genetic resistance to leaf spot caused by *Alternaria brassicae*. Euphytica 125:411-417.

- Sosnowski, M.R., E.S. Scott and M.D. Ramsey. 2006. Survival of *Leptosphaeria maculans* in soil on residues of *Brassica napus* in South Australia. *Plant Pathol.* 55:200-206.
- Thaher, N. 2011. Fungicide insensitivity in *Ascochyta rabiei* in Saskatchewan. M.Sc. Thesis. Guelph, ON: University of Guelph.
- Thaher, N.H., B.D. Gossen and M.R. McDonald. 2010. Baseline sensitivity of *Ascochyta rabiei* to penthiopryad, a new SDHI fungicide. *Phytopathology* 100 Suppl:S125.
- Thomas, P.L. and J.G. Menzies. 1997. Cereal smuts in Manitoba and Saskatchewan, 1989-1995. *Can. J. Plant Pathol.* 19:161-165.
- Turkington, T.K., K. Xi, J.P. Tewari, H.K. Lee, G.W. Clayton and K.N. Harker. 2005. Cultivar rotation as a strategy to reduce leaf diseases under barley monoculture. *Can. J. Plant Pathol.* 27:283-290.
- Xi, K., H.R. Kutcher, N.D. Westcott, R.A.A. Morrall and S.R. Rimmer. 1991. Effect of seed treatment and fertilizer coated with flutriafol on blackleg of canola (oilseed rape) in western Canada. *Can. J. Plant Pathol.* 13:336-346.
- Zadoks, J.C. and R.D. Schein. 1979. *Epidemiology and Plant Disease Management*. Oxford, UK: Oxford University Press. 427 pp.
- Zegada-Lizarazu, W. and A. Monti. 2010. Energy crops in rotation. A review. *Biomass Bioenergy* 35:12-25.

The development of new crops: case studies of niger and camelina

**William E. May¹, Eric N. Johnson², Guy P. Lafond¹ and
Christopher B. Holzapfel³**

¹Agriculture and Agri-Food Canada (AAFC), P.O. Box 760, Indian Head, SK S0G 2K0;

²AAFC, P.O. Box 10, Scott, SK S0K 4A0; ³Indian Head Agricultural Research Foundation,
P.O. Box 156, Indian Head, SK S0G 2K0

Email: william.may@agr.gc.ca

Camelina [*Camelina sativa* (L.) Crantz] and niger [*Guizotia abyssinica* (L.f.) Cass.] are two crops being investigated for their potential in North America. Three areas need to be considered: potential market, the economics of transporting the new crop to market or processor, and the agronomics of growing the new crop. Currently, camelina has a large potential market, but present use is very low. Niger has a small, but well established market. Camelina's transportation cost should be similar to canola; niger will need to be cleaned before shipping, and buyers may only want smaller amounts that may be uneconomic to ship. Camelina seeding rates should be at least 500 seeds/m². The appropriate seeding depth appears to be between 0 and 2.5 cm. Early spring seeding has been the most optimum time to seed camelina. Fall seeding has been done successfully, but more research is required to refine the techniques required to fall seed and to determine in which geographic areas it is most suited. The responsiveness of camelina to nitrogen depends on the yield potential of the area in which it is grown. Broadleaf weed control is a major concern in camelina, with tolerance to only trifluralin being identified. Niger appears to have tolerance to the broadleaf herbicides ethalfluralin, flucarbazone, and sulfentrazone. A seeding rate of 6.7 kg/ha is recommended. Since niger is very sensitive to frost, seeding should occur after the risk of frost has diminished. Currently, the development of camelina and niger are occurring in an almost opposite manner. Camelina is driven by a potential market, while niger is trying to fill a small, but already established market.

Additional keywords: crop management, fertility, markets, seeding, transportation

Introduction

Crop diversification can be an important management tool for farmers. Crop diversity enhances the management of plant disease and insect pests. Crop diversity may provide opportunities for different weed control strategies, in terms of herbicide selection and timing, and a better ability to prevent herbicide resistance in weeds. Greater crop diversity can mitigate economic risk since global market forces do not necessarily impact the profitability of all crops similarly in any given year. New crop development is necessary to enhance the agronomic and economic options available to producers.

When starting to evaluate the potential of a new crop for an agricultural region, there are three areas of major concern that need to be considered: potential market, the economics of transporting the new crop to market or processor, and the agronomics of growing the crop. All three are important in determining if and how quickly a new crop will develop. These factors also affect the expansion and contraction of existing crops. In this paper, the potential of camelina [*Camelina sativa* (L.) Crantz] and niger [*Guizotia abyssinica* (L.f.) Cass.] as new crops for North America are examined.

Potential markets

Camelina, a Brassicaceae oilseed, is also known as false flax, linseed dodder, or gold-of-pleasure (Putnam et al. 1993; Zubr 1997). Camelina has three potential markets: human consumption, biofuel, and as a crop that could be genetically modified to produce specialty oils for high-value industrial products. The human body requires omega-3 fatty acids (Ni Eidhin et al. 2003a), and oil extracted from camelina has a high level of α -linolenic acid, an omega-3 fatty acid (Abramovič and Abram 2005; Budin et al. 1995; Plessers et al. 1962; Zubr and Matthaus 2002). The high concentration of unsaturated fatty acids suggests that oxidative stability (tendency to rancidity) could be a concern with camelina oil; however, Abramovič et al (2007) determined that unrefined camelina oil is relatively stable due to its high content of antioxidant (phenolic and tocopherols) compounds. Camelina oil was more stable than fish and flaxseed (*Linum usitatissimum* L.) oil, which also contain long chain omega-3 fatty acids, but less stable than sunflower (*Helianthus annuus* L.), corn (*Zea mays* L.), sesame (*Sesamum indicum* L.), and olive (*Olea europaea* L.) oil (Ni Eidhin et al. 2003b).

Camelina is now currently available as cold-pressed culinary oil (Three farmers 2011), and researchers are looking at its potential to be included in salad dressings, mayonnaises, and oil-based spreads (Ni Eidhin and O’Beirne 2010). The possibility of creating high-value industrial products through genetic manipulation of this crop is being championed by Linnaeus Plant Sciences (see Grushcow (2011), this volume). In addition, camelina oil is being evaluated as a biofuel in the aviation industry. This market has a large potential, but is more sensitive to price and public perception of what is or is not an ecologically-friendly fuel.

Niger, also known as niger thistle, Nyjertm, noog, and ramtil, is an open-pollinated oilseed crop that has been cultivated in Ethiopia and India for several thousand years, accounting for 50% of the Ethiopian and 3% of Indian oilseed production (Getinet and Sharma 1996). The oil content of niger seed ranges from 30 to 50%, depending on environmental conditions during development (Nagaraj and Patil 2004). The major fatty acid is linoleic acid, with a range of 75 to 80% and 45 to 66% for niger grown in Ethiopia and India, respectively. Niger is used as a culinary oil in both India and Ethiopia, roasted or fried seeds eaten as a snack or used as a condiment, or used as bird feed in Europe and North America. Approximately 50,000 to 60,000 t of niger seed is imported into the United States (U.S.) annually. In the medium term, U.S. would be a logical market for niger produced in Canada. However, current regulations forbid the import of niger seed into the U.S. unless it has been heat-treated at a approved facility to control noxious weeds. At present, no approved facility exists in western Canada or in the U.S. near the border with Canada, thus limiting the market to processors in Canada who include niger in their bird seed mixes. There has been interest in obtaining non-sterilized niger seed, expressed by people raising young birds. Obviously, niger is starting with a small market that needs to be increased over time. One potential market would be the production of niger oil, as a traditional cooking oil, for people who have immigrated from Ethiopia or India.

Transportation costs

Camelina has a reasonable density, so shipping cost should be similar to that for canola, depending on volume being shipped and economies of scale. Currently, cold press capacity to crush camelina exists in Canada, and industrial-scale production is currently under development in the U.S. If

high-value industrial products created through genetic manipulation are marketed, then handling and processing facilities will have to be built; presumably, close enough to the area of production to minimize the cost of transportation. Currently, oil being produced for human consumption is processed on a small scale in the region it is grown, with the bottled oil sold in stores and shipped directly to consumers.

Niger is a small seed and would have to be cleaned prior to being shipped long distances, due to the high levels of plant material that are commonly in the harvested seed. Shipping niger in small quantities to niche markets, such as selling non-sterilized seed to customers raising small birds, can be costly; if this crop is to expand, a strategy to reduce transportation costs must be developed.

Camelina agronomy

The agronomy of camelina in North America has been examined in Ontario (Plessers et al. 1962), Alberta (Plessers et al. 1962), Minnesota (Putnam et al. 1993), Montana (McVay and Lamb 2008), Prince Edward Island (Urbaniak et al. 2008a), Nova Scotia (Urbaniak et al. 2008a) and Saskatchewan (Gugel and Falk 2006). Gugel and Falk (2006) reported that despite its small seed size, current farm equipment are suitable for growing and harvesting camelina with few modifications being required. McVay and Lamb (2008) developed an agronomic guide for camelina production in Montana. Currently, research is underway in western Canada to provide the information needed to create a camelina production guide for western Canada (Johnson et al. 2010b, 2010c). When camelina is considered as a new crop for western Canada, there are several issues around production practices that need to be addressed to provide guidance to growers.

Camelina seeding date, seeding rate and seeding depth

Information on the effects and importance of seeding date, seeding rate, and seeding depth on seed yield are necessary for the successful production of camelina in western Canada. The effect of seeding rate on seed yield was investigated by Urbaniak et al. (2008b) and McVay and Lamb (2008). Both studies found that seed yield increased with increasing seeding rate until an optimum plant density was reached, after which further increases in seeding rate had little or no positive effect on seed yield. Both studies found that seed yield plateaued when seeding rates exceeded 600 seeds/m². Johnson et al. (2010b) used a wide range of seeding rates and

found that seed yields no longer increased when seeding rate were increased beyond 500 seeds/m² (Fig. 1). This seeding rate resulted in approximately 150 plants/m²; in the Maritimes, this rate resulted in approximately 234 to 275 plants/m² (Urbaniak et al. 2008b).

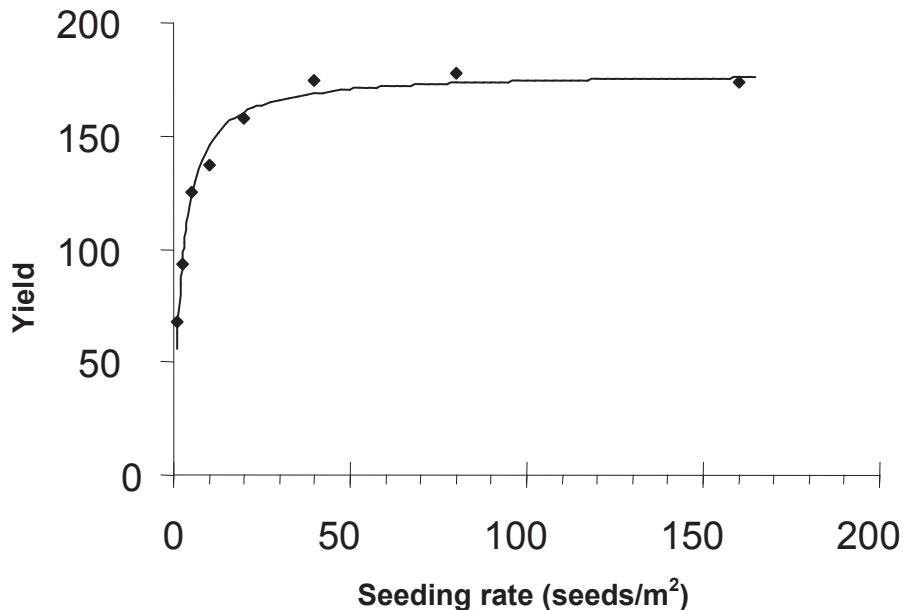


Figure 1. Relationship between planting density (viable seeds/m²) and camelina seed yield (mean of nine sites in Alberta and Saskatchewan, 2007-08).

Camelina has a small seed, and questions have been raised over how deep it should be seeded. Preliminary data from a study investigating the appropriate depth for seeding camelina using no-till seeding equipment, indicates that seeding depth can range from 0 (on the soil surface) to 2.5 cm below the soil surface in the seed row (May et al. 2010). For camelina seeded in the fall of 2009 at Indian Head, Saskatchewan, seeding at the deepest depth of 2.5 cm resulted in lower seed yields compared to the other three shallower depths; however, when seeded in the spring of 2010, seeding depth had no effect on seed yield (Table 1). Over two site-years in Montana, Johnson et al. (2010a) found that for fall-seeded camelina, there was no yield

difference between seed placed on the surface and packed and seed placed at a 1.3-cm depth on tilled ground. On tilled ground in the Maritimes, Urbaniak et al. (2008b) found that dropping seed onto the soil surface with a forage seeder and lightly packing the seed created a more even stand than a double-disk seed drill placing the seed at a depth of 1 cm; however, there was no difference in seed yield between the two treatments. On tilled ground in Minnesota, Robinson (1987) found that seeding at 2.5 cm had as good or better emergence than broadcast-seeding followed by packing or light incorporation. Robinson (1987) reported that a significant percentage of camelina seed placed at a depth of 5 cm successfully emerged. Broadcasting the seed was also discussed in two other papers, and its success or failure appears to depend on the environmental conditions under which it was conducted (McVay and Lamb 2008; Putman et al. 1993).

Table 1. The effect of seeding depth and seeding date on the plant density and seed yield of camelina at Indian Head, SK in 2010.

Seeding date	Depth	Plant density	Seed yield
	cm	no./m ²	kg/ha
Fall (Nov 9, 2009)	0	37	971
	0.6	34	986
	1.3	49	998
	2.5	35	754
Spring (May 7, 2010)	0	123	1273
	0.6	129	1299
	1.3	116	1321
	2.5	114	1319
LSD (P=0.05)		56	186
CV		48	6.8

Seeding date research can be broken down into spring seeding and fall or winter seeding. In Montana, early seeding in the spring resulted in the highest yields as seed yield tended to decline as seeding was delayed (McVay and Lamb 2008). A Saskatchewan study found that April and early May seeding dates generally gave higher yields than early June seeding dates (May et al. 2010). In the Maritimes, seeding dates ranging from early May to early June had no effect on seed yield (Urbaniak 2008b). Fall seeding of camelina has been studied in Europe (Zubr 1997), Minnesota (Gesch 2010; Putman et al. 1993), North Dakota (Johnson et al. 2010a), Montana (McVay

and Lamb 2008), and Saskatchewan (May et al. 2010). In all of these studies, camelina has successfully survived the winter; however, much more research is required in North America to determine how consistently and under what environmental conditions fall seeding of camelina can be used successfully by farmers. It is important to note that camelina seedlings are very sensitive to water pooling on the soil surface; in areas where this occurs, seedling mortality is very high. In addition, the term 'dormant seeding' must be used carefully, since May et al. (2010) observed that seed placed in unfrozen ground tends to start germinating if water is present in the soil, while emergence may not occur until spring. In Saskatchewan, a wide range of seeding dates from October 1 to May 30 have been used by farmers as they begin to grow camelina in western Canada; soil conditions have ranged from dry to wet and warm to frozen.

Camelina fertility

It has been widely stated that camelina is a crop that does not require high levels of nitrogen fertility; however, research tends to indicate that the nitrogen response of camelina depends on the crop's yield potential at the location it is grown. In Saskatchewan, Johnson et al. (2010c) reported that camelina yield plateaued with applied nitrogen rates between 110 and 120 kg/ha. In Montana, McVay and Lamb (2008) recommend that applied nitrogen plus residual soil nitrogen equal 90 to 100 kg/ha. Zuber (1997) reported that the optimum nitrogen fertilizer rate was 100 kg/ha in Denmark. Urbaniak et al. (2008a) looked at the nitrogen response of camelina at two sites in the Maritimes. At one site, seed yield peaked at 1,300 kg/ha with 60 to 80 kg/ha nitrogen. At the other location, seed yield appeared to increase as the nitrogen rate increased, reaching a yield of 1,950 kg/ha with the highest rate used of 120 kg/ha nitrogen. At Indian Head, SK in 2009, the seed yield of camelina increased with increasing nitrogen rates to 2,640 kg/ha at a rate of 200 kg/ha nitrogen. In 2010 at Indian Head, when seed yield was limited by excess precipitation and weeds, seed yield increased to 1,000 kg/ha at a nitrogen rate of 200 kg/ha (Fig. 2). Both responses were statistically significant ($P=0.0001$ in 2009 and $P=0.01$ in 2010).

There have not been many studies on the phosphorous, potassium, and sulphur requirements of camelina. Jackson (2008) reported that camelina will respond to phosphorus application when soil phosphate (P_2O_5) levels are less than 12 ppm. For camelina production in Denmark, Zubr (1997) recommended 30 kg/ha of phosphorous and 50 kg/ha of potassium. In

Montana, camelina was not responsive to sulphur applications (Jackson 2008).

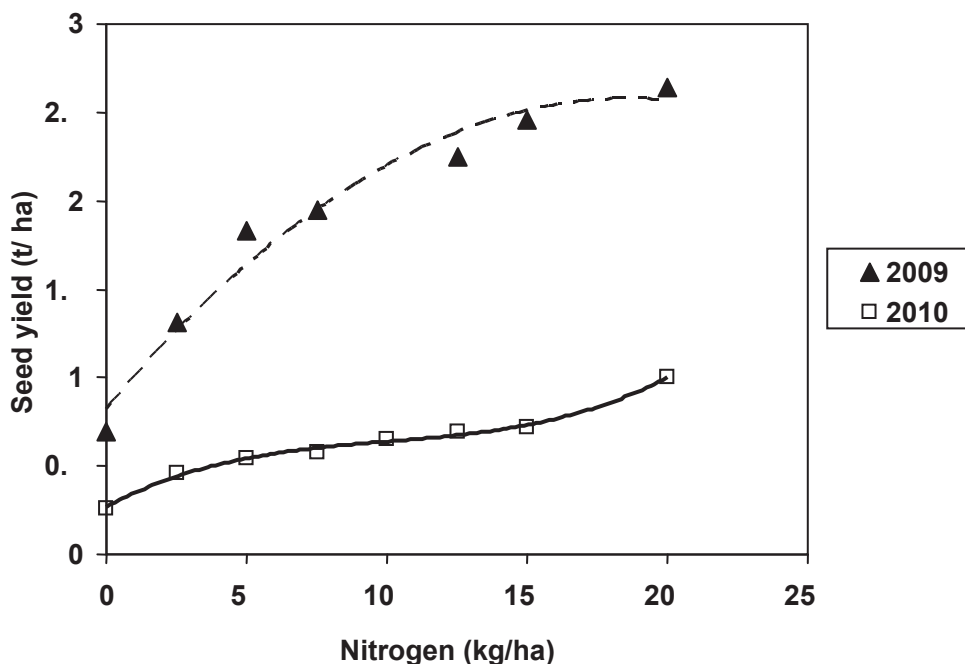


Figure 2. The effect of applied nitrogen fertilizer on the seed yield of camelina in high- and low-yielding environments (Indian Head, SK in 2009 and 2010).

Weed control in camelina

Weed control is a challenge for camelina production. Currently, quizalofop has been registered for control of annual grasses and volunteer cereals in camelina in Canada. There are no registered herbicides for broadleaf weed control; screening trials on the Prairies have failed to identify potential candidates except dinitroaniline herbicides such as trifluralin, which camelina appears to tolerate (Pearson and Walker 1999; Putnam et al. 1993; Zubr 1997); however, currently it is difficult to register dinitroaniline herbicides for new uses. The ability of camelina to germinate readily at relatively low temperatures and be seeded in the fall or early spring may provide the crop with a competitive advantage over weeds (Putnam et al. 1993; Zubr 1997); however, if adverse environmental conditions or a production problem prevent the development of a robust and thick canopy, there is no way to prevent broadleaf weed pressure from overwhelming the

crop. Resistance to sulfonylurea herbicides has been reported in a wild relative, *C. microcarpa* Andr. ex. DC. (Hanson et al. 2004); therefore, it may be possible to transfer that resistance into camelina. The incidence of *C. sativa* as a weed has been decreasing over the last few decades (Francis and Warwick 2009), probably due to the crop's susceptibility to most broadleaf herbicides currently being used in western Canada.

Niger agronomy

When examining the road blocks preventing the production and consistent seed yields of niger, weed control is the biggest limitation followed by a lack of cultivars that are more vigorous and competitive in the cool spring. Crop development of niger also presents challenges, since this crop is very sensitive to frost; thus, its production may be limited to geographic locations where the growing season is sufficiently long. Finally, the research community working on niger agronomy is small, especially in North America, thus limiting the amount and extent of agronomic information that can be quickly generated.

Weed control in niger

Due to the slow growth of niger in the spring, broadleaf weed control is very important. The tolerance of niger to several herbicides was tested (Kandel and Porter 2002). Niger was severely injured by bentazon, bromoxynil, dicamba, fomesafen, thifensulfuron, clopyralid, imazamethabenz, imazamox, nicosulfuron, acetochlor, isoxaflutole, flumetsulam and 2,4-D. The broadleaf herbicides that niger was found to be tolerant included ethalfluralin, flucarbazone, and sulfentrazone (Table 2; Kandel and Porter 2002; May et al. 2008). In addition, and not unexpectedly, tolerance to clethodim, a grass herbicide, looks to be very good (Kandel and Porter 2002). Preliminary data at Indian Head, SK also supports this conclusion. Field selection is very important since currently no herbicides have been found to control perennial weeds like Canada thistle [*Cirsium arvense* (L.) Scop.] and perennial sow-thistle (*Sonchus arvensis* L.). Niger appears to be very sensitive to bromoxynil. As observed over several years, niger is not a competitive weed in subsequent crops.

Table 2. Tolerance of niger to pre- and post-emergence applications of herbicides in 2007 at Indian Head, SK.

Treatment	Plant density		Injury Eight leaves	Injury 100% flower	NDVI ^z 5% flower	Seed yield
	g ai/ha	no./m ²	%	%	%	kg/ha
Weed-free check		67	0	0	0.7370	987
Ethalfluralin	1396	73	0	0	0.7496	868
Trifluralin	1700	88	0	2.0	0.7314	847
Flucarbazon	20	98	7.8	3.0	0.7238	847
Flucarbazon	30	79	17.5	11.0	0.6338	851
MCPA	562	120	55.0	36.3	0.5337	684
2,4-D	562	80	66.3	78.8	0.3633	302
Sulfentrazone	280	81	2.5	4.8	0.6941	790
Ethalfluralin + sulfentrazone	1396 210	103	0	0	0.7351	966
Ethalfluralin + sulfentrazone	1396 280	82	0	0	0.7525	881
Ethalfluralin + flucarbazon	1396 20	77	11.0	5.0	0.7036	853
Ethalfluralin + MCPA	1396 350	86	36.3	15.8	0.6594	760
Ethalfluralin + MCPA	1396 562	117	55.0	37.5	0.6059	743
Weedy check		76	0	0	0.7590	877
LSD (P=.05)		36	36	7.20	0.0790	138
CV		29	29	36	8.2	12
Treatment Prob(F)		0.1258	0.0001	0.0001	0.0001	0.0001

^z NDVI = normalized difference vegetation index.

Crop development of niger

Recently, a private plant breeder in Minnesota developed several cultivars of niger that mature and produce seed in the Northern Great Plains of North America. However, even with these new cultivars, niger is a long-season crop and very sensitive to frost. Therefore, the thermal time required to reach various stages of development is important in looking at areas where niger can be grown and in selecting higher-yielding cultivars. The growing degree-days (base temperature of 5 °C) required from seeding to flower was between 500 and 600, and from seeding to swathing was approximately 1,200. The seed yield appears to be related to the duration of the flowering period of niger, with a potential for higher seed yields as the flowering duration is increased (Fig. 3). The yield potential of niger in Saskatchewan ranges from 250 to 1,200 kg/ha.

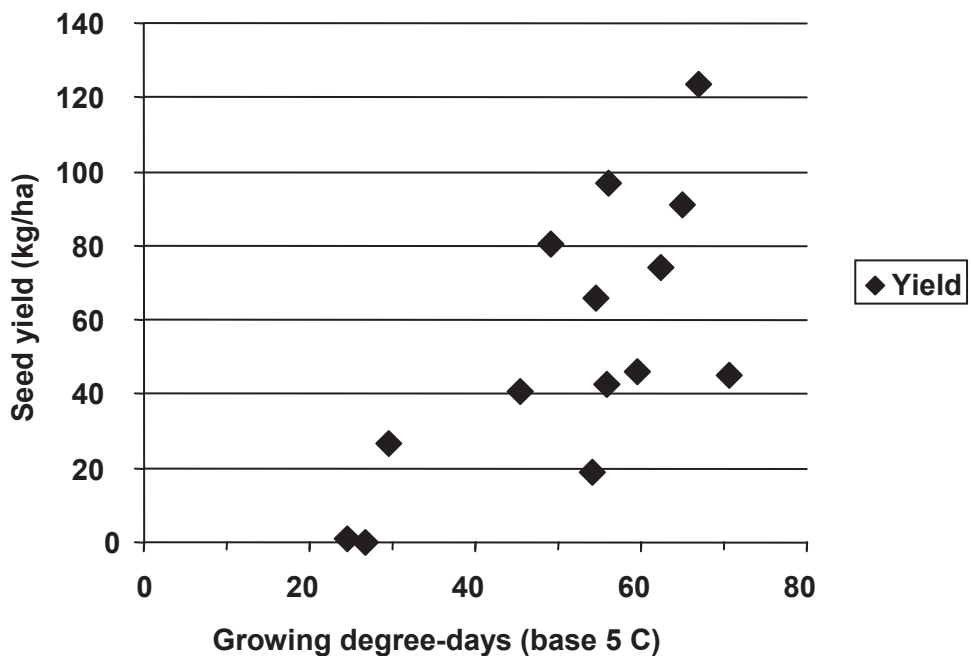


Figure 3. The growing degree-days accumulated during the flowering of niger at several locations and years.

Niger seeding rate, seeding date, and seeding depth

Niger is a small-seeded crop, and its 1000-seed weight can vary between 2 and 6 g. A high seeding rate is usually not recommended. A

seeding rate of 6.7 kg/ha was recommended for niger grown in Minnesota and North Dakota (Kandel et al. 2004), which corresponded to a plant density of 90/m². Getinet and Sharma (1996) reported that the recommended seeding rates were 5 to 10 kg/ha in Ethiopia and 5 to 8 kg/ha in India.

No seeding date research for North America could be found in the literature. As previously discussed, niger is a long-season crop and current cultivars are very sensitive to frost; thus, the current recommendation in Saskatchewan is to seed in late May after the threat of frost has past. Niger appears to be slow growing in cold soils and an earlier seeding date not only exposes the crop to potential frost, but provides a competitive advantage to weeds that are better adapted to cool soils.

In India and Ethiopia, niger is seeded by broadcasting the seed on the soil surface and lightly harrowing it or by seeding the crop in rows, usually 30 cm apart (Getinet and Sharma 1996). Two reports from India indicate that there is a significant yield increase when niger is seeded in rows versus broadcast on the surface (Khandwe and Sharma 2003; Thaker et al. 2004). Several approaches to seeding, using equipment available in North America, were suggested by Kandel et al. (2004). In research plots at Indian Head, SK, an air drill has been successfully used (May et al. 2008). Reduced plant stands have been observed when the seed opener placed seed directly over the seed row of the previous crop, when soil moisture levels are low, or when the seed is placed too deep in wet soil. No reports were found in the literature on the best seeding depth for niger.

Niger fertility

There is limited data on fertility of Niger. In North Dakota and Minnesota, nitrogen rates from 0 to 67 kg/ha were tested at 10 sites, concluding that increasing nitrogen only increased seed yield at one site and decreased seed yield at one site (Henson et al. 2004). This response is supported by production practices in India, where only 20 kg/ha of nitrogen is often recommended (Getinet and Sharma 1996; Khandwe and Sharma 2003; Thakur et al. 2004). Research on phosphorus and potassium have been very limited in North America, with Kandel and Porter (2002) stating that they have not seen any particular response to phosphorus and potassium, but not showing any data. In India, 20 to 40 kg/ha of phosphorus is recommended (Getinet and Sharma 1996), and this recommendation is supported by research (Dhange et al. 2009; Thakur et al. 2005).

Pollinators in niger

Since niger is an outcrossing species with a self-incompatibility mechanism (Getinet and Sharma 1996), research into pollination will be required. Niger yields are increased by pollinators, and the length of time needed to set seed was increased in their absence (Pastagia and Patel 2009a, 2009b). Consequently, good pollination is essential for this crop to be successful in a short season area like western Canada. As the size of niger fields are increased, research will need to examine a number of questions. Which wild pollinators are attracted to niger? How large an area of niger can wild pollinators successfully pollinate? If supplemental pollinators are economic, are honey bees or leaf cutter bees more effective? In addition, niger, tends to start flower before canola finishes flowering, so proximity to canola fields may have an effect on niger pollination and seed yield.

Harvesting niger

Niger blooms over an extended period of time, and the seed shatters after maturity; therefore swathing is recommended once the majority of flower buds have turned black and the earliest seed is starting to shatter. However, if frost occurs and damages this sensitive crop, the farmer must decide whether to swath immediately or direct cut in a few days. Direct-combining after frost has been successful if weed density is light; most weeds are less susceptible to frost and will still be green when niger is ready for harvest, making combining difficult. Henson et al. (2003) found that seed yields increased as swathing was delayed at some sites, but not all.

Comparison of the ongoing development of camelina and niger

The development of camelina and niger in western Canada is occurring in an almost opposite manner. Currently, the development of camelina is being driven by entrepreneurs who want to develop products from camelina and its oil, whether it is for food, fuel, or specialty products. There is a significant amount of funding and effort going into the development of this crop and its products. In turn, this has driven agronomic research in production practices even though the actual market consumption of camelina is quite small. The benefit of this approach is that farmers will have a better understanding of the potential benefits and pitfalls of growing camelina. The disadvantage is that if the entrepreneurs fail to be successful with their product development, much more research will have been carried out on camelina than is justified by the current size of the crop.

On the other hand, niger has an existing market that is met through imports. With the development of an adapted cultivar, the challenge becomes the development of production practices and marketing strategies to allow Canadian-grown niger to economically compete with the imported product. The current research effort is very small, and will only expand if local success can be achieved. The advantage of this is that few resources are allocated at the beginning of the development process; however, it may take much longer, if at all, to identify geographic regions where niger makes the most economic sense to grow.

Literature cited

- Abramovič, H. and V. Abram. 2005. Physico-chemical properties, composition and oxidative stability of *Camelina sativa* oil. Food Technol. Biotech. 43:63-70.
- Abramovič, H., B. Butinar and V. Nikolic. 2007. Changes occurring in phenolic content, tocopherol composition and oxidative stability of *Camelina sativa* oil during storage. Food Chem. 104:903-909.
- Budin, J., W. Breene and D. Putnam. 1995. Some compositional properties of camelina (*Camelina sativa* L. Crantz) seeds and oils. J. Am. Oil Chem. Soc. 72:309-315.
- Dhange, A.M., P.N. Karanjikar, A.L. Dhamak, A.B. Kasbeand and S.R. Barkule. 2009. Effect of land configurations and phosphorus levels on the yield of niger under rainfed condition. Asian J. Soil Sci. 4:328-330.
- Francis, A. and S.I. Warwick. 2009. The biology of Canadian weeds. 142. *Camelina alyssum* (Mill.) Thell.; *C. microcarpa* Andr. ex DC.; *C. sativa* (L.) Crantz. Can. J. Plant Sci. 89:791-810.
- Gesch, R.W. 2010. Winter camelina in the northern U.S. and the potential of its use in double-cropping. Proc. 2010 New Crops: Exploring Diversity, Preserving our Future. The Association for the Advancement of Industrial Crops, Fort Collins, CO.
- Getinet, A. and S.M. Sharma. 1996. Niger. *Guizotia abyssinica* (L. f.) Cass. Promoting the conservation and use of underutilized and neglected crops. 5. Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute, Rome.
- Gugel, R.K. and K.C. Falk. 2006. Agronomic and seed quality evaluation of *Camelina sativa* in western Canada. Can. J. Plant Sci. 86:1047-1058.
- Hanson, B.D., K.W. Park, C.A. Mallory-Smith and D.C. Thill. 2004. Resistance of *Camelina microcarpa* to acetolactate synthase inhibiting herbicides. Weed Res. 44:187-194.
- Henson, R.A., H.J. Kandel, B.K. Hanson, M.A. Halvorson, B.L. Johnson, D.G. LeGare and P.M. Porter. 2003. Effect of Swathing Time on Niger Thistle Yield.

- Carrington Research Extension Center. Carrington, ND: North Dakota State University. 3 pp. [Online] Available: <http://www.ag.ndsu.edu/CarringtonREC/agronomy-1/research-documents/production-management/03%20Swathing%20Time%20on%20Niger.pdf/view>.
- Henson, R.A., H.J. Kandel, B.K. Hanson, B.L. Johnson, M.A. Halvorson, D.G. LeGare and P.M. Porter. 2004. Niger Thistle Response to Nitrogen Fertilizer. Proc., ASA-CSSA-SSSA 2004 Annual Meeting, Seattle, WA.
- Jackson, G.D. 2008. Response of camelina to nitrogen, phosphorus, and sulfur. Bozeman, MT: Montana State University Extension Service. 2 pp.
- Johnson, B.L., M.T. Berti and P.J. Petersen. 2010a. Seeding depth and genotype influence on winter camelina in North Dakota. Proc. 2010 New Crops: Exploring Diversity, Preserving our Future. The Association for the Advancement of Industrial Crops, Fort Collins, CO.
- Johnson, E.N., K. Falk, H. Klein-Gebbinck, L. Lewis, C. Vera, Y. Gan, L.M. Hall, K. Topinka, S. Phelps and B. Davey. 2010b. Optimizing seeding rates and plant densities for *Camelina sativa*. Proc. Soils and Crops Workshop 2010. Saskatoon, SK: University of Saskatchewan.
- Johnson, E.N., K. Falk, S.S. Malhi, D. Leach, L.M. Hall, K. Topinka, W.E. May, S. Phelps and B. Davey. 2010c. Optimizing nitrogen rates in *Camelina sativa*. Proc. Soils and Crops Workshop 2010. Saskatoon, SK: University of Saskatchewan.
- Kandel, H. and P. Porter. 2002. Niger [*Guizotia abyssinica*] (L. f.) Cass.] Production in Northwest Minnesota. University of Minnesota Extension Service, MN. 32 pp.
- Kandel, H.J., P.M. Porter, B.L. Johnson, R.A. Henson, B.K. Hanson, S. Weisberg and D.G. LeGare. 2004. Plant population influences niger seed yield in the northern Great Plains. Crop Sci. 44:190-197.
- Khandwe, R. and R.C. Sharma. 2003. Yield and economics of niger, *Guizotia abyssinica* Cass., as influenced by new technologies on the farmers' fields. J. Oil. Res. 20:320-321.
- May, W.E., E.N. Johnson, K. Falk, Y. Gan, L. Hall, H. Klein-Gebbinck, G.P. Lafond, L. Lewis and C. Vera. 2010. Camelina agronomy research on the Northern Great Plains of Canada. Proc. ASA-CSSA-SSSA International Annual Meetings, Long Beach, CA.
- May, W.E., G.P. Lafond and C.B. Holzapfel. 2008. Weed control in niger (*Guizotia abyssinica*). Canadian Weed Science Society Annual Meeting, Banff, AB. [Online] Available: <http://www.weedscience.ca>.
- McVay, K.A. and P.F. Lamb. 2008. Camelina Production in Montana. Field Crops Extension Report No D-16. Bozeman, MT: Montana State University.
- Nagaraj, G. and H.S. Patil. 2004. Quality and composition of niger, *Guizotia abyssinica* (L.) Cass - a review. J. Oil. Res. 21:224-229.

- Ni Eidhin, D., J. Burke, B. Lynch and D. O'Beirne. 2003a. Effects of dietary supplementation with camelina oil on porcine blood lipids. *J. Food Sci.* 68:671-679.
- Ni Eidhin, D., J. Burke and D. O'Beirne. 2003b. Oxidative stability of ω 3-rich camelina oil and camelina oil-based spread compared with plant and fish oils and sunflower spread. *J. Food Sci.* 68:345-353.
- Ni Eidhin, D. and D. O'Beirne. 2010. Oxidative stability of camelina oil in salad dressings, mayonnaises and during frying. *Int. J. Food Sci. Technol.* 45:444-452.
- Pastagia, J.J. and M.B. Patel. 2009a. Behaviour of flower opening and pollination in niger, *Guizotia abyssinica* (L.f.) Cass, as affected by bee pollination. *Insect Environ.* 15:8-10.
- Pastagia, J.J. and M.B. Patel. 2009b. Effect of bee pollination on yield of niger, *Guizotia abyssinica* (L.f.) Cass. *Insect Environ.* 15:10-12.
- Pearson, N. and K.C. Walker. 1999. The performance of *Camelina sativa* in the UK. *Asp. Appl. Biol.* 56:249-255.
- Plessers, A.G., W.G. McGregor, R.B. Carson and W. Nakoneshny. 1962. Species trials with oilseed plants. II. Camelina. *Can. J. Plant Sci.* 42:452-459.
- Putnam, D.H., J.H. Budin, L.A. Field and W.M. Breene. 1993. Camelina: a promising low-input oilseed. Pages 314-322 in J. Janick and J.E. Simon, eds. *New Crops*. New York, NY: John Wiley & Sons.
- Robinson, R.G. 1987. Camelina: A Useful Research Crop and a Potential Oilseed Crop. Station Bulletin 579-1987. Minnesota Agricultural Experiment Station, University of Minnesota, MN. pp. 1-12.
- Thakur, N.S., M.R. Deshmukh, R.K. Reddy and R.S. Sharma. 2004. Evaluation of niger *Guizotia abyssinica* (Coss) productivity under resource constraints. *J. Oil. Res.* 21:200-201.
- Thakur, N.S., M.R. Deshmukh and R.S. Sharma. 2005. Studies on N and P fertilization of niger, *Guizotia abyssinica* (L.f.) Cass in Satpura Plateau Zone of Madhya Pradesh. *J. Oil Res.* 22:213-214.
- Three Farmers. 2011. Three Farmers Camelina Oil. [Online] Available: <http://www.threefarmers.ca>.
- Urbaniak, S.D., C.D. Caldwell, V.D. Zheljazkov, R. Lada and L. Luan. 2008a. The effect of cultivar and applied nitrogen on the performance of *Camelina sativa* L. in the Maritime provinces of Canada. *Can. J. Plant Sci.* 88:111-119.
- Urbaniak, S.D., C.D. Caldwell, V.D. Zheljazkov, R. Lada and L. Luan. 2008b. The effect of seeding rate, seeding date and seeder type on the performance of *Camelina sativa* L. in the Maritime provinces of Canada. *Can. J. Plant Sci.* 88:501-508.
- Zubr, J. 1997. Oil-seed crop: *Camelina sativa*. *Indust. Crops Prod.* 6:113-119.
- Zubr, J. and B. Matthaus. 2002. Effects of growth conditions on fatty acids and tocopherols in *Camelina sativa* oil. *Indust. Crops Prod.* 15:155-162.

Volunteer and feral crop plants and latent seed populations play an important role in novel trait containment

Rene C. Van Acker¹ and Muthukumar V. Bagavathiannan²

¹*Department of Plant Agriculture, University of Guelph, Guelph, ON N1G 2W1;*

²*Department of Crop, Soil and Environmental Sciences, University of Arkansas,*

Fayetteville, AR 72704

Email: vanacker@uoguelph.ca

Risks associated with the production of plants with novel traits are often linked to escape and movement of the novel trait either into wild and weedy populations or into areas of the agricultural supply chain where the trait is unintended and perhaps unexpected. The movement of novel traits in agriculture involves both the metapopulation and the latent population, and in some cases may involve a species complex. For crop species, a metapopulation includes cropped, volunteer, and feral subpopulations (including escaped populations that effectively serve as sinks or sources for traits) while a latent population includes any viable seed for that species anywhere within the agricultural supply chain. In the case of metapopulations, an assessment or a model of novel trait movement relies on a good understanding of the biology of volunteer and feral populations. In the case of latent populations, an assessment or model of novel trait movement also relies on a deep understanding of supply chain operations, processes, protocols, and equipment. Studying both metapopulations and latent populations can provide insights into and useful data for modeling and novel trait containment. To predict, prevent, and mitigate potential harm(s) arising from the escape and movement of novel traits, there is a need to better understand novel trait movement throughout agricultural supply chains.

Additional keywords: feral, genetically modified, metapopulation, novel traits, volunteer

Introduction

The advent of genetic engineering (GE) has heightened the awareness of the challenges and potential risks that can come with the development of novel traits in plants, although plants with novel traits (PNTs) are not necessarily GE. Many risks related to the release of PNTs are related to trait movement both from crop to wild or weedy species (interspecific) and from crop to crop (intraspecific) (Marvier and Van Acker 2005). This is especially true for the movement of traits within and among farming systems and agricultural supply chains (NRC 2004; Tolstrup et al. 2003).

The issue of containing traits and making sure they do not end up where they are not intended and/or wanted has become a key point in debates about PNTs. In cases where traits can be contained, regulators can be much more permissive about which traits are allowed in crop plants; on the other hand, in cases where traits cannot be readily contained, then regulators and technology developers need to be much more cautious about which traits are allowed, not only for widespread commercial release but also in plants that would be grown in small, contained plots. In either case, traits that are regulated must be contained and there is zero tolerance for trait escape.

In North America, we have over 20 yrs of experience with agricultural plant-based GE, which provides a tremendous wealth of examples and evidence that bear on the consideration of novel trait containment. In a recent review, Marvier and Van Acker (2005) emphasized two important points in the context of growing PNTs outside the laboratory environment and trying to contain traits:

- (1) When PNTs are grown outside at a commercial scale for any length of time, the movement of traits beyond their intended destinations (i.e., trait escape) is a virtual certainty. The risk of escape increases with scale of production (and associated equipment) and as the number of participants in the production and handling increases.
- (2) It is unlikely that one can fully retract novel traits once they have escaped into the environment (which includes agricultural supply chains) and as such, in situations where the escape is a problem, the problem becomes persistent.

These points support the need for great caution and care in the production of PNTs that require any sort of containment, including situations where there is a hope or expectation of coexistence and commercial segregation, and especially for situations where the trait is still regulated. Trait movement can be complex, in particular, when it occurs within large

agricultural supply chains that involve many actors and many living elements across a living landscape. Traits may reside in and move among living populations of plants including feral and volunteer plants and among latent populations in seed that may exist in a myriad of places within the production and supply chain. In any case, the role of volunteer and feral populations and latent seed populations in trait persistence and trait movement can be substantive and needs to be well understood for trait risk assessment purposes and for the consideration of commercial coexistence or segregation schemes.

Examples of trait escape

The most extensive area of cultivation of GE crops has been North America, and it is in North America where there have been a number of documented cases of novel trait escape (Marvier and Van Acker 2005). Among all documented cases, intraspecific trait movement in canola (*Brassica napus* L.) has been the most common, although this has been in the context of commercial production of canola varieties that have been deregulated and have unconfined release status. In western Canada, where canola is grown on a large number of hectares, canola varieties with novel traits are grown on the majority of those hectares (Van Acker et al. 2004). There has been so much intraspecific trait escape in canola that farmers in this region have come to expect the appearance of unintended traits in their canola (Friesen et al. 2003). Even after only four seasons of commercial production of herbicide-resistant canola (1995-1998), Hall et al. (2000) found that the specific traits encoding for different herbicide-resistance traits were stacking within individual volunteer canola plants, giving rise unintentionally to multiple herbicide-resistant volunteer canola plants. By the 1999 season, only 5 yr after the initiation of commercial production of GE herbicide-resistant canola in western Canada, farmers commonly began to complain about the appearance of volunteer glyphosate-resistant canola in their fields, even when they had not intentionally sown glyphosate-resistant canola in these fields in the previous year. Many of these farmers suspected that the non-glyphosate-resistant certified canola seed they were using had adventitious presence (AP) of glyphosate-resistant canola seed. Independent testing of certified canola seedlots from western Canada revealed that the majority of tested seedlots contained at least trace amounts of GE herbicide-resistance traits. In fact, the majority of seedlots tested by Friesen et al. (2003), and Downey and Beckie (2002) had foreign traits present at

detectable levels (above 0.01%), with one seedlot having an AP level of 4.9% suggesting AP occurred very early in the pedigree and perhaps even at the breeder seed level for that particular variety (Downey and Beckie 2002). The source of the AP for these seedlots was never determined, but could have resulted from inadvertent mechanical mixing of certified seedlots during harvest or handling, or pollen-mediated gene flow (PMGF) occurring in earlier generations of pedigreed seed production (i.e., Breeder or Foundation seed) (Friesen et al. 2003). The range in AP levels was significant, and it was apparent that some breeding companies were able to achieve very low AP levels. No followup was done to determine whether there were specific differences in practices among companies, but it was clear from the Friesen et al. (2003) study that AP level was associated with seed company. There is recent evidence that the AP of specific traits in certified seed can also occur in species such as wheat (*Triticum aestivum* L.) that are considered to be primarily self-pollinated and have very limited seed persistence. Gaines et al. (2007) showed that the non-GE trait conferring tolerance to imidazolinone (IMI) herbicides in wheat has been found in certified conventional wheat seedlots in the United States (U.S.) at levels of up to 11% only 2 to 3 yr after commercial release. This AP occurred despite the fact that certified seed is grown and handled under a strict segregation regime and wheat is considered a self-pollinating species (Waines and Hegde 2003).

It is important to consider cases of trait escape that involve human error because they are so unpredictable; human error is a critical factor in trait containment for agricultural crops because there is so much human involvement in the production, processing, transport, and storage. In the U.S., there have been a number of documented cases of trait escape involving human error. Perhaps the most famous of these is the 'Starlink' case where corn (*Zea mays* L.), engineered to express an insecticidal protein, was found in the human food supply chain, while it was only approved for use as animal feed. There was a considerable segregation oversight between food and feed streams in the U.S. bulk commodity handling systems, and consequently the insecticidal protein associated with Starlink corn was found in a number of processed foods in 2000 (Marvier and Van Acker 2005). Three yr after this discovery and after the execution of a massive recall effort, traces of the Starlink protein could still be found within both food and feed handling streams in the U.S. (USDA 2003), demonstrating the pervasiveness of the AP. This pervasiveness should not be surprising if the trait in question codes for a neutral trait and the GE traits commercialized to

date (herbicide and insect resistance) are neutral outside of farmed fields and are therefore not selected for or against in a portion of the metapopulation. Population genetics theory confirms that neutral traits can persist in the environment (Brûlé-Babel et al. 2006) at very low levels. The Starlink case showed not only that human error can result in problematic trait escape, but full retraction of traits (and their products) from complex and massive commercial food and feed systems is difficult, and perhaps impossible. Another example of human-mediated trait escape is the Prodigene case, also in the U.S., where corn genetically engineered to produce a vaccine that prevents diarrhea in pigs was discovered in a commercial grain elevator in Iowa (Gillis 2002). Upon investigation, the U.S. Department of Agriculture (USDA) found that the company who owned this GE corn (Prodigene) had failed to comply with U.S. federal regulations requiring that the company destroy volunteer GE corn growing in subsequent crops. This error required that 13,600 t of contaminated soybean [*Glycine max* (L.) Merr.] be destroyed to prevent further contamination of food or feed supply chains. This case demonstrated inadequate trait containment oversight on the part of the company (Prodigene). Between 2000 and 2008, the USDA documented six cases of regulated novel traits escaping beyond their intended containment spaces within the U.S. (USGAO 2009). The mechanism for escape ranged from cross-pollination and co-mingling of seed, to cross-pollination and uncontrolled volunteers, to misidentified seed. In the three remaining cases, the mechanism of escape has not been determined according to the USDA. In Canada, there have also been cases of human error leading to trait escape. A well-documented case was the inadvertent release of the GT200 event of RoundupReady® canola (resistance to glyphosate) in western Canada (Demeke et al. 2006; Pollack 2002). The company's response to the mistake was swift and effective, but the case demonstrated the possibility for these types of mistakes to occur and they highlight the challenges of trait containment and the diligence that must be employed in order to effectively contain traits. By the year 2000, the widely publicized Starlink case would have made this clear to the public and also to technology developers responsible for containing regulated events.

Transgene movement and containment

Any consideration of trait containment must be realistic with respect to the many complex routes of trait escape (Fig. 1). Traits can move from crop to crop via pollen (PMGF) and/or via seed (seed-mediated gene flow

and seed admixture). Trait movement via pollen and/or seed occurs within a complex of sub-populations which exist across agricultural landscapes including crop, volunteer, and feral sub-populations (Bagavathiannan and Van Acker 2009). If very strict containment practices are not in place, it may be possible for traits to move among these sub-populations, which together comprise a metapopulation. In this context, trait containment efforts must take into account all possible sub-populations and possible pollen and/or seed movement opportunities between them.

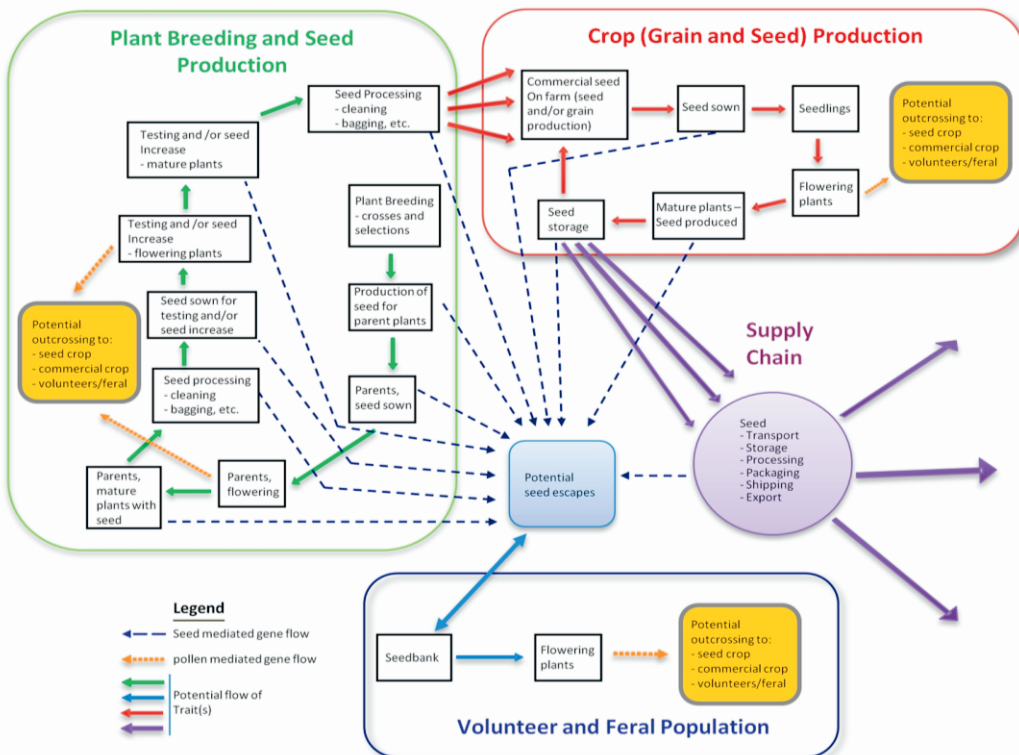


Figure 1: A simplified scheme of the possible routes of trait movement within an agricultural crop production system and leading to a supply chain (adapted from ideas and a schematic presented by S. Shirtliffe, University of Saskatchewan).

A functional metapopulation for a given trait within a given crop in a region makes containment difficult, because the points of trait escape and reception may be stratified in time and space (Van Acker et al. 2003). In this

type of scenario, it is challenging to predict when and from where the trait will arrive and it means that trait containment efforts must be comprehensive. The required stringency of a given trait-containment system will depend upon the threshold level and the facility of trait escape and movement. The latter depends on the nature of the crop species (e.g., it is most difficult to confine traits in species which are highly outcrossing and form persistent seedbanks; Tolstrup et al. 2003) and also on the complexity of the crop production and handling system (the supply chain). If threshold levels are high (e.g., 5%) and the crop in question is an obligate selfing species (a species which cannot outcross but only self-fertilize) that also does not produce a persistent seedbank, then containment will be much less difficult. It will also be less difficult for species that do not produce persistent volunteer or feral populations.

If we consider the elements that contributed to the movement of novel traits among canola crops in western Canada, it is apparent the role that volunteer and feral populations can play (Van Acker et al. 2004). For crops that are commonly grown in a region, appear frequently in rotation, have a great potential to volunteer, produce a persistent seedbank that allows for volunteers to appear in many subsequent years, and have some outcrossing ability, there will be great difficulty in containing novel traits. Canola is an example of this type of crop, and it is the nature of this crop that has lead European scientists to suggest that coexistence and commercial-trait containment is not practical for canola (Tolstrup et al. 2003). Even for species that are considered primarily selfing, such as spring wheat, the scale of production and frequency in rotation may compensate for low levels of outcrossing to affect a viable genetic bridge from volunteer to cropped populations (Van Acker et al. 2004). In addition, because volunteers for crops such as wheat have not been difficult to control in typical rotations in the past, there has been relatively little study of the biology and ecology of these volunteers (Willenborg and Van Acker 2008). Harker et al. (2005), for example, discovered that spring wheat could persist in some scenarios in western Canada for 2 to 3 yr, and empirical evidence from western Canada shows persistence for up to 5 yr (Van Acker et al. 2004).

The feral potential of crop species has been studied to a relatively limited extent (Gressel 2005), and the role of volunteer and feral crop plants in trait movement and containment has been studied to a very limited extent (Bagavathiannan and Van Acker 2008). Trait containment in the context of metapopulation dynamics creates a new need to consider the fertility of crop species. Feral potential varies tremendously among crop species and can

depend very much upon key traits including the ability to form a persistent seedbank (Watrud et al. 2004). Occurrence of feral populations has been reported for some crop species including canola (Garnier et al. 2006), radish (*Raphanus sativus* L.) (Snow and Campbell 2005), rye (*Secale cereale* L.) (White et al. 2006), cotton (*Gossypium hirsutum* L.) (Ellstrand et al. 1999), sugar beet (*Beta vulgaris* L.) (Sukopp et al. 2005) and sunflower (*Helianthus annuus* L.) (Massinga et al. 2003). Key feral traits include, but are not limited to: high levels of outcrossing, prolific seed production, seed dispersal, seed dormancy, discontinuous germination, rapid vegetative growth, tolerance to competition, and tolerance to biotic as well as abiotic stresses. The establishment of PNTs as ferals may be more likely if they possess traits of ecological significance (Wolfenbarger and Phifer 2000). There are some species that form very effective and persistent feral populations. One crop species of note in this regard is alfalfa (*Medicago sativa* L.) (Bagavathiannan and Van Acker 2009). Feral alfalfa populations are very common in North America, and have been shown to flower synchronously with both hay- and seed-cropped alfalfa; as an insect-pollinated species, there is very high potential for outcrossing between feral and cropped alfalfa. The feral success of alfalfa poses particular challenges for trait containment in this species, especially when feral populations reside on public lands including roadsides (Bagavathiannan et al. 2010). In fact, in some cases alfalfa was a constituent of species mixtures seeded to help stabilize municipal road verges and ditches. Fertility is less common for annual crop species, but is not impossible. Knispel et al. (2008), for example, confirmed that roadside populations of canola could successfully accumulate unintended traits, and as such act as both a source and a sink for traits within a broader metapopulation. With novel traits being conferred upon an increasing range of crop plants, there is a need to more deeply consider novel trait movement. For example, a virus-resistant plum (*Prunus domestica* L.) has been deregulated in the U.S., but the environmental impact statement (EIS) contains very little information on novel trait movement from this species. Investigation of the possible novel trait dynamics for plum reveals that there is both good potential for metapopulation dynamics and dynamics within a broad species complex, which includes a full range of common tender fruits (Cici and Van Acker 2011).

In the context of agricultural production systems and agricultural supply chains, the role of latent populations, in the form of seed, should not be underestimated as a source and sink for traits. Given the size and complexity of agricultural supply chains (Fig. 1), latent populations add

tremendous complication in terms of effectively preventing trait escape and movement. Segregation of specific traits and coexistence have not been attempted within generalized bulk commodity production and handling systems, likely because industry stakeholders recognize and respect the challenges involved in trait containment and perhaps are not willing to accept the liability (Miller 2009). Small-scale segregation is managed for seed and for higher-value specialty products. Without regulations or incentives, agricultural commodity handlers have little motivation to effectively segregate novel traits. To be effective, containment plans must encompass a broad range of characteristics (Van Acker 2003). These plans must be based on realistic, science-based, robust, and tested models of transgene movement (Gealy 2005). The plans also need to extend beyond individual fields or farms. Experience with the movement of the RoundupReady trait in canola in western Canada shows that canola existed as a metapopulation with respect to the RoundupReady transgene; transgene containment would have required a plan which encompassed the entire cropping system (not just the canola crop) and was operational across the entire region of western Canada, which is highly impractical, and in the case of a trait that had achieved unconfined release, not necessary. However, if containment is required, management for containment within a given field and for a given crop alone will be insufficient for effective containment. In this regard, there needs to be a specific recognition in these plans of the fact that in the absence of reliable genetic technologies for preventing PMGF (e.g. genetic use restriction technologies, GURTs; Van Acker et al. 2007b), transgene movement has to be controlled at reception points. This poses a particular challenge for trait containment when receptor crops are grown by non-adopters of particular PNTs, and it poses a special problem for regulated events or conditionally-deregulated events (e.g., Starlink). In this respect, as well, the plans must reflect a realistic expectation of commitment from farmers to implement the plan given the economic constraints and capacity limits, and the plans have to assume that the potential receptor is looking for the unwanted trait(s). The challenges in managing trait containment are many and they include the fact that the traits are typically invisible and their monitoring requires effective (and sometimes sophisticated) detection methods (Marvier and Van Acker 2005; Tolstrup et al. 2003). In particular, trait containment is highly dependent upon detection and eradication of the trait at reception points. This is a critical consideration for those devising containment plans, because the trait reception points may occur in the fields,

farms, equipment, and business operations of people not involved in the containment effort (Van Acker 2003).

Farming equipment is a common vector for the dispersal of seeds (Shirtliffe and Entz 2005), and it can be a common vector for the movement of crop seed from field to field. Trait movement can occur via equipment including planting, harvesting, seed cleaning, seed handling, and seed storage equipment. Each piece of equipment can act as a sink or a source for traits (often as seed), and in this respect, each piece of equipment could be considered an additional sub-population (latent population) for a given trait in a given crop. Cleaning farm equipment, as well as seed storage, transport, and handling facilities and equipment, including farm-based seed cleaning facilities, is a critical part of trait containment plans (Van Acker et al. 2007a). It can be difficult to completely clean some pieces of equipment, such as commercial combine harvesters. Farmers who wish to maintain their operations free from even trace amounts of a given trait might consider never using difficult-to-clean equipment, which they cannot assure to be free from unintended traits. This may preclude some farmers from renting equipment or from sharing equipment with farmers who do not assure their operations to be free from a given trait, and it means that farmers and supply chain participants must be aware of the handling of any and all crops and traits in facilities (and equipment) within any supply chain in which they participate; this would be especially critical in regard to the containment of regulated events (Van Acker et al. 2007a).

For farmers and others operating within agricultural supply chains wishing to avoid the presence of unwanted traits in their crops, on their farms, or in their commodities or processing facilities, there are a number of management practices that need to be implemented (Riddle 2004; Van Acker et al. 2007a). Given the complexity of mechanisms leading to trait escape (e.g., Fig. 1) and the stochasticity and persistence of escapes, those who wish to assure and deliver products free from given traits will need to employ all methods available to them in order to prevent the AP of traits (NRC 2004). Perhaps the most important management practice for farmers is to use clean seed. This is fundamental because even low-level presence of unintended trait(s) within a seedlot can result in significant levels in the harvested product (Friesen et al. 2003). For species which produce a persistent seedbank, a single seeding with an impure seedlot can lead to years of problematic trait presence via persistent, self-replicating and self-disseminating volunteers. The use of clean seed is even a problem for farmers who purchase certified seed because there are currently no

regulations requiring seed certification agencies to ensure the genetic purity of seed (ISF 2004), and in the context of trait containment, seed purity may require a new definition. Typically, seed was fit for purpose if it was relatively pure phenotypically (e.g., CSGA 2005), but seed purity assurance systems were not necessarily designed to deliver the type of seed purity levels required for facilitating novel trait containment. The European Union (EU) and New Zealand are the only jurisdictions, globally, in which discussions in this regard are ongoing within governments. The International Seed Federation has demonstrated that the costs of ensuring genetic purity rise exponentially as threshold levels for unintended traits decline below 1%, to the point where costs become prohibitive at thresholds below 0.5 to 0.3% (ISF 2001). If there is no regulation requiring seed certification agencies to guarantee genetic purity of seed, or if the genetic purity standards are set relatively high (e.g., above 0.5%), then farm-saved seed, or specialty suppliers may be the only option for farmers wanting to start with genetically-pure seed that is free from traits they do not want or whose presence will cause harm to their system or business. It is also clear that those working with and responsible for the containment of regulated traits must pay extra caution to prevent trait escape into seed sources (USGAO 2009).

The involvement of farmers and their attitudes towards trait containment protocols and the risk associated with the production of PNTs have not commonly been considered by regulators, yet cooperation among neighboring farmers may be a fundamental requirement for trait containment (Tolstrup et al. 2003). The human or cultural element of trait containment management is difficult to characterize and control, and this makes trait containment success difficult to manage. Collegial cooperation is impossible if neighbours are unaware of the possible presence of unwanted traits in their locale. Open communication among neighbours, either formal or informal, is an essential element of a successful trait containment strategy (Van Acker et al. 2007a).

Physical isolation is a traditional means for limiting PMGF in crop breeding programs, and it can be exploited as an aid to help limit trait escape or arrival. Physical isolation, however, is not an absolute protection from trait invasion and farmers must realize that traditional isolation distances were established to assure seed purity and not the prevention of novel trait escape and/or immigration *per se*. Isolation distance must be suited to the nature of the species and the threshold (Tolstrup et al. 2003). PMGF at low levels has been recorded at very long distances for species such as canola

(3 km) (Rieger et al. 2002) and creeping bentgrass (*Agrostis stolonifera* L.) (21 km) (Watrud et al. 2004). Pollen traps (or barriers) are sometimes promoted as an additional means for achieving physical isolation to limit PMGF, but in order to be effective, these pollen traps must be significantly taller than the targeted crop at the time of flowering (and/or pollen release) for that crop. In western Canada, corn has sometimes been used ineffectively as a pollen barrier to prevent pollen escape from confined GM wheat trials because in the cooler climate of western Canada, corn is only as tall as normal wheat plants at time of flowering (early July) (personal observation).

The effective transfer of traits between volunteers and cropped plants can be minimized by creating temporal isolation between these cohorts. A long and diverse crop rotation allows farmers to reduce seedbanks for volunteer species before that same species is cropped again within a given field. Isolation in time is traditionally used by plant breeders and seed growers to help them facilitate the maintenance of seed purity (CSGA 2005). A diverse crop rotation facilitates the detection of volunteers in subsequent crops and allows for effective rouging or control with herbicides. Although many common annual crop species do not produce persistent seedbanks, there are some exceptions. For example, in the United Kingdom, canola seed has been shown to persist for up to 12 yr (Lutman 2003). Of course, segregation in time is only effective if there has been a conscious effort to eliminate the impact of feral populations from being an effective source or sink for traits.

Conclusion

Risks associated with the production of PNTs are often linked to escape and movement of the novel trait either into wild type or weedy populations or into areas of the agricultural supply chain where the trait is unintended and perhaps unexpected. Studying both metapopulations and latent populations, and in some cases species complexes, can provide insights into and useful data for modeling and novel trait containment. There is an increasing base of knowledge on the mechanisms of trait movement and considerations in trait containment. However, the global asynchrony of deregulation of PNTs will drive an increasing need for trait containment based on an understanding of trait movement. With certain PNTs, there may also be a need for domestic trait containment. To date, much of the effort to understand trait movement has focused on PMGF. This paper makes plain

that robust and reliable trait containment requires an understanding of movement from all sources. In addition, the complexity of agroecosystems with regard to novel trait containment should not be underestimated by regulators, farmers, and supply chain constituents; proposals to require trait containment need to be prudent and realistic in this regard.

Literature cited

- Bagavathiannan, M.V., R.H. Gulden and R.C. Van Acker. 2010. Occurrence of feral alfalfa (*Medicago sativa* L.) populations along roadside habitats in southern Manitoba, Canada and their role in intraspecific novel trait movement. *Transgenic Res.* 19: doi 10.1007/s11248-010-9425-2.
- Bagavathiannan, M.V. and R.C. Van Acker. 2008. Crop ferality: implications for novel trait confinement. *Agric. Ecosys. Environ.* 127:1-6.
- Bagavathiannan, M.V. and R.C. Van Acker. 2009. The biology and ecology of feral alfalfa (*Medicago sativa* L.) and its implications for novel trait confinement in North America. *Crit. Rev. Plant Sci.* 28:69-87.
- Brûlé-Babel, A.L., C. J. Willenborg, L.F. Friesen and R.C. Van Acker. 2006. Modeling the influence of gene flow and selection pressure on the frequency of a GE herbicide-tolerant trait in non-GE wheat and wheat volunteers. *Crop Sci.* 46:1704-1710.
- Cici, S.Z.H. and R.C. Van Acker. 2011. Gene flow in *Prunus* species in the context of novel trait risk assessment. *Env. Biosafety Res.* 10:in press.
- [CSGA] Canadian Seed Growers Association. 2005. Regulations and Forms. [Online] Available: <http://www.seedgrowers.ca/regulationsandforms/index.asp?lang=e>.
- Demeke, T., D.J. Perry and W.R. Scowcroft. 2006. Adventitious presence of GMOs: scientific overview for Canadian grains. *Can. J. Plant Sci.* 86:1-23.
- Downey, R.K. and H.J. Beckie. 2002. Isolation Effectiveness in Canola Pedigreed Seed Production. Final Research Report. Saskatoon, SK: Agriculture and Agri-Food Canada. [Online] Available: <http://www.saskorganic.com/oapf/pdf/canola-study.pdf>.
- Ellstrand, N.C., H.C. Prentice and J.F. Hancock. 1999. Gene flow and introgression from domesticated plants into their wild relatives. *Ann. Rev. Ecol. Syst.* 30:539-563.
- Friesen, L.F., A.G. Nelson and R.C. Van Acker. 2003. Evidence of contamination of pedigreed canola (*Brassica napus*) seedlots in western Canada with genetically engineered herbicide resistance traits. *Agron. J.* 95:1342-1347.
- Gaines, T.A., C. Preston, P.F. Byrne, W.B. Henry and P. Westra. 2007. Adventitious presence of herbicide resistant wheat in certified and farm-saved seed lots. *Crop Sci.* 47:751-756.

- Garnier, A, A. Deville and J. Lecomte. 2006. Stochastic modeling of feral plant populations with seed immigration and road verge management. *Ecol. Model.* 197:373-382.
- Gealy, D.R. 2005 Gene movement between rice (*Oryza sativa*) and weed rice (*Oryza sativa*) – a U.S. temperate rice perspective. Pages 323-354 in J.B. Gressel, ed. *Crop Fertility and Volunteerism*. Boca Raton, FL: CRC Press.
- Gillis, J. 2002. Soybeans Mixed with Altered Corn; Suspect Crop Stopped from Getting into Food. *The Washington Post*, November 13, 2002. P. E01.
- Gressel, J. 2005. The challenges of ferality. Pages 1-7 in J.B. Gressel, ed. *Crop Fertility and Volunteerism*. Boca Raton, FL: CRC Press.
- Hall, L., K. Topinka, J. Huffman, L. Davis and A. Good. 2000. Pollen flow between herbicide-resistant *Brassica napus* is the cause of multiple-resistant *B. napus* volunteers. *Weed Sci.* 48:688-694.
- Harker, K.N., G.W. Clayton, R.E. Blackshaw, J.T. O'Donovan, E.N. Johnson, Y. Gan, F.A. Holm, K. Sapsford, R.B. Irvine and R.C. Van Acker. 2005. Glyphosate-resistant wheat persistence in western Canadian cropping systems. *Weed Sci.* 53:846-859.
- [ISF] International Seed Federation. 2001. Motion on Adventitious Presence of GM Material in non-GM Seeds. ISF position paper. [Online] Available: www.worldseed.org.
- [ISF] International Seed Federation. 2004. Coexistence of Genetically Modified, Conventional and Organic Crop Production. ISF position paper. [Online] Available: www.worldseed.org.
- Knispel, A.L., S.M. McLachlan, R.C. Van Acker and L.F. Friesen. 2008. Genetically engineered multiple herbicide resistance in escaped canola populations. *Weed Sci.* 56:72-80.
- Lutman, P.J.W. 2003. Coexistence of conventional, organic and GM crops – role of temporal and spatial behaviour of seeds. Pages 33-43 in B. Boelt, ed. *Proc. 1st European Conference on the Coexistence of Genetically Modified and Conventional and Organic Crops*. Flakkebjerg, Denmark: Danish Institute of Agricultural Sciences Research Centre.
- Marvier, M. and R.C. Van Acker. 2005. Can crop transgenes be kept on a leash? *Front. Ecol. Environ.* 3:93-100.
- Massinga, R.A., K. Al-Khatib, P. St. Amand and J.F. Miller. 2003. Gene flow from imidazolinone-resistant domesticated sunflower to wild relatives. *Weed Sci.* 51:854-862.
- Miller, J. 2009. Wheat co-existence issues from a processing perspective in K. Alcock and G. Spangenberg, eds. *Proc. Fourth International Conference on Coexistence between Genetically Modified (GM) and non-GM based Agricultural Supply Chains*. Melbourne, Australia. [Online] Available: <http://www.gmcc-09.com/wp-content/uploads/jmiller-gmcc-09.pdf>.
- [NRC] National Research Council. 2004. *Biological Confinement of Genetically Engineered Organisms*. Washington, DC: The National Academies Press.

- Pollack, A. 2002. Unapproved Canola Seed may be on Farms, Makers Say. The New York Times April 16, 2002. p. C4.
- Riddle, J.A. 2004. A Plan for Co-existence: Best Management Practices for Producers of GMO and Non-GMO Crops. St. Paul, MN: University of Minnesota. 4 pp.
- Rieger, M.A., M. Lamond, C. Preston, S.B. Powles and R.T. Roush. 2002. Pollen-mediated movement of herbicide resistance between commercial canola fields. *Science* 296:2386-2388.
- Shirliff, S. and M.H. Entz. 2005. Chaff collection reduces seed dispersal of wild oat (*Avena fatua*) by a combine harvester. *Weed Sci.* 53:465-470.
- Snow, A.A. and L.G. Campbell. 2005. Can feral radishes become weeds? Pages 193-208 in J.B. Gressel, ed. *Crop Fertility and Volunteerism*. Boca Raton, FL: CRC Press.
- Sukopp, U., M. Pohl, S. Driessen and D. Bartsch. 2005. Feral beets – with help from maritime wild? Pages 45-58 in J.B. Gressel, ed. *Crop Fertility and Volunteerism*. Boca Raton, FL: CRC Press.
- Tolstrup, K., S.B. Andersen, B. Boelt, M. Buus, M. Gylling, P.B. Holm, G. Kjellsson, S. Pedersen, H. Ostergard and S. Mikkelsen. 2003. Report from the Danish Working Group on the Coexistence of Genetically Modified Crops with Conventional and Organic Agriculture. Tjele, Denmark: Danish Institute of Agricultural Sciences. Plant Production no. 94. 275 pp.
- [USDA] United States Department of Agriculture. 2003. Starlink Test Results. November 19, 2003. Washington, DC: United States Department of Agriculture, Grain Inspection, Packers and Stockyards Administration.
- [USGAO] United States Government Accountability Office. 2009. GAO Report to the Committee on Agriculture, Nutrition, and Forestry, U.S. Senate. Genetically Engineered Crops: Agencies are Proposing Changes to Improve Oversight, but could Take Additional Steps to Enhance Coordination and Monitoring, November 2008. GAO-09-60. 103 pp.
- Van Acker, R.C. 2003. Potential Approaches for the Management of Herbicide Tolerant Crops. Canadian Food Inspection Agency, Plant Products Directorate, Plant Biosafety Office Technical Workshop on the Management of Herbicide Tolerant (HT) Crops Report. [Online] Available: www.inspection.gc.ca/english/plaveg/bio/consult/herbtolrepe.shtml#7.
- Van Acker, R.C., A.L. Brûlé-Babel and L.F. Friesen. 2003. An environmental risk assessment of Roundup Ready wheat: risks for direct seeding systems in western Canada. Report prepared for the Canadian Wheat Board, Winnipeg, MB. 33 pp. [Online] Available: <http://www.cwb.ca/en/topics/biotechnology/report/index.jsp>.
- Van Acker, R.C., A.L. Brûlé-Babel and L.F. Friesen. 2004. Intraspecific gene movement can create environmental risk: The example of Roundup Ready wheat in western Canada in B. Breckling and R. Verhoeven, eds. *Risk, Hazard, Damage – Specification of Criteria to Assess Environmental Impact of*

- Genetically Modified Organisms. Bonn (Bundesamt für Naturchutz) - Naturschutz und Biologische Vielfalt 1:37-47.
- Van Acker, R.C., N. McLean and R.C. Martin. 2007a. Development of quality assurance protocols to prevent GM-contamination of organic crops. Pages 466-489 in J. Cooper, U. Niggli, and C. Leifert, eds. Handbook of Organic Food Safety and Quality. Boca Raton, FL: CRC Press.
- Van Acker, R.C., A.R. Szumigalski and L.F. Friesen. 2007b. The potential benefits, risks and costs of genetic use restriction technologies. Can. J. Plant Sci. 87:753-762.
- Waines, J.G. and S.G. Hegde. 2003. Intraspecific gene flow in bread wheat as affected by reproductive biology and pollination ecology of wheat flowers. Crop Sci. 43:451-463.
- Watrud, L.S., E.H. Lee, A. Fairbrother, C. Burdick, J.R. Reichman, M. Bollman, M. Storm, G. King and P.K. Van de Water. 2004. Evidence for landscape-level, pollen-mediated gene flow from genetically modified creeping bentgrass with CP4 EPSPS as a marker. Proc. Natl. Acad. Sci. 101:14533-14538.
- White, A.D., D.J. Lyon, C. Mallory-Smith, C.R. Medlin and J.P. Yenish. 2006. Feral rye (*Secale cereale*) in agricultural production systems. Weed Technol. 20:815-823.
- Willenborg, C.J. and R.C. Van Acker. 2008. The biology and ecology of bread wheat and its implications for novel trait confinement. Can. J. Plant Sci. 88:997-1013.
- Wolfenbarger, L.L. and P.R. Phifer. 2000. Biotechnology and ecology – the ecological risks and benefits of genetically engineered plants. Science 290:2088-2093.

Index

- abiotic stress, 2, 3, 42, 44, 48
- abscisic acid, 40
- agricultural supply chain, 94, 107
- Agrostis stolonifera*, 106
- alfalfa, 102, 107
- Allee effects, 28
- Alternaria*, 48, 72, 73
- alternative crops, 40, 44
- ANCOVA, 26
- Arundo donax*, 36
- Ascochyta rabiei*, 67, 69, 73
- ascospores, 63
- Australia, 10, 20, 59, 63, 65, 71, 73, 109
- Australian Weed Risk Assessment, 16, 17
- Avena sativa*, 36
- azoxystrobin, 65, 67
- barley, 36, 37, 38, 42, 56, 63, 73
- Beta vulgaris*, 35, 102
- biodiesel, 6, 7, 8, 14, 45, 49, 56
- biodiversity, 2, 3, 7, 37, 46
- bioeconomy, 45, 50
- biofuel crops, 36, 50
- biofuels, 2, 31, 52
- bioplastics, 1
- bioplatforms, 39
- bioproducts, 2, 12
- biorefineries, 45
- blackleg, 55, 56, 57, 58, 59, 60, 62, 63, 64, 65, 66, 67, 68, 70, 71, 72, 73
- Brassica, 1, 3, 6, 10, 35, 36, 45, 49, 50, 51, 53, 55, 60, 61, 68, 69, 70, 71, 72, 73, 96, 108
- Brassica napus*, 3, 6, 35, 49, 50, 51, 55, 68, 69, 70, 71, 72, 73, 96, 108
- broadleaf weed control, 46, 48, 83, 84
- bromoxynil, 84
- C. microcarpa*, 10
- C. alyssum*, 10
- C. rumelica*, 10
- camelina, vii, 1, 4, 9, 10, 12, 13, 27, 36, 45, 46, 49, 57, 75, 76, 78, 79, 80, 81, 82, 83, 89, 90, 91, 92
- Camelina sativa*, 1, 6, 9, 14, 27, 31, 36, 51, 57, 73, 75, 76, 90, 91, 92, 93
- Canada, i, iii, vi, 1, 3, 4, 7, 10, 13, 14, 20, 32, 34, 35, 36, 37, 38, 39, 44, 45, 46, 49, 50, 51, 53, 55, 56, 57, 59, 61, 62, 63, 64, 65, 68, 69, 70, 71, 72, 73, 75, 77, 78, 79, 82, 83, 84, 89, 91, 92, 93, 96, 99, 101, 103, 106, 107, 108, 110
- Canadian Weeds Seeds Order, 36
- canaryseed, 36
- Cannabis sativa*, 36
- canola oil, 7
- castor bean, 6, 7, 8, 9, 13
- chickpea, 67, 69
- China, 8, 56, 72
- Cicer arietinum*, 67
- Cirsium arvense*, 67
- clubroot, 56, 57, 58
- CNTs, 2, 4, 36, 37, 38, 39, 43, 44, 45, 48
- Commelinaceae, 24, 31
- competitiveness, 2, 34, 42
- corn, 7, 35, 37, 39, 44, 51, 52, 77, 98, 106
- cotton, 35, 102
- cow cockle, 34, 36, 47, 50, 52
- creeping bentgrass, 106, 111
- crop diversity, 13, 76
- crop rotation, 55, 56
- cultivars, 2, 42, 44, 49, 56, 58, 59, 60, 61, 62, 63, 64, 65, 68, 71, 72, 84, 86, 87
- dayflower family, 24
- Demographic models, 19, 20, 28, 29, 30
- demography, 23, 26, 28, 31, 33
- deregulation, 107
- drought tolerance, 39
- environmental stochasticity, 29
- Ethiopia, 77, 87, 88
- Ethiopian mustard, 1, 45, 46, 49
- Fabaceae, 29
- fatty acids, 7, 9, 14, 76, 93
- feedstocks, 1, 2, 6, 7, 53
- feral, vii, 3, 94, 96, 99, 101, 106, 107, 108, 110
- fertility, 53, 82, 88
- field pennycress, 36
- fitness traits, 34, 35
- flax, 10, 14, 27, 38, 39, 46, 50, 51, 57, 60, 63, 72, 73, 76
- Flax Council of Canada, 38
- flucarbazone, 75, 84, 85
- forages, 63
- Freedom to Farm Act, 40
- frost, 75, 84, 86, 87, 89

- fungicide, 55, 65, 66, 67, 68, 70, 73
 GE. See genetic engineering
 gene pyramiding, 65
 genetic engineering, 95
 genetic use restriction technologies, 103, 111
 genetically engineered, 51, 98, 108, 111
 genetically modified, 2, 5, 48, 53, 76, 111
 genetically-enhanced, 2
 genotypes, 20, 30, 44, 57, 64
 germplasm, 8, 10, 12, 43, 44
Glycine max, 7, 35, 98
 glyphosate resistance, 38
 GM, 35, 38, 40, 42, 51, 52, 53, 106, 109, 110
Gossypium hirsutum, 35, 102
Guizotia abyssinica, 75, 76, 90, 91, 92, 93
 GURTs. See genetic use restriction technologies
Helianthus annuus, 77
Heliconia acuminata, 28
 hemp, 36
 herbicides, 41, 46, 47, 49, 67, 75, 83, 84, 85, 91, 97, 106
Hordeum vulgare, 36, 56
 hydroxy fatty acid, 6, 10
 hydroxylase gene, 9
 imidazolinone, 97, 109
 improved vigour, 34
 India, 8, 50, 51, 70, 77, 87, 88
 industrial, vii, 1, 6, 7, 12, 39, 46, 53, 76, 78
 inoculum, 58
 intermediate wheatgrass, 1
 interspecific, 95
 intraspecific, 95, 96, 107
 invasive populations, 25, 26
 invasive species, 16, 18, 19, 20, 22, 24, 25, 26, 33, 52
 invasiveness, vii, 2, 3, 5, 16, 19, 20, 22, 24, 26, 27, 28, 29, 30, 31, 32, 34, 35, 36, 37, 40, 42, 48, 53
 kochia, 40
Kochia scoparia, 40
 latent population, 94, 104
 legume family, 29
Lens culinaris, 36
 lentil, 36, 38
Leptosphaeria maculans, 55, 56, 61, 62, 68, 69, 70, 71, 72, 73
 Linnaeus Plant Sciences, 3, 6, 9, 12, 13, 14, 77
 linseed dodder, 76
Linum usitatissimum, 10, 38, 51, 57, 77
 Maritimes, 79, 80, 81, 82
 markets, 38, 40, 76, 78
 Material other than grain, 11
Medicago sativa, 102, 107
 metapopulation, 94, 98, 99, 100, 101, 103
 Minnesota, 78, 80, 81, 86, 87, 88, 91, 92, 109
Miscanthus x giganteus, 36
 MOG, 11
 monoculture, 37, 39, 48, 58, 62, 63, 73
 Montana, 13, 27, 31, 78, 80, 81, 82, 91, 92
 niger, vii, 4, 75, 76, 77, 78, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93
 Nitrogen, 41, 42, 43, 44, 49, 91
 nitrogen-use efficiency, 2, 34, 37
 noninvasive, 16, 20, 22, 24, 25, 26, 31
 nontransgenic, 41
 noog, 77
 North Dakota, 68, 69, 81, 87, 88, 91
 novel trait, vii, 94, 95, 96, 102, 105, 106, 107, 108, 111
 novel traits, vii, 3, 4, 34, 35, 49, 94, 95, 96, 98, 101, 102, 103
 nutrient-use efficiency, 35
 Nyjertm, 77
Olea europaea, 77
 olive, 77
 omega fatty acids, 11
 omega-3 fatty acids, 77
 outcrossing, 2, 39, 46, 89, 100, 101, 102
Panicum virgatum, 1
 pathogens, 55, 57, 58, 67, 69
 perennial sow-thistle, 84
Phalaris canariensis, 36
 phosphorous, 82
 phylogenetic sampling, 24
Pisum sativum, 36, 57
 plants with novel traits, 94, 95
Plasmodiophora brassicae, 56
 pleiotropic effects, 41
 plum, 102
 PNTs. See plants with novel traits
 pollinators, 89
 population dynamics, 28, 29, 32
 population growth, 16, 17, 19, 20, 22, 23, 24, 25, 27, 28, 29, 30, 31
 post-emergence applications, 85
 post-release monitoring, 2, 3, 4
 potassium, 41, 82, 88

- Prairie Carnation, 48
 Prairie provinces, 36
 prediction, 16, 17, 26, 30, 32
 prevention, 16, 17, 32, 106
 Provincial Noxious Weeds Acts, 36
Prunus domestica, 102
 pyraclostrobin, 65, 67, 69
 quizalofop, 83
 ramtil, 77
 regulators, 4, 36, 43, 48, 95, 105, 107
 resistance, 1, 34, 35, 37, 38, 41, 42, 46,
 47, 49, 55, 56, 58, 59, 60, 61, 62, 63,
 64, 66, 67, 68, 69, 70, 71, 72, 73, 76,
 84, 96, 98, 108, 109
 ricinoleic acid, 6, 8
 ricinolein, 7, 9
Ricinus communis, 6, 7
 Rilsan® Polyamide, 7
 rotation, 13, 37, 39, 49, 51, 55, 56, 57, 58,
 59, 60, 62, 63, 64, 67, 68, 69, 70, 73,
 101, 106
 Russian thistle, 40
Salsola tragus, 40
Saponaria vaccaria, 34, 36, 47, 49, 50, 52
 Saskatchewan, i, vi, 3, 39, 46, 48, 52, 53,
 58, 59, 61, 62, 65, 67, 68, 70, 72, 73,
 78, 80, 81, 82, 86, 87, 91, 100
Sclerotinia sclerotiorum, 56, 70
 sclerotinia stem rot, 56, 57, 58
 Second-generation crops with novel traits,
 35
 second-generation traits, 1, 2, 3, 34, 37,
 38, 48
 seed yield, 1, 42, 79, 80, 81, 82, 83, 86,
 88, 89, 91
 seed yields, 38, 79, 80, 84, 86, 89
 seedbank, 100, 106
 seeding, 14, 40, 45, 46, 51, 59, 65, 75,
 79, 80, 81, 86, 87, 88, 91, 93, 105, 110
 seeding date, 14, 79, 81, 87, 93
 seeding rates, 45, 75, 79, 87, 91
 seedling mortality, 81
 SG-CNTs, 35, See second-generation
 crops with novel traits
Sinapis alba, 36
Sonchus arvensis, 84
 soybean, 7, 35, 37, 39, 51, 98
 spring wheat, 36, 38, 44, 47, 101
 stinkweed, 36
 stress tolerance, 2, 10, 34, 35, 37, 43, 44
 strobilurin fungicides, 67, 69, 70
 sugar beet, 102
 sulfentrazone, 75, 84, 85
 sulphur, 82
 summerfallow, 38, 44
 sunflower, 77, 92, 102, 109
 switchgrass, 1
 TAG. See Triglycerides
Thinopyrum intermedium, 1
 thistle, 77, 84
Thlapsi arvense, 36
 time of flowering, 106
 tocopherols, 11
 tocopherols, 11, 76, 93
 trait containment, vii, 94, 95, 98, 99, 100,
 101, 102, 103, 104, 105, 107
 trait escape, 95, 96, 97, 99, 100, 103,
 104, 106
 trait-based models, 16, 19, 20
 transgene containment, 103
 transgene movement, 37, 51, 103
 transgenic crops, 4, 35, 52
 transportation, 36, 75, 78
 trifluralin, 75, 83
 Triglycerides, 7
Triticum aestivum, 36, 56, 97
 Tuljapurkar's approximation, 29
 U.S. Farm Policy, 40
 value-enhanced crops, 35
 vector, 104
 vital rates, 21, 22, 23, 29
 volunteer, 4, 10, 48, 58, 59, 63, 83, 94,
 96, 98, 99, 101, 106
 volunteer glyphosate-resistant canola, 97
 weed control, 83, 84, 92
 weediness, vii, 2, 3, 4, 16, 19, 22, 24, 27,
 30, 37, 40, 46
 wheat, 36, 37, 38, 49, 51, 56, 59, 60, 63,
 68, 97, 101, 106, 108, 109, 110, 111

