*Flyleaf*

AN ABSTRACT OF THE DISSERTATION OF

Gabriel D. Flick for the degree of Doctor of Philosophy in Crop Science presented on March 21, 2019.

Title: Brassicaceae Seed Persistence and Dormancy in the Willamette Valley

Abstract approved:

---

Carol A. Mallory-Smith

Producers in the Willamette Valley of Oregon are interested in growing canola as a rotational crop in grass seed and cereal rotations. Many other Brassicaceae seed crops also are produced within the valley. Concerns of seed persistence and volunteer contamination of Brassicaceae seed crops prompted the investigation of seed persistence and dormancy. Brassicaceae crop production can result in substantial input of crop seeds into the soil seed bank. These seeds may enter secondary dormancy, a condition which develops in mature dispersed seed, and form a persistent seed bank. Laboratory, greenhouse, and field studies were conducted over 4 years to determine if seed persistence of canola was different from that of other Brassicaceae crops produced in the valley. Surveys of seed loss were conducted during harvest. Canola seed loss was not different from daikon radish, turnip, or forage rape. In depth of emergence studies, canola and turnip seed emergence were similar; daikon radish emerged from greater depths. In field tillage trials, radish seedling emergence was greater than canola or turnip in deep tillage treatments. Seed persistence was not

affected by crop type, but tillage treatments were significant. There were no seeds remaining in the no-till treatment after 38 months. The greatest number of seeds were recovered from the deep tillage treatment.

The viability of radish seeds buried in pods declined at a steady rate over 30 months regardless of burial depth. However, the total number of persistent seeds was greater at deeper depths. Incubation of seeds in polyethylene glycol (PEG) resulted in a broad range (0-20% in 2016 and 0-60% in 2017) of secondary dormancy induction. Canola and daikon radish have similar potentials for secondary dormancy induction and very few (<3%) turnip seeds were induced into secondary dormancy. Kale, collard, and Michihili Chinese cabbage had similar secondary dormancy potentials as canola in 2016. No seeds of Crimson Giant radish were induced into secondary dormancy. A relationship between temperature during seed production and dormancy potential was found. Seeds produced in 2017, which was cooler than average, had greater levels of secondary dormancy. NCED gene expression in osmotically stressed seeds was similar among species. Differences in relative expression and secondary dormancy induction indicated that NCED expression alone cannot predict dormancy potential. Studies were conducted evaluating the effect of 2,4-D and MCPA applications and timings on seed germination and root and stem growth. Applications of 2,4-D and MCPA in greenhouse studies resulted in reductions in canola root and stem growth. When applied pre-emergence, 2,4-D and MCPA reduced canola emergence by 45% and 60%, respectively, compared to untreated controls. In a field trial, 2,4-D reduced emergence of Brassicaceae seedlings by more than 90% when rainfall occurred within 1 wk of application. Canola did not persist to a greater extent than any of the other Brassicaceae crops with which it was compared. The secondary

dormancy induction potential of canola was similar to that of other Brassicaceae crops which are produced in the Willamette Valley.

# Brassicaceae Seed Persistence and Dormancy in the Willamette Valley

by
Gabriel D. Flick

A DISSERTATION

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Presented March 21, 2019
Commencement June 2019

Doctor of Philosophy dissertation of Gabriel D. Flick presented on March 21, 2019.

APPROVED:

Major Professor, representing Crop Science

Head of the Department of Crop and Soil Science

Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Gabriel D. Flick, Author

# ACKNOWLEDGEMENTS

I would like to sincerely thank my major professor, Dr. Carol Mallory-Smith, for her advice, mentorship, immense number of edits, and patience with my farming addiction. I would also like to thank the members of my graduate committee: Dr. Andrew Hulting, Dr. Thomas Chastain, Dr. Edward Peachey, and Dr. Russell Ingham for their support and direction. A thank you goes out as well to Dan Curtis, and Kyle Roerig, for their friendship and assistance with field work, and Kevin Pompe for his advice and instruction on all things mechanical. To Dr. Laurent Deluc's lab, and especially Dr. Satanarayana Gouthu: your time, patience, humor, and expertise were appreciated more than you know.

A huge thank you to my fellow graduate students for all the laughs, encouragement, ideas, long-days, late-nights, beers, and friendship. Pete: thank you for allowing me to become 'Uncle Gabe', giving me a place to do laundry, eat a home cooked meal, practice my home improvement skills, and for continually reminding me that, "you are not special." I cannot imagine going through the last six years with anyone else.

To my parents, who have always encouraged me, pushed me, supported me, loved me, and worked tirelessly to keep the farm running in my absence; thank you. And, a special thank you to Stephanie for all of your patience, love, and support.

We made it.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF TABLES

# Brassicaceae Seed Persistence and Dormancy in the Willamette Valley

## CHAPTER 1

## General Introduction

The Willamette Valley of Western Oregon is home to diverse agricultural crop production. More than 170 different crops are produced on just over 760,000 ha of farmable land (ODA 2019). This broad diversity is due in part to the knowledgeable grower community as well as the mild, Mediterranean climate (CsB). Annual rainfall in the valley increases with elevation and ranges from 940 mm to 1150 mm across the majority of the valley with measurements exceeding 1700 mm in the foothill regions with much of the precipitation occurring over the cool winter months (Taylor et al. 1993). Winter temperatures rarely drop below -17 C and much of the valley floor has a growing season between 150 to 180 d. Dry, warm summers, with daily maximums seldom exceeding 32 C, allow for the production of high-quality seed which requires very little to no artificial drying (Brewer 2005).

Soils in the Willamette Valley vary from well-drained alluvial deposits to poorly drained or steep hillslopes (Oregon State Land-Use Planning Committee 1941). Bottom and terraced soils account for 335,000 ha of the valley floor and support diverse crops often with irrigation. Around 400,000 ha of cultivated ground in the valley are classified as hillslopes. These areas are characterized by steep slopes, poor drainage, lack of irrigation, and production is limited to grass seed, cereal crops, pasture, and tree production.

Between 141,000 to 162,000 ha of grass seed are produced annually with estimated farm gate sales to be $228,464,000 (Anderson 2017). Grass seed producers have expressed the need to find a broadleaf rotational crop which would disrupt pest, weed, and disease cycles, slow the development of herbicide resistance, diversify cultural practices, and provide economic returns while utilizing their current production equipment (K Hadley, personal communication). Despite the large diversity of crops grown within the valley, few rotational options exist for grass seed that is produced on ground classified as hillslopes or which lacks irrigation.

Canola (*Brassica napus* L.) has been suggested as a rotational option and has demonstrated high yield potential in Willamette Valley trials (Ferguson et al. 2016). Canola, which is marketed as a commodity crop, gives producers the option of when to sell and at what price. In addition, growers receive payment within 30 days of delivery which is economically important for some enterprises. However, interest in the production of canola as a rotation crop within cereal and grass seed rotations raised concerns about the potential for seed contamination of specialty seed crops due to volunteers arising from long-term seed bank persistence, increased pest pressure, and gene flow that would reduce seed purity (Loberg 2013).

Vegetable and specialty seeds have been produced in Oregon in some capacity since the early decades of the twentieth century (Schudel 1952). *Brassica* seed crops have been grown in the Willamette Valley for at least eight decades (Hyslop and Schoth 1937). Vegetable seed production increased when World War II interrupted the trade of seeds from Europe and necessitated a significant increase in domestic production. A vegetable production survey in 1943 reported 2,700 ha of vegetable seed production with a farm gate value of $2,500,000 (Oregon Vegetable Seed

Industry 1943). Oregon's potential to produce high-quality seed was recognized, and production peaked in 1944 at nearly 4,000 ha (Schudel 1952). The end of conflicts allowed foreign trade to resume and Oregon's seed acreage decreased. In the decades to follow seedsmen and growers steadily expanded and increased production. In 1980, a collection of seedsmen and companies formed the Willamette Valley Specialty Seed Association (WVSSA) (Loberg 2013).

Oregon produced 5,700 ha of vegetable seed with a farm gate value of $27,429,748 in 2012, roughly 3,200 ha of which was produced in the Willamette Valley (National Agricultural Statistics Service 2014). The specialty seed industry is a significant sector of the agricultural community in the Willamette Valley, and the area is recognized worldwide for its high-quality seed. International markets are critical for the sale of Oregon's seed, and it is important that those markets be preserved. Therefore, research regarding the impact of canola production on current cropping systems and specialty seed production in the Willamette Valley is needed and is valuable for multiple stakeholders.

## Origin and Development of Canola

The history and origins of many members of the Brassicaceae family have been widely discussed and debated. Similar morphological characteristics exist in multiple genera and species, which are diverse in geographical distribution (OECD 2012). These similarities caused early taxonomists some difficulty and led to multiple classification schemes, misclassifications, and subsequent reclassifications (Hedge

1976). The development of karyotyping in the early twentieth century allowed cytological studies which enabled the development of the botanical relationships for economically important *Brassica* species in the 1930s (U 1935). Observations of chromosomal pairing indicated that three amphidiploid species were derived from the interspecific crossing of three diploid progenitors. Confirmation of these relationships was achieved by the artificial synthesis of *B. napus* (Jourdan et al. 1988; U 1935), *B. juncea* (Prakash 1973) and *B. carinata* (Sarla and Raut 1988) and later through DNA confirmation with SCAR markers (Iñiguez-Luy et al. 2006). The primary center of origin for many of the Brassicaceae, including *B. rapa,* was postulated to be the Irano-Turanian region, an area spanning from the eastern shores of the Mediterranean through Afghanistan and northward across Kazakhstan (Hedge 1976). Eastern central Asia was suggested as a secondary center of origin for *B. rapa* where archeological excavations have unearthed seeds dating back 6000 to 7000 years (OECD 2012). *Brassica oleracea,* known for its leafy cole vegetable derivatives, was originally confined to the Mediterranean region (Kimber and McGregor 1995). Comparatively, *B. napus* is of a more recent origin; developing along the Mediterranean coast, the overlap of the two primary regions containing its progenitor species (OGTR 2016).

The utilization of *Brassica* as an oil source is thought to have first occurred in the middle ages; however, a determination of the species cultivated is unclear (Appelqvist and Ohlson 1972). Heresbach wrote in 1570 (as cited by Kimber and McGregor 1995) on the production of winter rape for lamp oil, soap making, and as a cheap cooking fat in the Rhineland area of Germany in the late 16$^{th}$ century. The slow-burning and nearly odorless characteristics of rapeseed oil continue to make it a choice for sanctuary lamps in some churches today. Land dedicated to the production

of oilseed rape peaked in Europe during the 1860s, then declined steadily until the early twentieth century (Appelqvist and Ohlson 1972).

The advent of the steam engine created a new demand for rapeseed oil when engineers discovered that the oil clung to water-washed metal components in a superior manner to other lubricants (Boulter 1983). This property led to its adoption as the favored lubricant for naval and marine engines of all capacities. As war broke out in the European theater, imports of rapeseed oil from Asia and Eastern Europe to North America were blockaded thus stimulating domestic rapeseed production. Rape production had existed since the pioneering days of both the U.S. and Canada but only as fodder or pasture varieties. It was in 1936 that immigrant farmer Fred Solvonik introduced the oilseed type of *B. rapa* from Poland (Bell 1982). This seed would become the material later sent to Canadian research farms and facilities just prior to the outbreak of World War II by T.M. Stevenson who noted that the crop grew vigorously and yielded well when grown during the long-day Canadian summers. Commercial production of *B. rapa* oilseed rape was not undertaken until 1943 (Anstey 1986). One year later, field selected seed of Argentine rapeseed stock (*B. napus*) was acquired and became the basis of the breeding program of W. J. White (Bell 1982).

It should be noted that variation exists between the two main species of *Brassica* which are called rapeseed. Most biennial, winter rapeseed produced is *B. napus* or Argentine rape, and this is true in the Willamette Valley. In regional trials, *B. napus* varieties yield 15-20% more than *B. rapa* varieties (Canola Council of Canada 2016; Daun 1983). However, *B. rapa* varieties are less prone to shattering when mature and typically reach maturity in fewer days making them more suitable

as spring rape (Hang et al. 1982). This becomes an important factor for northern production regions which have fewer consecutive frost-free days. *B. napus* varieties are commonly taller, have darker seed, and have a higher oil content than *B. rapa* varieties (Hang et al. 1982; SeedTec 1990). The pollination characteristics of the two species are also different. *B. napus* is about 70% self-pollinated, while *B. rapa* is 100% cross-pollinated. The seeds of *B. napus* are also about 0.4 mm larger in diameter.

By 1950, production of rapeseed had almost disappeared from the Canadian prairies. Conversion to diesel power reduced marine demand, government price supports had been eliminated, and what remained were a few processing facilities and the need to find a suitable rotation crop for Canada's farmers.

There are several factors which spurred on the efforts and resources committed to revitalizing the production of rapeseed and its subsequent transformation into canola. The first, and undoubtedly most influential, was the desire by Canada's government officials to secure the nation's food supply for its citizens and soldiers in the aftermath of world conflict and the threat of the Cold War (Busch et al. 1994). Secondarily, the need to meet the increasing demand for protein sources as animal feed; and more as an afterthought, a rotational crop for the predominantly grain based systems of the prairies. The harsh Canadian winters precluded the production of many common edible-oil plants except sunflower, soybean, and rape. Rape stood out because spring varieties matured and were harvestable before early winter frosts and snows, allowing the crop to be grown across a much larger geographical region than the other crops. It was also a crop that could be grown and harvested with the available machinery on most farms. However, rape was not

without undesirable characteristics. The seed grown for industrial oil matured unevenly resulting in a greenish-yellow oil with a strong odor. Significant processing was required to produce oil suitable for edible use. There were also concerns regarding erucic acid, a 22-carbon monounsaturated fatty acid with regards to human consumption and glucosinolates restricted meal use in animal rations, relegating its value to little more than a fertilizer.

Engineering and chemical process improvements during the early 1950s by workers at the National Research Council in Ottawa, Canada, proved that through refining, hydrogenation, bleaching, and deodorizing rapeseed oil could be substituted for soybean oil in edible products (Anstey 1986). The first licensed rapeseed in Canada was released in 1954 under the name 'Golden' boasting higher yields and even maturity. By 1955, margarine and salad oils were being commercially produced, but a temporary halt was caused by health concerns that were raised in 1956 (Boulter 1983). Work by Dr. Kenneth Carroll, while pursuing the development of new pharmaceuticals from long chain fatty acids, determined that erucic acid increased adrenal cholesterol in rats (Carroll 1953). The erucic acid proportion of total fatty acids in rapeseed produced at the time was around 55% in both *B. napus* and *B. rapa* species. Because rapeseed oil was used in such small quantities in the Canadian diet, processing for food products was allowed to continue.

Two separate breeding programs were in operation in Canada headed by R. K. Downey and B. R. Stefansson. Progress in the search for lower erucic acid genetics was slow at first. Improvements to gas-liquid chromatography and the development of the half-seed method, allowed oil analysis to be conducted on one half of a seed while the other half could be used to produce a plant if it contained desirable

characteristics (Downey and Harvey 1963). This method accelerated the screening process tremendously and in 1960 a plant was found which had zero erucic acid and was later released as the variety ‘Liho’. ‘Oro’ was released in 1968 as the first Low Erucic Acid Rapeseed (LEAR) and it contained only 1.2% erucic acid (Daun 1983). However, ‘Oro’ was not considered commercially successful as it never gained market share due to its lower agronomic and processing qualities.

In 1970, The Canadian Food and Drug Directorate revisited the issue of erucic acid after studies released from French and Dutch workers confirmed the earlier results of Dr. Carroll. A call for a national change to LEAR varieties led to over 74% of new crop samples meeting the directorates standards by 1972 (Daun 1983). The discovery of low glucosinolate levels by a visiting Polish scientist in the *B. napus* variety ‘Bronowski’ led to the development of ‘double low’ varieties of rapeseed released in 1974 and 1977 to fill both the *B. napus* and *B. rapa* markets, respectively (Bell 1982). The development of these varieties and their greatly changed chemical properties prompted the creation of the trademark “canola” in 1979. To be considered canola a variety’s oil profile must contain no more than two percent erucic acid and the meal, after defatting, must have less than 30 μmol/g of glucosinolates (Shahidi 1990). Except for a select few varieties which are tailored for specific markets, such as high erucic acid varieties whose oil is used for lubricants and slip-agents, most canola varieties are of the “Double Low” type. Development of “Triple Low” varieties, those which are also low in fiber, have been bred for improved utility of the meal in animal rations.

Although rapeseed oil had been used in food products since the 1950s in Canada, it was not until 1977 that the Canada Department of Health and Welfare declared that canola oil was safe for human consumption (Anstey 1986). European countries followed in 1979 but the United States FDA did not declare canola oil GRAS (Generally Recognized as Safe) until 1985. Following the GRAS declaration, the demand for edible canola oil increased. At that time U.S. grain producers were struggling with large production carryovers, depressed prices, and sagging exports. In the Pacific Northwest, rapeseed production in 1986 increased fourfold over the previous year (Karow 1986).

## Rapeseed and Canola Production in the Pacific Northwest and Willamette Valley

Rape has been grown in the Willamette Valley for over three-quarters of a century. It was considered a valuable pasture and companion crop as well as a good seed producer for valley farmers (Hyslop and Schoth 1937). The biennial variety 'Dwarf Essex' was found to be high-yielding and produced very desirable pasture stands which could be grazed and overwintered to produce seed the following season or to be grazed and tilled under as a soil amendment. Plots left for seed were hand harvested and threshed to be sold to the local market, as no other outlets for seed or processing for oil had been developed. Between the years of 1936 and 1943 rape seed was harvested on around 80 ha annually (Thomas et al. 1944). A new crop project began in 1960 at Oregon State University which evaluated many oilseed species for their adaptation to Oregon (Karow 1986). Vegetable oils were of great interest to

reduce import dependence and because of their increasing value in industrial processes and the isolation of their novel chemical constituents. Crambe was identified as a good source of erucic acid; however, rape was pursued because of its higher yield potential in the Willamette Valley.

The first winter rape variety and agronomic trials were conducted between 1966 and 1968 at Oregon State University, and a breeding program was initiated in 1969 (Calhoun et al. 1975). The primary goal was to develop a high-erucic acid, low glucosinolate, cold-tolerant variety for production in western Oregon. This goal was partially realized with the release of 'Indore' which met the desired chemical profile and yielded equally as well as 'Dwarf Essex' but was slightly less cold tolerant (Calhoun et al. 1983). Commercial success was never realized as Coast Trading Company Inc., who held the exclusive rights to distribution, folded due to financial difficulties (Karow 1986). Limited varietal and agronomic trials were continued at Oregon State into the mid-1980s. In 1979, a funding request was submitted to the Pacific Northwest Regional Commission for research into the development of oilseeds as alternative crops for the Pacific Northwest (University of Idaho et al. 1979). This joint research effort between Oregon State University, University of Idaho, and Washington State University researchers provided the broad base needed to test varieties and agronomic traits across the region before funding was discontinued in 1981 (Karow 1986). In the late 1980s and early 1990s, Seedtec INTL a division of Mitsubishi INTL produced several thousand acres of canola seed in the Willamette Valley (SeedTec 1990). Small and scattered amounts of canola seed were produced until the mid-2000s (T Chastain, personal communication).

Production of canola in the three Pacific Northwest states has been controlled by production districts since the mid-1980s. Idaho and Washington recognized the increasing production of rapeseed for both industrial and edible markets and proposed and passed legislation in 1986 to create districts to separate the two market classes (edible and industrial) of rapeseed being grown (1986 Amendments 1986, AP 1986). Twelve districts in Washington were finalized in 1988 (USDA 1996) and modified in 2008 because of increasing isolation concerns from seed producers (Inglis et al. 2013). Three new *Brassica* Seed Production Districts were created with general rules on growing, transporting, and processing applying to all of them. In 2012, District 2 was amended to exclude the production of winter canola (*B. napus* var. *biennis*) as the region had become a premier area for the production of spring canola stock seed.

Idaho's six districts initially separated edible and industrial oil production with District IV (Ada, Canyon, Gem, Owyhee, and Payette counties) being designated for rapeseed stock seed production only. A final rule prohibited the production of all rapeseed in District IV to protect the vegetable seed industry from feared contamination (Kephart and Murray 1990). In 2014, districts were consolidated to form two districts (IDAPA 2014). District II includes all land within the boundaries of Ada, Canyon, Gem, Owyhee (north of Murphy) and Payette counties. No rapeseed can be produced in District II. District I is all of Idaho not included in District II. Industrial and edible rapeseed are allowed in District I with restrictions. Industrial rapeseed in District I must have an isolation distance of 1.6 km from edible rapeseed. Industrial rapeseed growers must have written approval from farmers whose fields border the field being planted to industrial rapeseed.

In 1987, authority was given to the Oregon Director of Agriculture to create control areas (ORS 570.405). In 1989, ORS 570.450 allowed for the creation of rapeseed control districts. Twelve districts were identified, and six of them were activated in 1990 (Figure 1). In 2005, concerns were raised by the specialty seed industry about the increasing interest and incentives to produce canola for biofuel in the Willamette Valley (Karow 2012). The Oregon Department of Agriculture (ODA) after several hearings agreed to change the control districts. The original twelve were eliminated and replaced with four restricted zones, and the remaining area of the state was considered a general production area (Figure 2). The four restricted zones were the Willamette Valley (Benton, Clackamas, Marion, Linn, Lane, Polk, Washington, and Yamhill counties), Central Oregon (Crook, Deschutes and Jefferson counties), Northeast Oregon (Baker, Union, and a portion of Wallowa county), and the Malheur Strip (a 4.8 km wide strip of land along a portion of the Idaho border). The production of *Brassicas* for oil is prohibited except in the Northeast District, but *Brassica* seed production is allowed providing fields are pinned and isolated according to industry standards.

Continued interest in canola caused the ODA to request an allocation of funds from the Oregon Legislature's Emergency Board to support research by Oregon State University in 2006 (Karow 2012). An ODA budgetary allocation was then put in place through 2009 (ODA Oilseeds Grant) to continue research (Karow 2008). Research on gene flow between canola and specialty *Brassica* seeds, seed bank persistence of canola, insect and pathogen pressures, and alternative crops for grass seed producers were conducted (Karow 2010). In 2009, an advisory committee was formed by the ODA to review canola regulations (Coba 2009). However, after many

discussions, no consensus was reached. In 2009, the borders of the Willamette Valley protected district were redrawn to form a rectangle covering a majority of the Valley (Figure 3).

In 2012, the WVSSA, Specialty Seed Growers of Western Oregon (SSGWO), Willamette Valley Oilseed Producers Association, biofuel industry, vegetable growers, clover seed growers, OSU, Farm Bureau, State Board of Ag, and ODA were brought together again to discuss the coexistence of canola with specialty seed production (Karow 2012). In August of 2012, a temporary rule was issued by the ODA modifying the existing boundaries of the Willamette Valley protected district to allow the planting of canola in areas of the valley not historically planted to specialty *Brassica* seed (NASDA 2012). Shortly after issuance, a petition was filed by several members of the WVSSA and allied groups to stay the temporary rule pending judicial review (Mortenson 2012). In 2013, House Bill 2427 was passed and provided funding to assess the potential for co-existence between canola and other Brassicaceae seed crops in the Willamette Valley (HB 2427 2013).

Under the bill, 202 ha of canola were allowed to be grown under a special research permit within the Willamette Valley. Oregon State University was asked to review all of the available published materials and historical data on canola and specialty seed production and present it in a report to the legislature in November of 2017 (Mallory-Smith et al. 2017). Additionally, field monitoring for volunteers, insect pests, and diseases was to be conducted to help develop recommendations on the coexistence and production of canola in the Willamette Valley. In 2015, House Bill 3382 was passed which extended the production of 202 ha of canola under permit from 2017 to 2019 and asked the University to review published materials and

historical data on canola and specialty seed production in France, England, and New Zealand, as well as review the current canola production systems in Washington and Idaho (HB 3382 2015).

## Volunteer Persistence and Control

Crop domestication reduced seed shattering as reproductive structures mature (Tang et al. 2013). However, most crops in the Brassicaceae family remain prone to shattering; the reduction of which has been a primary goal for breeding programs around the world (Kadkol et al. 1984; Wang et al. 2007). Shattering can lead to increased seed rain and more seeds on the soil surface increasing the chances of seed persistence. Proper harvest timing has a significant effect on yield and oil quality (Brown et al. 1999). Average canola losses due to shattering can exceed 20% of the final yield in some years (Child et al. 1998) and be as high as 50% under poor harvest conditions (MacLeod 1981). Losses of 2 to 6% have been reported in recent years from canola producing regions (Gulden et al. 2003; Price et al. 1996). Harvest losses in the Willamette Valley from 12 to 53% of total yield have been reported (Quinn 2010). High loss potential coupled with small seed size could equate to a substantial seed rain of 2,400 to 9,100 seeds $m^{-2}$. This considerable seed rain together with seed persistence can lead to volunteer contamination of future crops.

Experiments and surveys of Brassicaceae seed persistence have varying results. Seedbank persistence of canola beyond two years was reported to be virtually nonexistent in Australia (Hails et al. 1997). In Canada, canola seed was reported to

persist at least 5 yr after harvest (Legere and Simard 2001). Workers in the U.K. recovered viable seeds 11 yr after burial (Lutman et al. 2003). The ability of canola to enter a state of secondary dormancy under certain environmental stresses contributes to seed persistence (Pekrun et al. 1998). Secondary dormancy is a condition that is induced or develops in mature seeds which are already dispersed (Bewley and Black 1994). Unlike many other plant genera, members of the commonly produced *Brassica* genus do not generally display primary dormancy (Lutman 1993).

The initiation and maintenance of dormancy are related to the plant hormone abscisic acid (ABA) (Taiz and Zeiger 2010). ABA is an important hormone which regulates plant growth and stomatal function, especially during periods of abiotic stress. ABA also has been found in seed tissues which have initiated active growth, suggesting that dormancy regulation was influenced by the balance of several endogenous hormones (Wareing and Saunders 1971). The isolation of ABA-deficient mutants of arabidopsis (*Arabidopsis thaliana* L. heynh.) helped confirm the theory that the balance of and sensitivity to phytohormones was more critical than their absolute concentrations (Koornneef et al. 1982). In *B. napus*, high levels of dormancy have been associated with increased biosynthesis and accumulation of ABA and a concomitant decrease in $GA_1$ (Gulden et al. 2004). A wide range of secondary dormancy potential exists among varieties of *B. napus* (Momoh et al. 2002; Pekrun et al. 1997), but no work has been completed examining the differences between *B. napus* and other Brassicaceae vegetable species.

Secondary dormancy can develop under high osmotic pressure, the absence of light, and high temperatures (Pekrun et al. 1998). Many of these factors are present in soils tilled shortly after harvest. Although tillage is commonly thought of as a means

to control germinated weed seedlings and to bury crop residues, it also moves seeds vertically within the soil profile, especially when inversion implements such as the moldboard plow are used. Plowing shifted a majority of seeds from the soil surface to deeper soil layers and reduced emergence the year following seed rain (Gruber et al. 2010). The depth of seed burial has a significant impact on seedling emergence. Seeds left on the soil surface are less likely to become part of the seedbank as they either germinate or succumb to mortality through pathogen and predator attack (Mohler and Galford 1997). Controlling the introduction of seed into the seedbank is critical to reducing the persistence of Brassicaceae crops.

The need to reduce persistent seed and volunteers has become more critical as disease pressure from black leg (*Leptosphaeria maculans* (Desm.) Ces. & De Not. [syn. *Plenodomus lingam* (Tode) Höhn.]) in the Willamette Valley has increased (Ocamb 2014). It is important to control volunteers before winter so that the availability of host tissue is minimized. The mild, wet winters of the Willamette Valley are a critical time for the sporulation and development of black leg (Claassen 2016). The immediate burial of infected residues by deep tillage, which also buries seeds on the soil surface, has been suggested as a control measure (Ocamb and Mallory-Smith 2015). Unfortunately, for reasons described earlier, this process also contributes to the induction of secondary dormancy.

Seed producers have noted that applications of phenoxy herbicides such as 2,4-D at the maximum labeled rate to fallow ground after harvest of Brassicaceae crops significantly reduced the number of persistent volunteers (T Hake, personal communication). Several studies reported that 2,4-D prevented germination of seed or resulted in abnormal seedlings that failed to mature (Allard et al. 1946; Hamner et al.

1946; Mitchell and Brown 1947). Phenoxy herbicides are commonly used in grass and cereal rotations within the Willamette Valley and offer the potential to be utilized to provide residual control of volunteers.

The objectives of the studies described herein were to evaluate the differences in seed persistence and volunteerism among canola, radish, and turnip, which is critical to determining the potential impacts of introducing canola into regional rotations. A survey of harvest loss was conducted to determine seed rain potential among the species. Studies evaluating seed fate and the maximum depth of emergence were conducted in the greenhouse. The fate of radish seeds buried in pods was examined under field conditions over 30 months. An assessment of annual tillage treatment effect on seed emergence and persistence was conducted over three years among the three crops to help develop best management practices for growers. Secondary dormancy induction potential was studied and *in situ* ABA production was evaluated through gene expression analysis using quantitative real-time PCR. Volunteer control using phenoxy herbicides was also evaluated in the greenhouse and under field conditions.

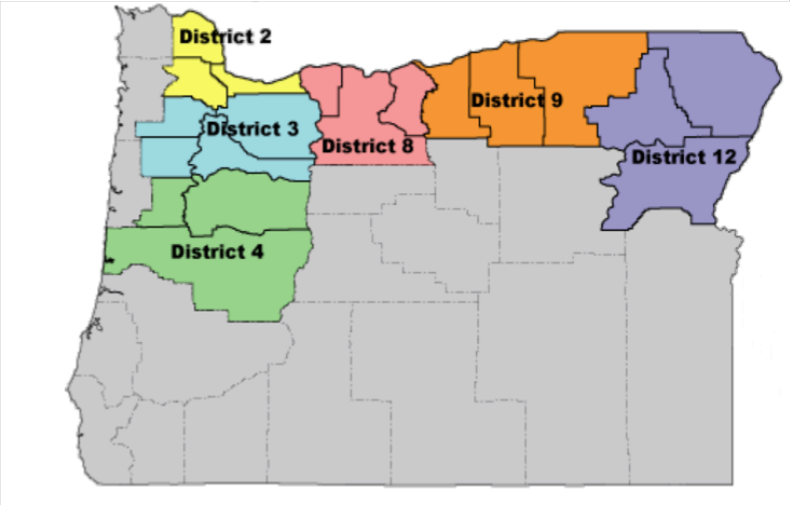


Figure 1.1 – Map of Oregon displaying the six rapeseed production districts which were activated in 1990. Adapted from Karow (2012).

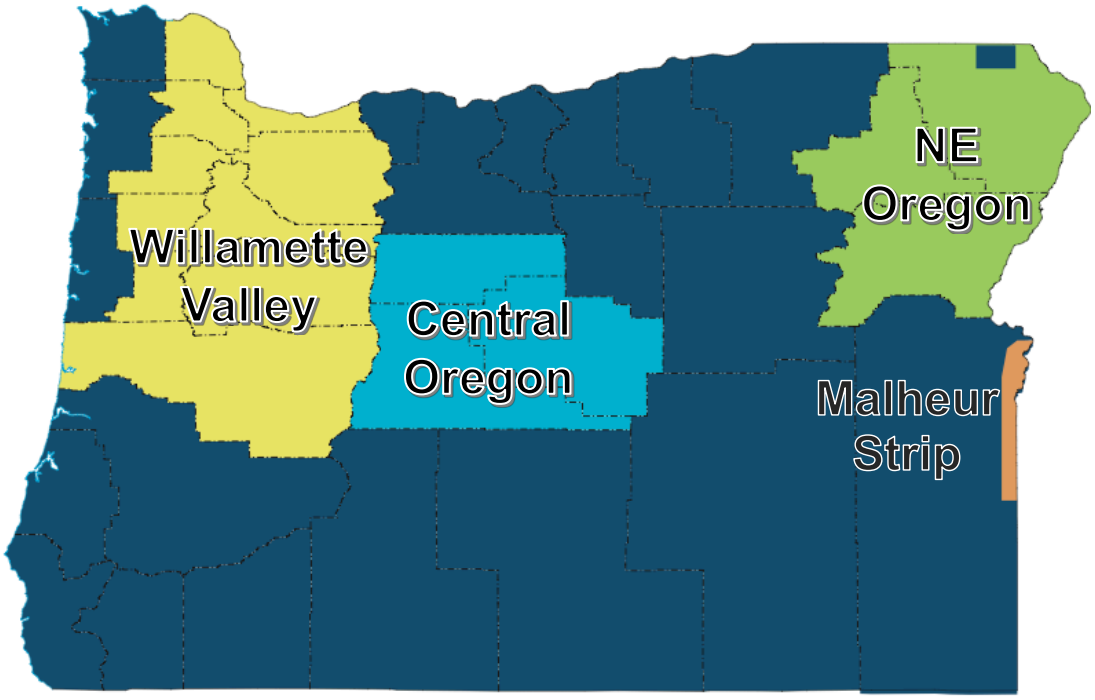


Figure 1.2 – Map of Oregon displaying 2005 modifications to the rapeseed production districts. The 12 districts were replaced with a general production zone (dark blue) encompassing all of Oregon not within four protected districts. Adapted from Karow (2012).

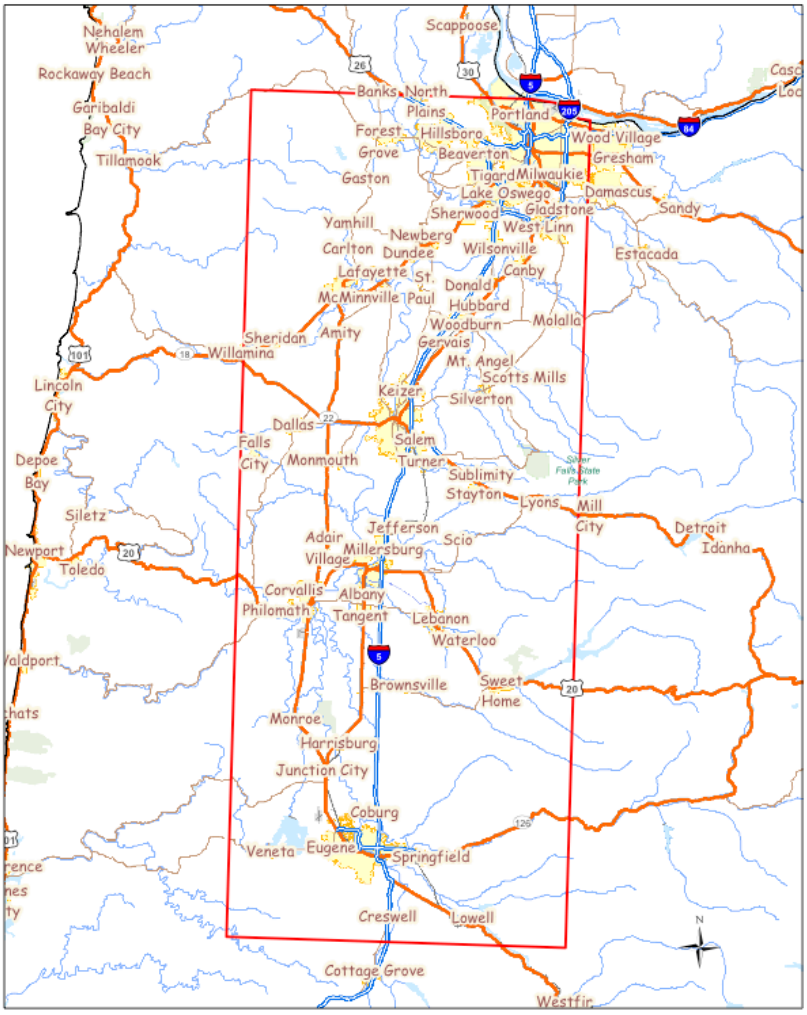


Figure 1.3 – Map of the Willamette Valley protected district after revision in 2009. Adapted from Karow (2012).

# CHAPTER 2

## Brassicaceae Seed Bank Persistence Under Different Tillage Treatments in Western Oregon

Gabriel D. Flick and Carol A. Mallory-Smith[1]

[1]Department of Crop and Soil Science, 107 Crop Science Building

Oregon State University, Corvallis, OR 97331

Formatted for submission to Weed Science for publication

## Abstract

The persistence of Brassicaceae seeds in the seed bank has been studied in most major canola production regions. Recent interest in canola production within the Willamette Valley of Oregon presents a unique coexistence challenge because the region produces many Brassicaceae vegetable seeds. Concerns of vegetable seed contamination arising from volunteer canola prompted an investigation of seed persistence by crop species and tillage treatment. Studies examining harvest loss, differences in depth of emergence, and seed persistence of canola, radish, and turnip were conducted. A field study including three tillage treatments (no-tillage, shallow tillage to 10 cm, and deep tillage to 20 cm) was conducted from 2014 to 2017 to determine tillage treatment effects on seed persistence. Another study evaluated the persistence of seeds buried in intact radish pods. Harvest seed losses of four Brassicaceae crops ranged from 97 to 345 kg $ha^{-1}$ but were not different from one another. In greenhouse studies, seeds buried at deeper depths were more likely to persist, and radish seedlings emerged from deeper depths than canola or turnip. The viability of radish seeds buried in pods declined at a constant rate regardless of depth and 16% of seeds buried at 20 cm were viable after 30 months. In the tillage study, there were no differences among crops within a tillage treatment; however, there were differences among tillage treatments. No seeds of any crop persisted under the no-tillage treatment. Deep tillage resulted in the greatest seed persistence for all crops. The percentage of seed recovered from the seed bank was 3.4, 1.6, and 1.4% of the original seed spread for canola, radish, and turnip, respectively, under the deep tillage treatment. Based on these studies, seed persistence was not different for these three

Brassicaceae crops but there were differences based on the tillage treatment. Growers concerned about seed persistence should avoid using deep tillage.

# Introduction

Brassicaceae seed crops have been grown in the Willamette Valley of Oregon for at least eight decades (Hyslop and Schoth 1937). The region experiences mild, wet winters and dry, warm summers which allow for the production of high-quality seed with little to no artificial drying (Brewer 2005). The Willamette Valley and similar regions in western Washington produce over 50% of the Unites States' supply of Brassicaceae vegetable seeds (Schreiber and Ritchie 1995). A majority of the world supply of cool season grass seed also is grown in the Willamette Valley with over half of the available acres of cropland devoted to its production (Anderson 2017). Interest in the production of canola (*Brassica napus* L.) as a broadleaf rotational crop in cereal and grass seed rotations raised concerns of increased pest pressure, seed persistence, and contamination of existing Brassicaceae seed production through admixture or cross-pollination. Anecdotal observations from industry personnel and producers suggested that there were differences in the persistence of Brassicaceae crop seeds after harvest.

A key aspect of crop domestication was the reduction of seed shattering as reproductive structures mature (Tang et al. 2013). However, most crops in the Brassicaceae family remain prone to seed shattering. Average canola losses due to shattering can exceed 20% of final yield in some years (Child et al. 1998) and be as high as 50% under poor harvest conditions (MacLeod 1981). Losses of 2 to 5% are commonly reported across Europe (Price et al. 1996). In Canada, losses of approximately 6% or 3,000 seeds $m^{-2}$ have been reported (Gulden et al. 2003). One

previous study conducted in the Willamette Valley reported canola harvest losses of 12 to 53% of total yield equating to 2,400 to 9,100 seeds $m^{-2}$ (Quinn 2010). No reports of harvest losses were found for radish (*Raphanus sativus* L.) or turnip (*Brassica rapa* L.).

Once shed, seeds of many crop species do not persist in the soil beyond three years (Rampton and Ching 1966). The domestication of crop plants to fit production systems has resulted in the reduction of dormancy and consequently most crop species do not constitute a serious weed threat in the form of volunteers (Bewley 1997). However, clover (*Trifolium* spp.), tobacco (*Nicotiana tabacum* L.), celery (*Apium graveolens* L.), and Kentucky bluegrass (*Poa pratensis* L.) seeds were viable after 39 yr of burial in jars (Toole and Brown 1946). The practice of burying seeds in jars or mesh bags, has been criticized for not accurately representing depletion rates (Van Mourik et al. 2005); therefore, studies which examine seed fate under field conditions are needed.

Experiments and surveys of Brassicaceae seed persistence report varying results. Seed bank persistence of canola beyond two years was reported to be virtually nonexistent (Hails et al. 1997). In Southern Australia, seed recovery dropped rapidly after 1 yr, and 3.5 yr after harvest all recovered seeds failed to germinate (Baker and Preston 2008). In Canada, canola seeds were reported to persist at least 5 yr after harvest (Legere and Simard 2001). Existence of volunteers on roadsides from varieties not produced for 8 to 9 yr in France was confirmed by Pessel et al. (2001) who deduced that populations were not from descending populations, but persistent seeds. Workers in the U.K. recovered viable seeds 11 yr after burial and also reported that burial depth had a pronounced effect on seed persistence (Lutman et al. 2003).

Pekrun et al. (1998) observed that seeds incorporated at a shallower depth (10 cm or less) were less likely to persist as compared to those at depths of 20 cm. It was postulated that this difference was due to the dormancy breaking effect of alternating temperatures at shallow depths. Many herbaceous species, especially those prone to forming persistent seed banks, utilize temperature fluctuations as a means of depth sensing (Thompson and Grime 1983). Therefore, it has been assumed that seeds buried in deeper soil layers where conditions are more constant will persist longer in the seed bank (Burnside et al. 1977).

Secondary dormancy is a condition that is induced or develops in dispersed mature seeds (Bewley and Black 1994). The ability of canola to enter a state of secondary dormancy under certain environmental stresses contributes to seed persistence (Pekrun et al. 1998). Unfavorable conditions such as high osmotic pressure, absence of light, or temperatures that are too high or low for optimum germination are known to promote the development of secondary dormancy. When tillage is performed immediately following harvest several of these conditions occur.

Although tillage is commonly thought of as a means to control germinated weed seedlings or to bury crop residues, it also moves seeds vertically within the soil profile, especially when inversion implements such as a moldboard plow are used. Plowing shifted a majority of seeds from the soil surface to deeper soil layers and reduced emergence the year following seed rain (Gruber et al. 2010). In another study, nearly 75% of linseed (*Linum usitatissimum* L.) and lucerne (*Medicago sativa* L.) seeds recovered after two 20 cm cultivations were found in the deepest 8 cm of the soil profile (Soriano et al. 1968). However, in other studies over time vertical distribution of seeds became more uniform under conventional moldboard plowing

systems than under reduced or no-tillage systems where seeds build up near the soil surface (Clements et al. 1996; Hoffman et al. 1998).

Depth of seed burial had a significant impact upon seedling emergence, and seedlings from seeds of greater size and weight emerge from greater depths (Benvenuti et al. 2001). Seeds left on the soil surface are less likely to become part of the seed bank as they either germinate, decay or are predated upon (Mohler and Galford 1997). Deeper burial of seeds provides a degree of protection from predators and pathogens while also increasing seed to soil contact. However, as the depth of seed placement increased the energy required for emergence also increased (Morton and Buchele 1960) and overall emergence declined (Benvenuti et al. 2001).

Understanding the differences and similarities among crops is critical to determining the potential impacts of introducing canola into the Willamette Valley. Seed bank persistence is directly correlated to the magnitude of harvest seed loss and influenced by environmental and cultural post-harvest management. The objectives of this study were to examine harvest losses, emergence characteristics, and long-term seed bank persistence under regional conditions. In order to draw comparisons, two Brassicaceae crops, radish and turnip, which are produced on larger fields (10 to 40 ha), were compared with canola. Forage rape (*Brassica napus* L.) was included only in the harvest loss evaluations.

## Materials and Methods

**Harvest Loss Study.** From 2014 to 2016, 11 fields of canola, forage rape, radish, and turnip seed were sampled after harvest to determine seed rain. Three pairs of samples were taken by randomly placing a 0.09 $m^2$ quadrat within the harvester separator shoe trail and outside of it. A vacuum (Shop-Vac 5015 Shop-Vac Corporation, Williamsport, PA) was used to collect seeds and residue within the quadrats. Samples were hand separated, and a germination test conducted by plating 50 randomly selected seeds on a moistened 90 mm blue germination blotter (Anchor Paper Company, Saint Paul, MN) in a 90 mm petri dish. Samples were placed in a germinator (SSG2-22 Hoffman Manufacturing Inc., Corvallis, OR) set to 25 C with a 12 h photoperiod. Additional water was added as needed. Germination counts (defined as the protrusion of the radical more than 2 mm through the testa) were taken 5 d after imbibition. Header width and harvester separator shoe width were used to calculate seed loss $ha^{-1}$ using the following equation:

$$\frac{\sum_{i=1}^{n_1} Y_i}{n_1} \times 10{,}000 \times \left(\frac{SW}{HW}\right) + \frac{\sum_{i=1}^{n_2} X_i}{n_2} \times 10{,}000 \times \left(1 - \frac{SW}{HW}\right)$$

Where *Y* is the number of viable seeds $m^{-2}$ in the harvester shoe trail, *X* is the number of viable seeds per $m^{-2}$ outside of the harvester shoe trail, *SW* is the separator shoe width, and *HW* is the header width. Data were subjected to ANOVA by PROC GLM is SAS. Means were separated by Tukey's HSD at $P \leq 0.05$.

**Greenhouse Seed Burial Study.** Canola 'Sitro', daikon radish, and turnip 'Purple Top White Globe' seeds were collected during harvest from fields within the Willamette Valley between June 25 and August 15, 2016. Samples were hand cleaned and stored in the dark at room temperature (22±2 C) until use. Plastic pots 15 cm in diameter and 18 cm deep were filled with a silt-loam field soil to within 2 cm of the planned burial depth. Coarse sand with a particle size of 0.35 mm (Unimin Corporation, Emmett, ID) was placed in the pots to achieve the desired burial depth and to allow for easier recovery of seeds. Twenty-five seeds of either canola, radish, or turnip were placed in each pot at one of six depths: 2.5, 5, 7.5, 10, 12.5, or 15 cm. Seeds were covered with 1 cm of sand and the remainder of the pot filled with field soil. The experiment was conducted in a greenhouse with a temperature regime of 20/15 C with natural light. Pots were randomly placed in shallow 5 cm deep trays filled with 2 cm of water. Soil was completely moistened through capillary action within 10 h; water was added to the trays as needed to keep soil moist. Buried seeds were never below the water level. Seedlings were counted and removed as they emerged. After four weeks, seeds were exhumed. Seed coats and germinated seeds, dead or degraded seeds, and intact seeds were counted. Recovered intact seeds were placed on moistened blue blotter paper and placed in a germination chamber at 25 C with a 12 h photoperiod. Seeds which germinated within 14 d were considered quiescent. Seeds that had not germinated after 14 d were exposed to a dormancy breaking treatment of gibberellic acid (GA) at a concentration of 300 ppm. GA treated seeds that germinated after 7 d were considered to be dormant. Those not germinated were counted as non-viable.

The study was a completely randomized design with four replications and was repeated. Recovery rates were less than 100%, so data were converted to proportions of recovered seed and arc sin transformed then tested for homogeneity of variance using PROC GLIMMIX in SAS 9.4. Results indicated heteroscedasticity assumptions were not met; therefore, runs were analyzed separately. Pots with seed recovery rates of less than 80% (3 canola and 9 turnip pots) were removed from analysis. Means separation at the 0.05 significance level was determined using PROC GLM and Tukey's HSD.

**Radish Pod Burial Study.** Daikon radish pods were collected from fields in the Willamette Valley in August 2014 and 2015 after windrowing but before harvest. Twenty-one cm diameter by 35 cm deep round pots with four 1.5 cm holes drilled down the side of the pot every 7 cm at 90-degree spacings for drainage were placed in the field in holes on September 14 and 10, 2014 and 2015, respectively. Ten pods were placed at 2.5, 10, and 20 cm depths within each pot on top of fiberglass coated mesh insect screen (Phifer Inc. Tuscaloosa, AL) to assist with removal. Pots were filled with field soil removed from the burial hole, so the soil layer in the top of the pot was level with the field surface. Thirty pods were randomly selected, and the seeds removed and counted to determine the average seeds per pod, 5.4 in 2014 and 5.6 in 2015. These numbers were used to determine an average seed count per pot.

Three pots were exhumed at predetermined times of 3, 6, 9, 12, 18, 24, and 30 months after burial. The soil was washed through a 3 mm screen and intact seeds removed. Recovered seeds were placed on moistened blue blotter paper and placed in a germination chamber at 25 C with a 12 h photoperiod. Seeds that did not germinate after 14 d were exposed to gibberellic acid at a concentration of 300 ppm. Germinated

seeds, as defined previously, were counted as viable and un-germinated seeds as non-viable. Data were log transformed to meet assumptions of normality and subjected to ANCOVA by PROC GLM is SAS. Contrasts in PROC GLM were used to separate regression coefficients of viable seed recovered by depth at $P \leq 0.05$. Means of viable seed recovered at 30 months were separated by Tukey's HSD at $P \leq 0.05$.

**Tillage Study.** A tillage study was established on the Oregon State University Hyslop Research Farm (44°38'01"N, 123°11'26"W), Corvallis, OR, on August 27, 2014. Soil in the trial area was a Woodburn silt-loam (fine-silty, mixed, mesic Aqualtic Argixerolls). The trial area had been fallowed for several years and had not contained a Brassicaceae crop in the previous 20 years. The soil was plowed, roller-harrowed, and rolled to create a firm surface similar to a post-harvest field.

Canola, radish, and turnip seed were collected during harvest from fields within the Willamette Valley from June to August 2014. Samples were hand cleaned and stored in the dark at room temperature (22 ± 2 C) until use. Based on harvest loss collections and previous data (Pari et al. 2012; Price et al. 1996; Quinn 2010), 32,300 canola seeds, 35,700 turnip seeds, or 26,800 radish seeds were spread by hand within each 2.44 m by 4.88 m plot.

Tillage treatments were applied immediately after seeds were spread and repeated at one-year intervals. A Great Plains 706NT no-till drill (Great Plains Ag, Salina, KS) was pulled across the no-till treatments, a Kuhn HRB 182 rotary harrow (Kuhn North America Inc., Broadhead, WI) was used for shallow tillage to a depth of 10 cm, and a three-bottom John Deere 825 moldboard plow (Deere & Company, Moline, IL) was used for deep tillage to a depth of 20 cm. A Brillion M-1241 Pulvi-

Mulcher (Landoll Farm Equipment, Brillion, WI) was pulled across plowed plots twice in opposite directions to smooth the soil surface after plowing.

Counts were taken as seedlings emerged following tillage and rainfall events. Counts were taken either for the whole plot or within 1 $m^2$ quadrats depending on seedling density. Plots were sprayed following each count with either 0.907 kg ai $ha^{-1}$ glyphosate plus 0.17% v/v R-11 non-ionic surfactant or 0.454 kg ai $ha^{-1}$ glyphosate plus 0.03 kg ai $ha^{-1}$ pyrasulfotole plus 0.17 kg ai ha $^{-1}$ bromoxynil plus 0.47% v/v crop oil concentrate to prevent any seed production within the study plots.

Thirty-eight months after trial establishment soil cores, which were 6 cm deep and 10.5 cm in diameter, were taken in 6 cm increments. Fourteen cores were taken from each increment; no-till treatments were sampled to 6 cm, shallow tillage treatments to 12 cm and deep tillage treatments to 24 cm. The number of cores taken per increment was chosen to represent a total surface sampling area equivalent to 1% of the total plot area (Medd 1992). Cores from the same depth and plot were homogenized and placed in 3.8 L plastic bags (S.C. Johnson and Son Inc. Racine, WI) and stored at 5 ± 2 C until processed.

Samples from radish plots were washed through a 1,000 micron screen (W.S. Tyler Mentor, OH) while canola and turnip samples were washed through a 589 micron screen to separate seeds and organic matter from soil using a water-spray machine (Kovach et al. 1988). The washed material was mixed with an 11 M potassium carbonate solution to separate seeds by flotation (Tsuyuzaki 1994). Washed material was stirred for 15 s and the process repeated until all organic material was removed. Brassicaceae crop seeds were separated from organic matter under a stereo dissecting microscope, rinsed with water, plated on blue blotter paper

in 90 mm petri dishes and placed in a germination chamber at 25/5 C with a 12 h photoperiod. After 7 d, non-germinated seeds were incubated in 1% tetrazolium chloride solution for 18 h and viability determined by examining staining patterns following the ISTA protocol (Leist et al. 2011).

A randomized complete block split-plot design with tillage as the main factor and crop as the subplot factor was utilized. Data were log transformed to meet assumptions of normality and subjected to ANOVA by PROC GLM is SAS. Means were separated by Tukey's HSD at $P \leq 0.05$.

## Results and Discussion

**Harvest Loss Study.** Average losses for canola and forage rape were 286 and 345 kg $ha^{-1}$, respectively and were similar to other reports. Average harvest losses in kg $ha^{-2}$ and losses as a percent of average yield were not different among crops (Table 2.1). A previous survey of canola fields in the Willamette Valley, reported losses from 71 to 260 kg $ha^{-1}$ representing 12 to 53% of yield (Quinn 2010). These results fall well within European reports of losses ranging from 250 up to 770 kg $ha^{-1}$ (Pari et al. 2012, Price et al. 1996). In a recent Canadian survey of canola production fields across the Northern Great Plains, losses up to 5.9% of yield or 4281 seeds $m^{-2}$ were reported (Cavalieri et al. 2016). Published data on harvest losses for radish or turnip seed for comparison were unavailable.

Radish pods have much thicker fruit walls and do not naturally dehisce like other crops (Warkentin 1985). In fact, radish pods can prove difficult to thrash and

partially or wholly un-thrashed pods are often expelled from the cleaning shoe of the harvester. Contrastingly, canola pods thrash easily and the reduction of their dehiscence has been a primary objective for breeders (Kadkol et al. 1984; Wang et al. 2007).

When making comparisons of harvest losses, it should be kept in mind that there are differences in 1,000 seed weights as well as yields. Turnip seed fields in the Willamette Valley yield around 1500 kg $ha^{-1}$ whereas winter canola fields yield upward of 3500 kg $ha^{-1}$ (K Hadley, personal communication). Forage rape and daikon radish seed fields yield on average 2800 kg $ha^{-1}$ and 1500 kg $ha^{-1}$, respectively (M Crawford and H Kuenzi, personal communication). For this reason, losses have been reported as seeds $m^{-2}$ and as a percentage of average yield to allow for a more equal comparison, particularly, when considering losses as they effect seed bank input and subsequent volunteer recruitment (Table 2.1).

**Greenhouse Seed Burial Study.** A majority of seedlings from seeds buried at 2.5 cm emerged in the first 7 d after study establishment. Emergence decreased as depth increased with no emergence occurring deeper than 12.5 cm (Figure 2.1). Emergence and seed loss of canola and turnip were similar within runs. Radish emergence from 7.5 cm in run 1 and 10 cm in run 2 were greater compared to canola or turnip at the same depth.

Seed loss (the sum of seeds germinated but not emerged, dead seeds, and seeds which were not recovered), accounted for 98% and 88% of seeds buried deeper than 10 cm in runs one and two, respectively. Only two viable canola seeds (0.32% of total planted at 10 cm) were recovered in run 1. Sixteen canola (2.6%), five radish (0.8%), and one turnip (0.16%) seed were recovered from run two.

No viable seeds were recovered from depths less than 10 cm, while at depths greater than 10 cm, viable seeds were recovered. Comparisons among the three depths from which viable seeds were recovered revealed there was no interaction between crop and depth within either run.

Crop species was the only significant factor in run two; no significance was found in run one. Results indicate that among the three crops, canola seeds had greater persistence than turnip seeds buried deeper than 10 cm, but canola and radish were not different (Figure 2.2).

**Radish Pod Burial Study.** In the pod burial study, viable seed recovery followed a negative trend over time across all depths (Figure 2.3). Regression coefficients of transformed data (non-transformed data presented for clarity) were not different for the three depths indicating that seed decay was constant regardless of burial depth. The notable difference between depths were the intercept values which reflect the number of seeds that germinated and emerged or died during the first three months after burial. A majority of seeds within pods buried at 2.5 cm germinated during the first three months. After 30 months, pods buried at 2.5 and 10 cm had fewer viable seeds than those buried at 20 cm. This result agrees with the results of the greenhouse burial study showing that seeds buried at deeper depths have a greater potential to persist.

**Tillage Study.** Emergence during the first six weeks after treatment (initial emergence) accounted for more than one-third of seed spread in no-till plots, and over half of seed spread in shallowly tilled plots regardless of species (Figure 2.4). Far less initial emergence was recorded from deep tilled plots; however, emergence beyond the first six weeks was greater in these plots, most specifically in radish plots. There

was no interaction between crop species and tillage treatments after 38 months. There were no differences among crops within a tillage treatment; however, there were differences among tillage treatments. No seeds of any crop persisted under the no-tillage treatment, and deep tillage resulted in the greatest seed persistence for all crops (Table 2.2). The percentage of seed recovered from the seed bank was 3.4, 1.6, and 1.4% of the original seed spread for canola, radish, and turnip, respectively, under the deep tillage treatment. Based on this 38-month study, seed persistence is not different for these three Brassicaceae crops but is affected by the tillage treatment applied.

Tillage has long been known to affect the rate of seed bank exhaustion (Brenchley and Warington 1933; Chepil 1946). One report indicated seed loss increased from 22% per year in undisturbed plots to 36% per year in plots disturbed four times (Roberts and Dawkins 1967). In contrast, deep tillage such as moldboard plowing has been shown to bury seeds and prolong persistence (Cheam and Lee 2009). Results from seed and radish pod burial studies, and tillage studies included in this current research indicate that seeds buried deeper than 10 cm persist longer than seeds buried at shallower depths.

Two and one-half to 10 times the amount of viable seeds were recovered from buried undisturbed radish pods compared to radish seed buried and subjected to annual tillage treatments. Although few studies have been conducted on cultivated radish in relation to seed persistence, wild radish (*R. raphanistrum* L.) has been the subject of numerous studies. One study found that 44% of wild radish seeds remained viable after three years of burial at 10 cm (Code et al. 1987). In another study, over 18% of wild radish seeds were viable after 5 yr in cultivated soil (Roberts and Boddrell 1983).

Radish crop seeds buried in pods had a half-life of around 18 months which is similar to results reported by Chancellor (1986) for wild radish, but about half as long as the wild radish populations studied by Tricault et al. (2018). While wild radish was found to have a short half-life compared to other weed seeds, viable seed was found after 20 years under sod demonstrating the ability of wild radish seeds to persist for long periods of time (Chancellor 1986). Considering the high harvest losses per ha reported here, even a low percentage of persistent seeds could result in volunteers for many years. This result highlights the importance of keeping seeds near the soil surface where they can germinate, degrade or be consumed.

Researchers in Europe concluded that reduced or delayed tillage decreased the number of seeds introduced into the seed bank (Lutman et al. 2003; Pekrun et al. 1998). Delayed tillage leaves crop residues and seeds untouched on the soil surface for a period of time, usually months, after harvest before tillage. The same research also indicated that zero-tillage may be the best strategy for reducing persistence. The current study supports this conclusion, as volunteer canola was not found to persist under no-till treatments whereas seeds persisted beyond three years under shallow and deep tillage.

Studies have shown that the diversity of arthropod species is affected by tillage regime (Brust and House 1988). Under conventional tillage there was a 40% reduction in consumption of weed seeds placed on the soil surface compared to no-tillage treatments. Arthropods actively transport seeds into underground nests, burrows and cracks (Zhang et al. 1997). Predator consumption is one explanation for the high percentage of unaccounted for seed from no-till plots in the tillage study. The greatest percentage of unaccounted for seed was in deep tillage plots. Germination

without emergence, which accounted for a large number of seeds in the greenhouse burial study at the three deepest depths, could account for a large percentage of the unaccounted for seeds. The lowest percentage of unaccounted for seed was in shallow tillage treatments; however, these treatments also had the greatest percent germination.

A close relationship exists between seed weight and the depth from which a seedling can emerge (Benvenuti et al. 2001). In the greenhouse study, no emergence of canola or turnip was found when seeds were buried deeper than 7.5 cm. Radish seeds, which are nearly three times heavier than canola, emerged at depths up to 10 cm in the greenhouse (Figure 2.1). In the tillage study, corresponding results were noted where radish emergence was greater in the deep tillage treatment (Figure 2.4).

As burial depth increases, an increasing proportion of seeds do not germinate and contribute to the seed bank (Baskin and Baskin 1998). If these remaining viable seeds have the ability to enter a state of secondary dormancy then a persistent seed bank is formed. The present greenhouse burial study agrees with previous studies and underscores the importance of keeping seeds near the soil surface to prevent the creation of a persistent seed bank.

It has been observed that loses from the seed bank occur at a constant rate. This constant depletion indicated that germination, predation, and losses to disease were more important than losses related to deterioration and aging (Rice 1989). Results of the pod burial studies reported in this paper confirm previous conclusions by demonstrating no difference in seed bank depletion rates of radish seed among three different burial depths over 30 months.

The results of the studies reported in this paper demonstrate that despite differing harvest losses seed bank persistence does not depend on which of the three crops was grown, but upon the tillage treatment applied following harvest. Seed bank depletion of radish seeds buried in pods occurred at a consistent rate regardless of depth. Radish seedlings emerged from deeper depths than canola and turnip. Producers concerned about seed bank persistence should avoid using deep tillage and attempt to reduce seed loss before and during harvest through proper harvest timing and combine adjustment. Future work comparing multiple cultivars of each crop species which are regionally produced could help to determine the effect of genetics on seed persistence.

## Literature Cited

Anderson N (2017) Willamette Valley Field Crops: Grass Seed. Oregon State University. https://valleyfieldcrops.oregonstate.edu/grass-seed. Accessed February 6, 2019

Baker J, Preston C (2008) Canola (*Brassica napus* L.) seedbank declines rapidly in farmer-managed fields in South Australia. Aust J Agric Res 59:780-784

Baskin JM, Baskin CC (1998) Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. London: Academic Press. 666 p

Benvenuti S, MacChia M, Miele S (2001) Quantitative analysis of emergence of seedlings from buried weed seeds with increasing soil depth. Weed Sci 49:528–535

Bewley J (1997) Seed germination and dormancy. Plant Cell 9:1055–1066

Bewley JD, Black M (1994) Seeds: Physiology of Development and Germination. 2nd edn. New York: Plenum Press. 445 p

Brenchley WE, Warington K (1933) The weed seed population of arable soil: II. Influence of crop, soil and methods of cultivation upon the relative abundance of viable seeds. J Ecol 21:103-127

Brewer DH (2005) Oregon Seed Production. Silverton, OR: OSU Seed Certification Services. 74 p

Brust GE, House GJ (1988) Weed seed destruction by arthropods and rodents in low-input soybean agroecosystems. Am J Altern Agric 3:19–25

Burnside OC, Wicks GA, Fenster CR (1977) Longevity of shattercane seed in soil across Nebraska. Weed Res 17:139–143

Cavalieri A, Harker KN, Hall LM, Willenborg CJ, Haile TA, Shirtliffe SJ, Gulden RH (2016) Evaluation of the causes of on-farm harvest losses in canola in the Northern Great Plains. Crop Sci 56:2005-2015

Chancellor RJ (1986) Decline of arable weed seeds during 20 years in soil under grass and the periodicity of seedling emergence after cultivation. J Appl Ecol 23:631–637

Cheam A, Lee S (2009) Inversion ploughing: Effects of long-term deep burial on weed seed reserves. Department of Agriculture and Food, Western Australia. 5 p

Chepil WS (1946) Germination of weed seeds: II. The influence of tillage treatments on germination. Sci Agric 26:347–357

Child RD, Chauvaux N, John K, Ulvskov P, Onckelen HAV (1998) Ethylene biosynthesis in oilseed rape pods in relation to pod shatter. J Exp Bot 49:829–838

Clements DR, Benott DL, Murphy SD, Swanton CJ (1996) Tillage effects on weed seed return and seedbank composition. Weed Sci 44:314–322

Code G, Walsh M, Reeves T (1987) Effect and depth and duration of burial of wild radish seed on seed viability and seedling emergence. Pages 136–138 *in* Proceedings of the Weed Seed Biology Workshop. Orange, Australia

Gruber S, Bühler A, Möhring J, Claupein W (2010) Sleepers in the soil—vertical distribution by tillage and long-term survival of oilseed rape seeds compared with plastic pellets. Eur J Agron 33:81–88

Gulden RH, Shirtliffe SJ, Thomas AG (2003) Harvest losses of canola (*Brassica napus*) cause large seedbank inputs. Weed Sci 51:83–86

Hails RS, Rees M, Kohn DD, Crawley MJ (1997) Burial and seed survival in *Brassica napus* subsp. *oleifera* and *Sinapis arvensis* including a comparison of transgenic and non-transgenic lines of the crop. Proc Biol Sci 264:1–7

Hoffman ML, Owen MDK, Buhler DD (1998) Effects of crop and weed management on density and vertical distribution of weed seeds in soil. Agron J 90:793–799

Hyslop GR, Schoth HA (1937) Rape Extension Bulletin 499. Federal Cooperative Extension Service. 4 p

Kadkol GP, Macmillan RH, Burrow RP, Halloran GM (1984) Evaluation of *Brassica* genotypes for resistance to shatter. I. Development of a laboratory test. Euphytica 33:63–73

Kovach DA, Thill DC, Young FL (1988) A water-spray system for removing seed from soil. Weed Technol 2:338–341

Legere A, Simard MJ (2001) Presence and persistence of volunteer canola in Canadian cropping systems. Pages 143-148 *in* Brighton Crop Protection Conference, Weeds. Brighton, UK: British Crop Protection Council

Leist N, Krämer S, Jonitz A, eds. (2011) ISTA Working Sheets on Tetrazolium Testing Volume 1: Agricultural, Vegetable and Horticultural Species with Supplements 2011. International Seed Testing Association (ISTA). 176 p

Lutman PJW, Freeman SE, Pekrun C (2003) The long-term persistence of seeds of oilseed rape (*Brassica napus*) in arable fields. J Agric Sci 141:231–240

MacLeod J (1981) Harvesting. Pages 107–119 *in* Oilseed Rape Book. A manual for growers, farmers and advisors. Cambridge: Cambridge Agricultural Publishing

Medd R (1992) Techniques for monitoring seedbanks of annual grass weeds on arable soils. Pages 333–335 *in* Proceedings of the First International Weed Control Congress. Melbourne, Australia: Weed Science Society of Victoria

Mohler CL, Galford AE (1997) Weed seedling emergence and seed survival: separating the effects of seed position and soil modification by tillage. Weed Res 37:147–155

Morton CT, Buchele WF (1960) Emergence energy of plant seedlings. Agric Eng 41:428–31, 453–55

Pari L, Assirelli A, Suardi A, Civitarese V, Del Giudice A, Costa C, Santangelo E (2012) The harvest of oilseed rape (*Brassica napus* L.): The effective yield losses at on-farm scale in the Italian area. Biomass Bioenergy 46:453–458

Pekrun C, Hewitt JDJ, Lutman PJW (1998) Cultural control of volunteer oilseed rape (*Brassica napus*). J Agric Sci 130:155–163

Pessel D, Lecomte J, Emeriau V, Krouti M, Messean A, Gouyon PH (2001) Persistence of oilseed rape (*Brassica napus* L.) outside of cultivated fields. Theor Appl Genet 102:841–846

Price JS, Hobson RN, Neale MA, Bruce DM (1996) Seed losses in commercial harvesting of oilseed rape. J Agric Eng Res 65:183–191

Quinn MP (2010) Potential Impacts of Canola *(Brassica napus* L.) on *Brassica* Vegetable Seed Production in the Willamette Valley of Oregon. Ph. D dissertation. Corvallis, OR: Oregon State University. 79 p

Rampton HH, Ching TM (1966) Longevity and dormancy in seeds of several cool-season grasses and legumes buried in soil. Agron J 58:220–222

Rice KJ (1989) Impacts of seed banks on grassland community structure and population dynamics. Pages 211-230 *in* MA Leck, VT Parker, RL Simpson, eds. Ecology of Soil Seed Banks. San Diego, CA: Academic Press Inc.

Roberts HA, Boddrell JE (1983) Seed survival and periodicity of seedling emergence in eight species of Cruciferae. Ann Appl Biol:301–304

Roberts HA, Dawkins PA (1967) Effect of cultivation on the numbers of viable weed seeds in soil. Weed Res 7:290–301

Schreiber A, Ritchie L (1995) Washington Minor Crops. Richland, WA: Washington State University. 325 p

Soriano A, Zeiger E, Servy E, Suero A (1968) The effect of cultivation on the vertical distribution of seeds in the soil. J Appl Ecol 5:253-257

Tang H, Cuevas HE, Das S, Sezen UU, Zhou C, Guo H, Goff VH, Ge Z, Clemente TE, Paterson AH (2013) Seed shattering in a wild sorghum is conferred by a locus unrelated to domestication. Proc Natl Acad Sci 110:15824–15829

Thompson K, Grime JP (1983) A comparative study of germination responses to diurnally-fluctuating temperatures. J Appl Ecol 20:141–156

Toole EH, Brown E (1946) Final results of the Duvel buried seed experiment. J Agric Res 72:201–210

Tricault Y, Matejicek A, Darmency H (2018) Variation of seed dormancy and longevity in *Raphanus raphanistrum* L. Seed Sci Res 28:34–40

Tsuyuzaki S (1994) Rapid seed extraction from soils by a flotation method. Weed Res 34:433–436

Van Mourik TA, Stomph TJ, Murdoch AJ (2005) Why high seed densities within buried mesh bags may overestimate depletion rates of soil seed banks: appraisal of the seed bag burial method. J Appl Ecol 42:299–305

Wang R, Ripley VL, Rakow G (2007) Pod shatter resistance evaluation in cultivars and breeding lines of *Brassica napus, B. juncea* and *Sinapis alba*. Plant Breed 126:588–595

Warkentin H (1985) Combine attachment for harvesting radish seeds and the like. U.S. Patent. No. 4,524,780. Washington, DC: U.S. Patent and Trademark Office

Zhang J, Drummond FA, Liebman M, Hartke A (1997) TB163: Insect Predation of Seeds and Plant Population Dynamics. Orono, ME: Maine Agricultural and Forest Experiment Station. 32 p

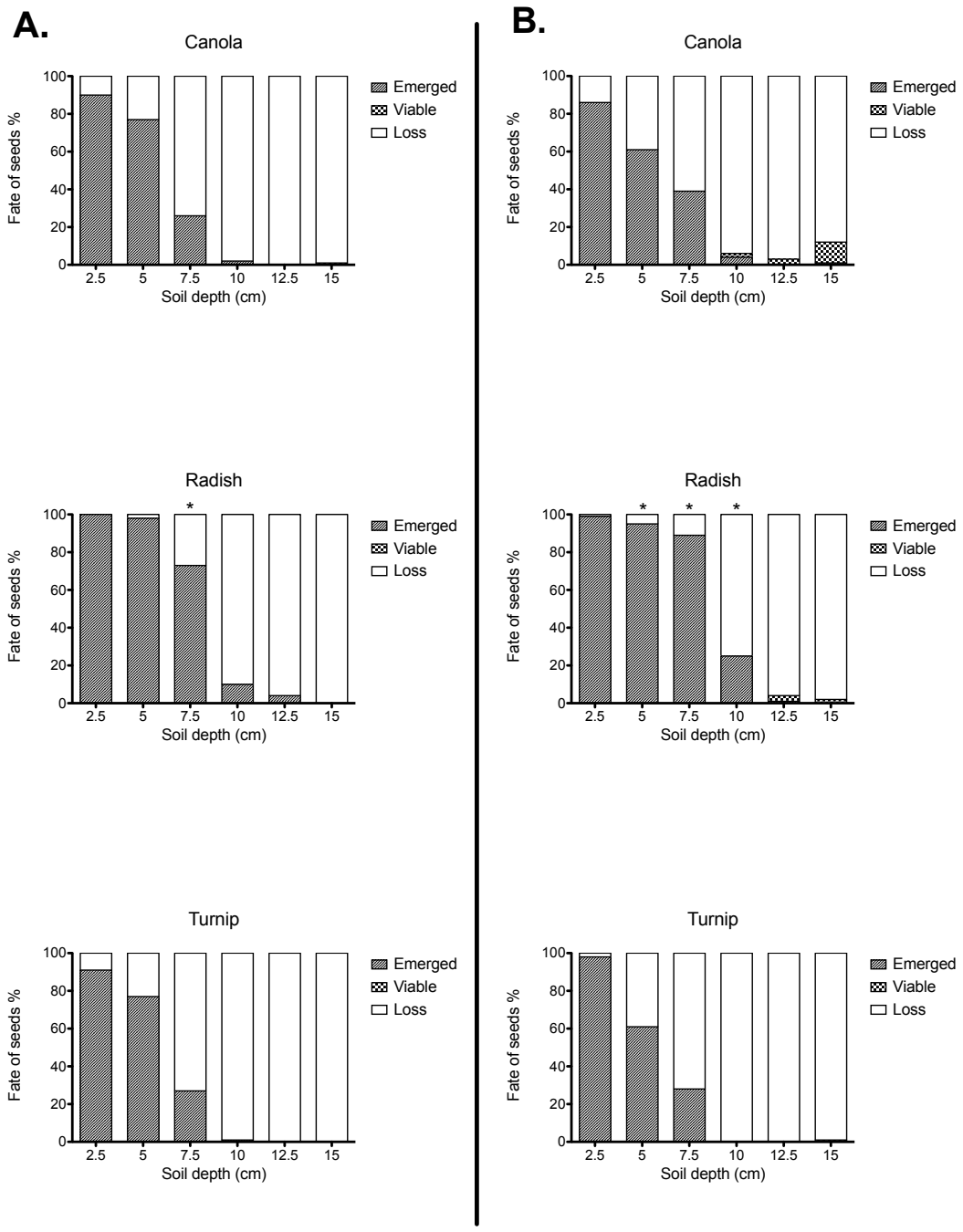


Figure 2.1 – Fate of seeds from run 1 (A.) and run 2 (B.) at different soil burial depths after 28 days in a greenhouse study. Asterisks above bars indicate differences among crops within a depth.

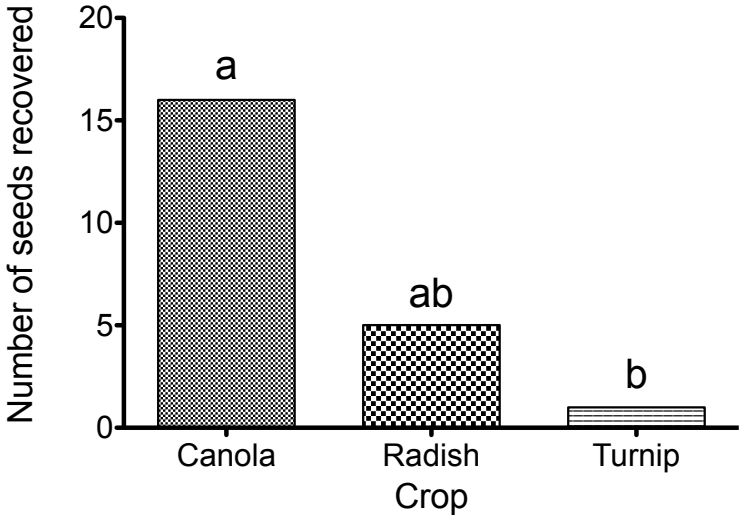


Figure 2.2 – Total seed recovered from all depths in run 2. Bars which have the same letter are not different according to Tukey's HSD (P≤0.05).

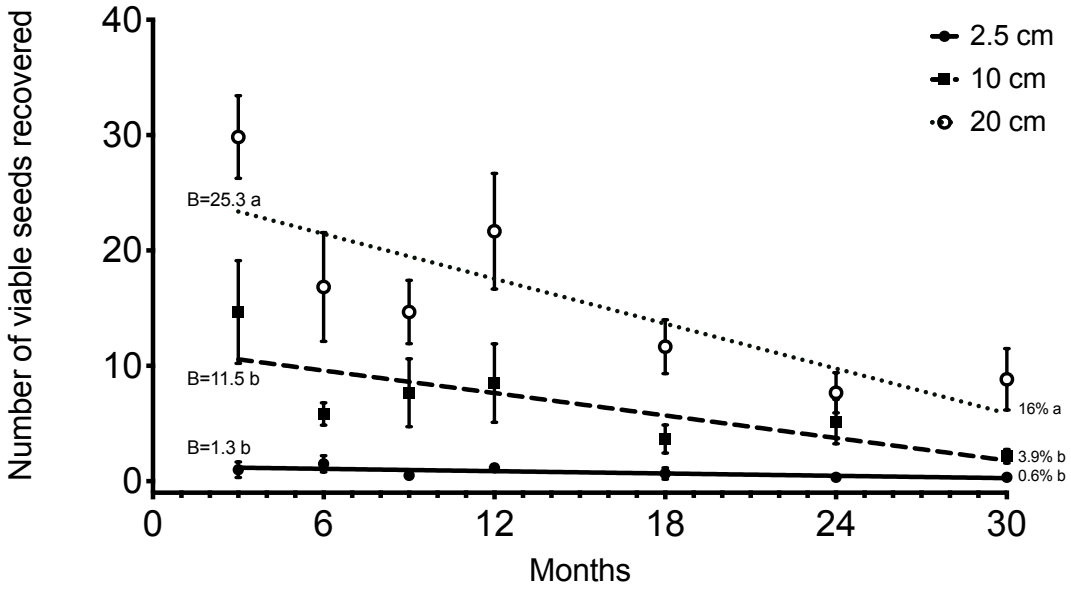


Figure 2.3 – Viable radish seeds recovered over 30 months after burial at three different depths. Intercept values for each regression are given near the Y axis, values with the same letter are not different according to Tukey's HSD (P≤0.05). Percentage viable buried seed recovered after 30 months is displayed on the lower right side of the chart area, and percentages with the same letter are not different according to Tukey's HSD (P≤0.05).

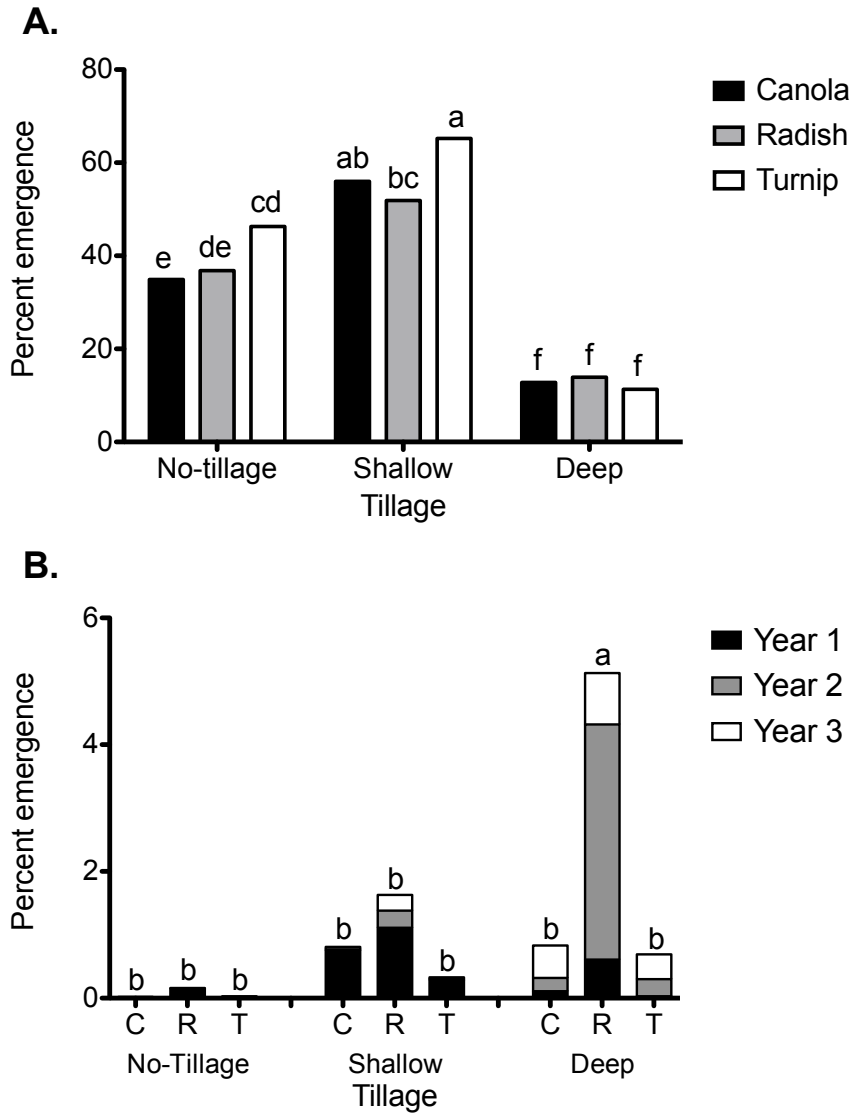


Figure 2.4 – The percentage of the initial amount of seed spread which was accounted for in germination counts (A). Initial counts were taken 30 days after seed was spread and tillage treatments were applied. Shallow tillage was equivalent to a 10 cm incorporation, and deep tillage was equivalent to a 20 cm incorporation depth. Figure 4-B displays emergence during year 1 which includes counts taken during the first year after treatment except for the initial count as well as emergence during years 2 and 3. Bars with the same letter are not different according to Tukey's HSD ($P \leq 0.05$).

Table 2.1 – Range and average harvest losses of fields surveyed from 2014 to 2016 in the Willamette Valley of Oregon.

| | Harvest losses | | | |
|---|---|---|---|---|
| Crop | Range | Average | Seeds $m^{-2}$ | % of average yield |
| | ----------Kg $ha^{-1}$---------- | | | |
| Canola | 130-635 | 286 a[a] | 5925 | 8.2 a |
| Forage rape | 189-500 | 345 a | 7147 | 12.3 a |
| Radish | 107-166 | 151 a | 1065 | 10.1 a |
| Turnip | 33-250 | 97 a | 3634 | 6.5 a |

[a] Means within a column followed by the same letter are not different according to Tukey's HSD test at ($P \leq 0.05$).

Table 2.2 – Fate of seeds under different tillage treatments after 38 months. Tillage treatments were applied annually.

| Crop and Treatment | Fate of seed | | |
|---|---|---|---|
| | Germinated | Unrecovered | Recovered from seed bank |
| Canola | ----------%---------- | | |
| No-till | 35.0 de | 65.0 bc | 0 c[a] |
| Shallow | 56.9 ab | 43 ef | 0 c |
| Deep | 13.6 f | 82.6 a | 3.4 a |
| Radish | | | |
| No-till | 36.9 cde | 63.0 bcd | 0 c |
| Shallow | 53.5 abc | 46.0 def | 0.5 b |
| Deep | 19.0 ef | 79.2 ab | 1.6 a |
| Turnip | | | |
| No-till | 46.3 bcd | 53.7 cde | 0 c |
| Shallow | 65.5 a | 34.3 f | 0.1 bc |
| Deep | 12.0 f | 86.5 a | 1.4 a |

[a] Percentages within a column followed by the same letter are not different according to Tukey's HSD test at ($P \leq 0.05$).

# CHAPTER 3

## Secondary Dormancy Induction in Several Brassicaceae Species

Gabriel D. Flick and Carol A. Mallory-Smith[1]

[1]Department of Crop and Soil Science, 107 Crop Science Building

Oregon State University, Corvallis, OR 97331

Formatted for submission to Weed Science for publication

## Abstract

Secondary dormancy is a condition known to develop in mature, dispersed seeds leading to the creation of persistent seed banks. Volunteers which arise from these persistent seeds can contaminate future crops and negatively affect seed purity. Studies were designed to determine secondary dormancy induction potential of several Brassicaceae species and elucidate contributing factors. Polyethylene glycol (PEG) was used under laboratory conditions to simulate osmotic stress. Secondary dormancy induction was different for seeds of some species from different harvest years. Canola was not different from daikon radish or forage rape either year. Secondary dormancy was low (<3%) in turnip and no dormant seeds were induced in red radish. A positive relationship was found between mean germination time and dormancy induction. Trends for expression of NCED6 and NCED9 genes were similar among crops at certain points in time, but relative expression was different among crops. The results of the studies indicate that the environment during seed production plays a role in the manipulation of seed characteristics which influence secondary dormancy, and that NCED expression alone did not predict secondary dormancy potential among related crops.

## Introduction

Persistence of canola (*Brassica napus* L.) seed has been documented by several researchers, and seedlings have been observed five years after harvest in Canada (Legere and Simard 2001). Lutman et al. (2003) recovered viable canola seeds 11 yr after burial in the UK. Persistence is known to be associated with the ability of canola seeds to develop secondary dormancy (Gulden et al. 2004; Pekrun et al. 1998). Relatives of canola in the Brassicaceae family such as wild radish (*Raphanus raphanistrum* L.) and wild turnip (*B. rapa* L.) also have been shown to persist in the soil seed bank (de Jong et al. 2013; Roberts and Boddrell 1983).

Persistent seed banks are defined as: seeds of a species which remain viable and germinate over a time period greater than one year (Thompson and Grime 1979). Seeds have evolved different mechanisms and strategies for survival, many of which employ some form of dormancy (Baskin and Baskin 1998). Many species develop primary dormancy on the mother plant and remain dormant until a period of after-ripening has been completed. However, crops that are members of the *Brassica* genus do not generally display primary dormancy (Lutman 1993). Secondary dormancy is a condition that is induced or develops in mature, dispersed seeds (Bewley and Black 1994). High osmotic pressure, absence of light, and high temperatures are known to promote the development of secondary dormancy (Pekrun et al. 1998). During harvest, when seed shatter occurs from mechanical or environmental disturbance, the conditions necessary for the induction of secondary dormancy occur in the soil matrix.

Canola seeds did not enter secondary dormancy while exposed to light during osmotic stress treatment in polyethylene glycol (PEG), which has been shown under laboratory conditions to mimic soil matric potentials (Pekrun et al. 1997). When tillage is performed shortly after harvest, seeds are moved into deeper soil layers where light is absent. The specific genetic-molecular controls of dormancy are still being elucidated, but several phyto-hormones have been implicated.

The first suggestion of interplay between germination promoting and inhibiting substances was made by Villiers and Wareing (1960) and a working model implicating both gibberellins (GA) and abscisic acid (ABA) was presented eight years later by Amen (1968). The presence of ABA in seed tissues, which had begun active growth, promoted the idea that dormancy regulation was influenced by the balance of several endogenous hormones simultaneously (Wareing and Saunders 1971). The isolation of ABA deficient arabidopsis (*Arabidopsis thaliana* L.) mutants helped confirm the theory that the balance of and sensitivity to phyto-hormones was more critical than their absolute concentrations (Koornneef et al. 1982).

ABA is an important plant hormone which regulates plant growth and stomatal function, especially during periods of abiotic stress (Taiz and Zeiger 2010). It is also critical to the initiation and maintenance of dormancy (Bewley et al. 2013; Seo et al. 2009). The first committed step in ABA synthesis is the cleavage of 9-cis epoxycarotenoids by the rate-limiting enzyme 9-cis-epoxycarotenoid dioxygenase (NCED) (Kende and Zeevaart 1997; Schwartz 1997). The multi-gene NCED family has undergone extensive functional analysis in arabidopsis. Two of these genes, NCED6 and NCED9, have been found primarily in seed tissues (Cadman et al. 2006; Lefebvre et al. 2006; Tan et al. 2003). Factors related to ABA induction during seed

maturation negatively regulate downstream GA biosynthesis (Seo et al. 2006). Overexpression of arabidopsis NCED6 suppressed germination indicating that ABA expression could be a singular determinant of dormancy (Martinez-Andujar et al. 2011; Nonogaki et al. 2014).

A wide range of secondary dormancy potential has been reported among varieties of *B. napus* (Momoh et al. 2002; Pekrun et al. 1997), but there are no reports of studies which examined the differences between *B. napus* and other Brassicaceae crop species. The two main objectives of this research were to determine: 1). if factors influencing the creation of persistent seed banks such as differences in secondary dormancy induction potential exists among commonly grown Brassicaceae crop seeds within the Willamette Valley, and 2). if differences in NCED gene expression exist between these closely related species and if those differences could be used to predict secondary dormancy induction.

## Materials and Methods

**Plant material.** 'Sitro' canola, daikon and 'Crimson Giant' radish, 'Purple Top White Globe' turnip (*B. rapa* var. *rapa* L.), 'Barsica' forage rape (*B. napus* L.), 'Georgia Southern' collard (*B. oleracea* var. *acephala* DC.), and 'Premier' kale (*B. oleracea* var. *acephala* DC.), and 'Michihili' Chinese cabbage (*B. rapa* L. ssp. *chinensis* (L.) Hanelt) seeds were harvested from fields grown within the Willamette Valley during 2016. Canola, radish, turnip, and Chinese cabbage also were collected in 2017. Samples were cleaned and stored in the dark at -20 C until use.

**Dormancy induction.** The procedure for secondary dormancy induction described by Weber et al. (2010) was followed. Fifty seeds with four replicates of each crop tested were placed on two layers of Whatman No. 2 filter paper discs and 8 ml of the appropriate concentration of polyethylene glycol (PEG) 6000 added to each dish for a specified incubation temperature. The concentration of PEG was calculated using an equation derived by (Michel et al. 1983) for each test temperature:

$$\psi_{PEG} = 1.29[PEG]^2T - 140[PEG]^2 - 4[PEG]$$

Where T = temperature of incubation and PEG = the amount of PEG in grams per gram of water. Solution $\psi$ of -1.5 MPa was verified with a Wescor HR-33 thermocouple psychrometer (Wescor Inc., Logan, UT). Petri dishes were randomly placed in sealed 3.8 L bags to limit evaporation and placed in boxes covered with black plastic film to exclude light. Boxes were placed in growth chambers (SSG2-22 Hoffman Manufacturing Inc., Corvallis, OR) set to either 20 or 30 C constant temperature and seeds were incubated for 4 wk in darkness. After 4 wk, ungerminated seeds were transferred under a green safety light to new petri dishes containing a double layer of filter paper and 6 ml of deionized water. Petri dishes were opened under a green safety light on days 2, 4, and 14, and germinated seeds removed. Seeds were considered germinated when the radicle protruded through the testa more than 2 mm. After germinated seeds were removed on day 4, remaining seeds were transferred to new petri dishes with 6 ml of fresh deionized water to reduce the potential effects of exudates from germinating seeds. After 7 d, a 12 h photoperiod was introduced. Ungerminated seeds at 14 d were considered to be dormant. Previous

work (Weber et al. 2010), and preliminary studies (data not shown) indicate that the viability of Brassicaceae seeds does not decline during incubation. The study was a completely randomized design and was repeated.

Material for gene expression was collected following the same protocol for seeds of canola, radish, and turnip. Seeds were incubated in PEG 6000 for 0, 3, 7, 14, or 28 d then transferred under a green safety light to new petri dishes containing a double layer of filter paper and 6 ml of deionized water. Three replicates of 50 seeds were removed at 0, 3, 6, 9, or 12 hours after imbibition. After removal, seeds were placed in 15 ml disposable sample containers (Meter Group Inc. Pullman, WA), wrapped in foil and frozen at -80 C.

**Germination pattern.** Viability and speed of germination tests were conducted. Fifty seeds with four replicates of each crop tested were placed in 90 mm petri dishes on two layers of Whatman No. 2 filter paper discs and 6 ml of deionized water added. Samples were placed in a germinator set to 20 C with a 12 h photoperiod and a completely randomized design was used. Germination was monitored over a 10 d period. The final germination percentage (FGP), mean germination time (MGT), and the germination index (GI) were calculated with the following equations:

FGP = total number of germinated seeds/number of seeds tested x 100

MGT = $\Sigma f \cdot x / \Sigma f$ where f is the number of seeds germinated on day x (Orchard 1975)

GI = (5 x n1) + (4 x n2) +…+ (1 x n5) where n1, n2, …, n5 are the number of germinated seeds on the first, second, and subsequent days until the fifth day; 5, 4,…, and 1 are weights given to the number of germinated seeds on each day (Reddy et al. 1985)

**RNA isolation and cDNA synthesis.** Seeds (50-100 mg dry weight) of each crop were placed into 2 ml microcentrifuge tubes with two 4.5 mm zinc-plated steel air-gun shot balls (Daisy Outdoor Products Rogers, AR). Samples were frozen in liquid nitrogen and cryogenically ground for 1 minute at 30 Hz with a Retsch MM 400 (Retsch GmbH. Haan, Germany). Total RNA extraction was performed using the protocol described by (Reid et al. 2006) with minor modifications. A phenol-chloroform extraction step was added in addition to chloroform:isoamyl alcohol (24:1) extractions prior total RNA precipitations to remove excess protein contaminants. DNA digestion was performed by DNase treatment (RNase-free DNase, Qiagen, Valencia, CA) following NaOAc precipitation. The quality and concentration of extracted RNA was assessed with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Integrity of total RNA was verified by running an aliquot of the sample on 1% agarose gels. First-strand cDNA was synthesized from 2 μg of total RNA using M-MuLV Reverse Transcriptase (New England Biolabs Inc. Ipswich, MA) and oligo $(dT)_{18}$ primers (Integrated DNA Technologies Inc. Skokie, IL) in a 20 μl reaction volume. Five microliters of 10x diluted cDNA were used as a template for each RT-PCR reaction.

**Gene identification and primer design.** Sequences for genes of interest were selected from the EnsemblPlants database (https://plants.ensembl.org) for *A. thaliana, B. napus, and B. rapa.* Sequences for *R. sativus* were obtained from the Radish Genome Database version 1.0 (http://radish-genome.org). NCED6 for *R. sativus* was not annotated and was found by blasting the genome using the sequence of *ATNCED6* (AT3G24220). Primers for reference genes and genes of interest were

designed using Primer3 software (http://bioinfo.ut.ee/primer3-0.4.0/) so that known isoforms of each gene were amplified by one primer set (Table 3.1).

**Quantitative Real Time PCR analysis.** NCED6 and NCED9 transcript levels were analyzed for each crop species by quantitative real time PCR (qRT-PCR). qRT-PCR was performed with Sso Advanced Universal SYBR Green Supermix (Bio-Rad, Hercules, CA) on a CFX96 Real-Time System attached to a C1000 Touch Thermal Cycler (Bio-Rad). Reactions were setup in 15 µl volumes containing 250 nM of each primer, 5 µl of template cDNA (~5 ng), and 7.5 µl 2X SYBR Green master mix. Samples of three biological replicates of each time point for each species were analyzed and each PCR reaction was performed in duplicate. The amplification data of target genes were normalized against the reference gene, ACTIN7, in each species (He et al. 2017). The reaction conditions were: heat activate/denature at 95 C for 3 minutes, followed by 95 C for 10 s, 60 C for 10 s (39 cycles). Agarose gels were used to verify the specificity of each primer, and melting curve analysis was used to assess specific amplification during qRT-PCR reactions. Relative expression of target genes was calculated using the double delta Ct method and results are expressed relative to the level of expression in untreated dry seed at 0 h imbibition. The primers used for expression studies are presented in Table 3.1.

**Statistical Analysis.** Data from secondary dormancy induction studies were arc sin transformed to meet normality and homoscedasticity assumptions for ANOVA in SAS (Statistical Analysis Systems, version 9.4, SAS Institute Inc., Cary, NC); however, non-transformed data are presented for clarity. Means were separated using

Tukey-Kramer's LSD ($P \leq 0.05$). Pearson correlation coefficients were calculated in Prism 8 (GraphPad Software, San Diego, CA).

## Results and Discussion

There was no interaction between crop type and temperature of incubation for seeds harvested in 2016. Temperature of incubation was significant for four crops, and more seeds incubated at 30 C entered secondary dormancy compared to those incubated at 20 C (Figure 3.1). There was a broad range (0 to 20%) of secondary dormancy induction among the eight crops tested. Only 1% of turnip seeds were induced into dormancy at either temperature. No seeds harvested in 2016 or 2017 of 'Crimson Giant' radish were induced into dormancy. In 2017, there was an interaction between crop type and temperature of incubation. As in 2016, secondary dormancy induction of canola and daikon radish were similar (Figure 3.2). After 'Crimson Giant' radish, turnip displayed the least amount of dormancy induction both years.

It has been suggested that a relationship exists between speed of germination and secondary dormancy induction (Momoh et al. 2002). Many of the standard methods for measuring the speed of germination were discussed by Al-Mudaris (1998). Mean germination time (MGT), final germination percentage, and germination index were proposed as the three factors that best measured germination. These three factors are shown for each crop tested in Table 4. MGT was found to have a moderate positive correlation with the percentage of secondary dormancy

induction in seeds incubated at 30 C in 2016 and 20 C in 2017 (Figure 3.3). A greater MGT value indicates a slower germination time, and crops which germinate more slowly also tend to show greater secondary dormancy induction potential.

The incubation of lettuce seeds at high temperatures resulted in increased thermodormancy (Chiwocha et al. 2003) and more rapid development of dormancy in several weed species compared to lower temperatures (Pons 1991). Secondary dormancy induction in 2016 was greater at 30 C vs 20 C, a result which was not observed in 2017. However, secondary dormancy induction was greater in 2017 overall, suggesting that environmental factors during each year influenced the predisposition of the seed to enter secondary dormancy.

The environmental conditions that parental plants are subject to during seed production have substantial effects on progeny characteristics (Baskin and Baskin 1998; Penfield and MacGregor 2017). Length of photoperiod (Munir et al. 2001), intensity of light (He et al. 2014), nitrogen level (Karimmojeni et al. 2014), and drought stress (Anchez et al. 1981; Eslami et al. 2010; Peters 1982) during seed development influence progeny dormancy levels. However, the single greatest environmental factor influencing seed dormancy characteristics appears to be temperature.

Temperature reductions as small as 1 C during seed development were reported to increase dormancy levels in arabidopsis (Springthorpe and Penfield 2015). Differences in temperature during the vegetative growth prior to seed production were reported to affect seed germinability and dormancy in several species (Chen et al. 2014; Sawhney et al. 1985; Thomas and Raper 1975). A comparison of the average daily temperatures for 2016 and 2017 in the Willamette Valley, showed up to

a 4 C difference during the vegetative and reproductive stages of Brassicaceae crops from which study seed was harvested (Figure 3.4). The average daily temperature in 2016 from January to June was warmer than 2017, but cooler from June through mid-September. The higher temperatures in 2016 coupled with lower than average precipitation in 4 of 6 months during flowering and seed development could have led to stressful conditions and therefore to seed with lower dormancy potential (Figures 3.1 and 3.2).

Lower temperatures during seed maturation led to an increase in ABA levels and a decrease in GA in dry mature seeds of arabidopsis (Kendall et al. 2011). It has also been demonstrated that ABA reduced the ability of *B. napus* to absorb water under osmotic stress (Schopfer and Plachy 1984). It is conceivable that higher endogenous ABA content could increase the induction of secondary dormancy among seeds exposed to high osmotic pressures, and also retard the speed of germination by slowing imbibition. Additionally, it would follow that crops produced under irrigation could be subject to lower drought and temperature stress producing seed with a higher potential to enter secondary dormancy.

Investigations of the effects of environmental stresses at the molecular level, have confirmed that many of the same gene families regulate both primary and secondary dormancy (Chiang et al. 2011; Footitt et al. 2011; Kendall et al. 2011). Considering the critical role which ABA plays in primary dormancy induction (Martinez-Andujar et al. 2011; Nonogaki et al. 2014) transcript expression could indicate the potential for secondary dormancy induction. Trends for NCED6 expression in the three-day osmotic stress treatment were similar among canola, radish, and turnip (Figure 3.5). Canola and daikon radish exhibited similar potentials

for secondary dormancy induction; however, canola NCED6 expression 6 h after imbibition was 7-fold higher. Turnip exhibited a very low potential to enter secondary dormancy (<3%) yet expressed NCED6 at levels similar to canola. NCED9 expression did not follow the same expression trend as NCED6, and expression was much lower relative to that of NCED6. Differences in expression between the two genes was found by (Martinez-Andujar et al. 2011), but no clear reason for this difference has been found.

Two factors could potentially explain the discrepancy between gene expression and dormancy induction. First, expression levels were calculated relative to expression in dry untreated seed which does not account for differences among crops in stored mRNA transcripts which accumulate during maturation. Second, there is growing evidence that genes such as *DELAY OF GERMINATION1* (DOG1) play an important role in regulating dormancy (Chiang et al. 2011). Therefore, expression of NCED does not guarantee increased endogenous levels of ABA or that the sensitivity of the seed remains constant.

Large levels of variation in secondary dormancy induction potential exist among species, and even varieties within species. Year-to-year environmental conditions during seed production appear to play a role in determining characteristics which affect seed persistence. This work provides evidence from field collected seeds that supports the current understanding of the relationship of temperature during seed production and dormancy. The preliminary molecular work reported here is a starting point for further investigations which should take into account the large array of interacting genes and their relationship to environmental conditions. Further studies examining a wider array of varieties and their response to cultural practices in the

field such as irrigation timing could be useful in creating practical management strategies to reduce secondary dormancy.

## Literature Cited

Al-Mudaris MA (1998) Notes on various parameters recording the speed of seed germination. J Agric Rural Dev Trop Subtrop 99:147–154

Amen RD (1968) A model of seed dormancy. Bot Rev 34:1–31

Anchez RA, Eyherabide G, Miguel L de (1981) The influence of irradiance and water deficit during fruit development on seed dormancy in *Datura ferox* L. Weed Res 21:127–132

Baskin JM, Baskin CC (1998) Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. London, UK: Academic Press. 666 p

Bewley JD, Black M (1994) Seeds: Physiology of Development and Germination. 2nd edn. New York: Plenum Press. 445 p

Bewley JD, Bradford KJ, Hilhorst HWM, Nonogaki H (2013) Seeds: Physiology of Development, Germination and Dormancy 3rd edn. New York: Springer. 392 p

Cadman CSC, Toorop PE, Hilhorst HWM, Finch-Savage WE (2006) Gene expression profiles of arabidopsis Cvi seeds during dormancy cycling indicate a common underlying dormancy control mechanism. Plant J 46:805–822

Chen M, MacGregor DR, Dave A, Florance H, Moore K, Paszkiewicz K, Smirnoff N, Graham IA, Penfield S (2014) Maternal temperature history activates flowering locus T in fruits to control progeny dormancy according to time of year. Proc Natl Acad Sci 111:18787–18792

Chiang GCK, Bartsch M, Barua D, Nakabayashi K, Debieu M, Kronholm I, Koornneef M, Soppe WJJ, Donohue K, Meaux JD (2011) DOG1 expression is predicted by the seed-maturation environment and contributes to geographical variation in germination in *Arabidopsis thaliana*. Mol Ecol 20:3336–3349

Chiwocha SDS, Abrams SR, Ambrose SJ, Cutler AJ, Loewen M, Ross ARS, Kermode AR (2003) A method for profiling classes of plant hormones and their metabolites using liquid chromatography-electrospray ionization tandem mass spectrometry: an analysis of hormone regulation of thermodormancy of lettuce (*Lactuca sativa* L.) seeds. Plant J 35:405–417

Eslami SV, Gill GS, McDonald G (2010) Effect of water stress during seed development on morphometric characteristics and dormancy of wild radish (*Raphanus raphanistrum* L.) seeds. Int J Plant Prod:10

Footitt S, Douterelo-Soler I, Clay H, Finch-Savage WE (2011) Dormancy cycling in arabidopsis seeds is controlled by seasonally distinct hormone-signaling pathways. Proc Natl Acad Sci 108:20236–20241

Gulden RH, Thomas AG, Shirtliffe SJ (2004) Relative contribution of genotype, seed size and environment to secondary seed dormancy potential in Canadian spring oilseed rape (*Brassica napus*). Weed Res 44:97–106

He H, de Souza Vidigal D, Snoek LB, Schnabel S, Nijveen H, Hilhorst H, Bentsink L (2014) Interaction between parental environment and genotype affects plant and seed performance in arabidopsis. J Exp Bot 65:6603–6615

He M, Cui S, Yang X, Mu G, Chen H, Liu L (2017) Selection of suitable reference genes for abiotic stress-responsive gene expression studies in peanut by real-time quantitative PCR. Electron J Biotechnol 28:76–86

de Jong TJ, Tudela Isanta M, Hesse E (2013) Comparison of the crop species *Brassica napus* and wild *B. rapa*: characteristics relevant for building up a persistent seed bank in the soil. Seed Sci Res 23:169–179

Karimmojeni H, Bazrafshan AH, Majidi MM, Torabian S, Rashidi B (2014) Effect of maternal nitrogen and drought stress on seed dormancy and germinability of *Amaranthus retroflexus*. Plant Species Biol 29:E1–E8

Kendall SL, Hellwege A, Marriot P, Whalley C, Graham IA, Penfield S (2011) Induction of dormancy in arabidopsis summer annuals requires parallel regulation of dog1 and hormone metabolism by low temperature and cbf transcription factors. Plant Cell 23:2568–2580

Kende H, Zeevaart J (1997) The five “classical” plant hormones. Plant Cell 9:1197–1210

Koornneef MLJ, Brinkhorst-van der Swan DLC, Karssen CM (1982) The isolation of abscisic acid (aba) deficient mutants by selection of induced revertants in non-germinating gibberellin sensitive lines of *Arabidopsis thaliana* (L.) Heynh. Theor Appl Genet 61:385–393

Lefebvre V, North H, Frey A, Sotta B, Seo M, Okamoto M, Nambara E, Marion-Poll A (2006) Functional analysis of arabidopsis NCED6 and NCED9 genes indicates that ABA synthesized in the endosperm is involved in the induction of seed dormancy. Plant J 45:309–319

Legere A, Simard MJ (2001) Presence and persistence of volunteer canola in Canadian cropping systems. Pages 143-148 *in* Brighton Crop Protection Conference, Weeds. Brighton, UK: British Crop Protection Council

Lutman PJW, Freeman SE, Pekrun C (2003) The long-term persistence of seeds of oilseed rape (*Brassica napus*) in arable fields. J Agric Sci 141:231–240

Martinez-Andujar C, Ordiz MI, Huang Z, Nonogaki M, Beachy RN, Nonogaki H (2011) Induction of 9-cis-epoxycarotenoid dioxygenase in *Arabidopsis thaliana* seeds enhances seed dormancy. Proc Natl Acad Sci 108:17225–17229

Michel BE, Wiggins OK, Outlaw WHJ (1983) A guide to establishing water potential of aqueous two-phase solutions (polyethylene glycol plus dextran) by amendment with mannitol. Plant Physiol 72:60–65

Momoh EJJ, Zhou WJ, Kristiansson B (2002) Variation in the development of secondary dormancy in oilseed rape genotypes under conditions of stress. Weed Res 42:446–455

Munir J, Dorn LA, Donohue K, Schmitt J (2001) The effect of maternal photoperiod on seasonal dormancy in *Arabidopsis thaliana* (Brassicaceae). Am J Bot 88:1240–1249

Nonogaki M, Sall K, Nambara E, Nonogaki H (2014) Amplification of ABA biosynthesis and signaling through a positive feedback mechanism in seeds. Plant J 78:527–539

Orchard TJ (1975) Estimating the parameters of plant seedling emergence. Seed Sci Technol 5:61–69

Pekrun C, Hewitt JDJ, Lutman PJW (1998) Cultural control of volunteer oilseed rape (*Brassica napus*). J Agric Sci 130:155–163

Pekrun C, Lutman PJW, Baeumer K (1997) Induction of secondary dormancy in rape seeds (*Brassica napus* L.) by prolonged imbibition under conditions of water stress or oxygen deficiency in darkness. Eur J Agron 6:245–255

Penfield S, MacGregor DR (2017) Effects of environmental variation during seed production on seed dormancy and germination. J Exp Bot 68:819–825

Peters NCB (1982) Production and dormancy of wild oat (*Avena fatua*) seed from plants grown under soil waterstress. Ann Appl Biol 100:189–196

Pons TL (1991) Induction of dark dormancy in seeds: its importance for the seed bank in the soil. Funct Ecol 5:669-675

Reddy LV, Metzger RJ, Ching TM (1985) Effect of temperature on seed dormancy of wheat. Crop Sci 25:455–458

Reid KE, Olsson N, Schlosser J, Peng F, Lund ST (2006) An optimized grapevine RNA isolation procedure and statistical determination of reference genes for real-time RT-PCR during berry development. BMC Plant Biol 6:27-38

Roberts HA, Boddrell JE (1983) Seed survival and periodicity of seedling emergence in eight species of Cruciferae. Ann Appl Biol 103:301–304

Sawhney R, Quick WA, Hsiao AI (1985) The effect of temperature during parental vegetative growth on seed germination of wild oats (*Avena fatua* L.). Ann Bot 55:25–28

Schopfer P, Plachy C (1984) Control of seed germination by abscisic acid: ii. effect on embryo water uptake in *Brassica napus* L. Plant Physiol 76:155–160

Schwartz SH (1997) Specific oxidative cleavage of carotenoids by VP14 of maize. Science 276:1872–1874

Seo M, Hanada A, Kuwahara A, Endo A, Okamoto M, Yamauchi Y, North H, Marion-Poll A, Sun T, Koshiba T, Kamiya Y, Yamaguchi S, Nambara E (2006) Regulation of hormone metabolism in arabidopsis seeds: phytochrome regulation of abscisic acid metabolism and abscisic acid regulation of gibberellin metabolism. Plant J 48:354–366

Seo M, Nambara E, Choi G, Yamaguchi S (2009) Interaction of light and hormone signals in germinating seeds. Plant Mol Biol 69:463–472

Springthorpe V, Penfield S (2015) Flowering time and seed dormancy control use external coincidence to generate life history strategy. https://elifesciences.org/articles/05557. Accessed March 1, 2019. 17 p

Taiz L, Zeiger E (2010) Plant Physiology 5th edn. Sunderland, MA: Sinauer Associates Inc. 782 p

Tan B-C, Joseph LM, Deng W-T, Liu L, Li Q-B, Cline K, McCarty DR (2003) Molecular characterization of the arabidopsis 9-cis epoxycarotenoid dioxygenase gene family. Plant J 35:44–56

Thomas T, Raper C (1975) Seed germinability as affected by the environmental temperature of the mother plant. Tob Sci 19:98–100

Thompson K, Grime JP (1979) Seasonal variation in the seed banks of herbaceous species in ten contrasting habitats. J Ecol 67:893–921

Villiers TA, Wareing PF (1960) Interaction of growth inhibitor and a natural germination stimulator in the dormancy of *Fraxinus excelsior* L. Nature 185:112–114

Wareing PF, Saunders PF (1971) Hormones and dormancy. Annu Rev Plant Physiol 22:261–288

Weber EA, Frick K, Gruber S, Claupein W (2010) Research and development towards a laboratory method for testing the genotypic predisposition of oilseed rape (*Brassica napus* L.) to secondary dormancy. Seed Sci Technol 38:298–310

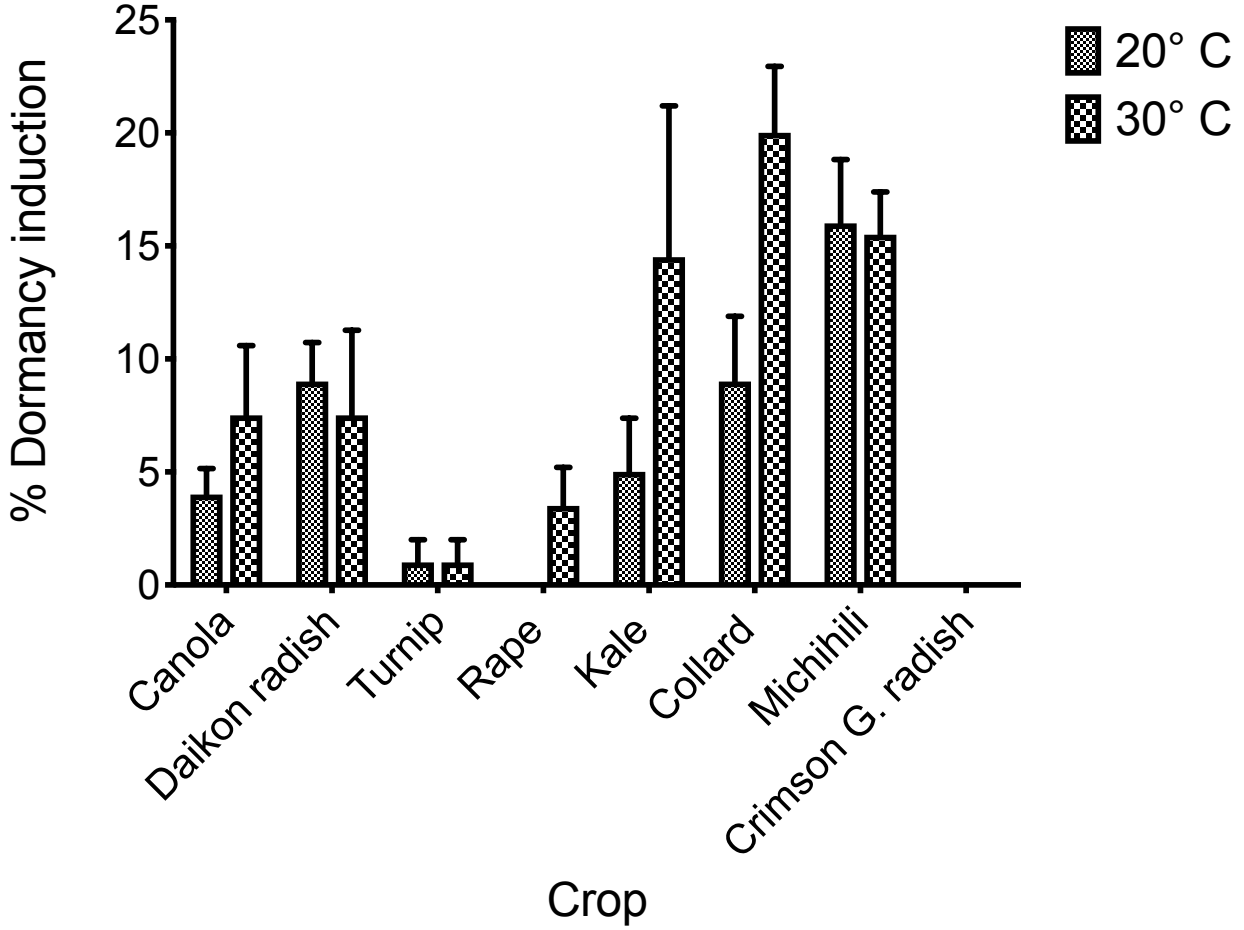


Figure 3.1 – Secondary dormancy induction of 2016 harvested seed incubated at 20 and 30 C for 28 d in PEG solution. No seeds of 'Crimson Giant (G.)' radish were induced into secondary dormancy. Bars indicate the standard error of the mean.

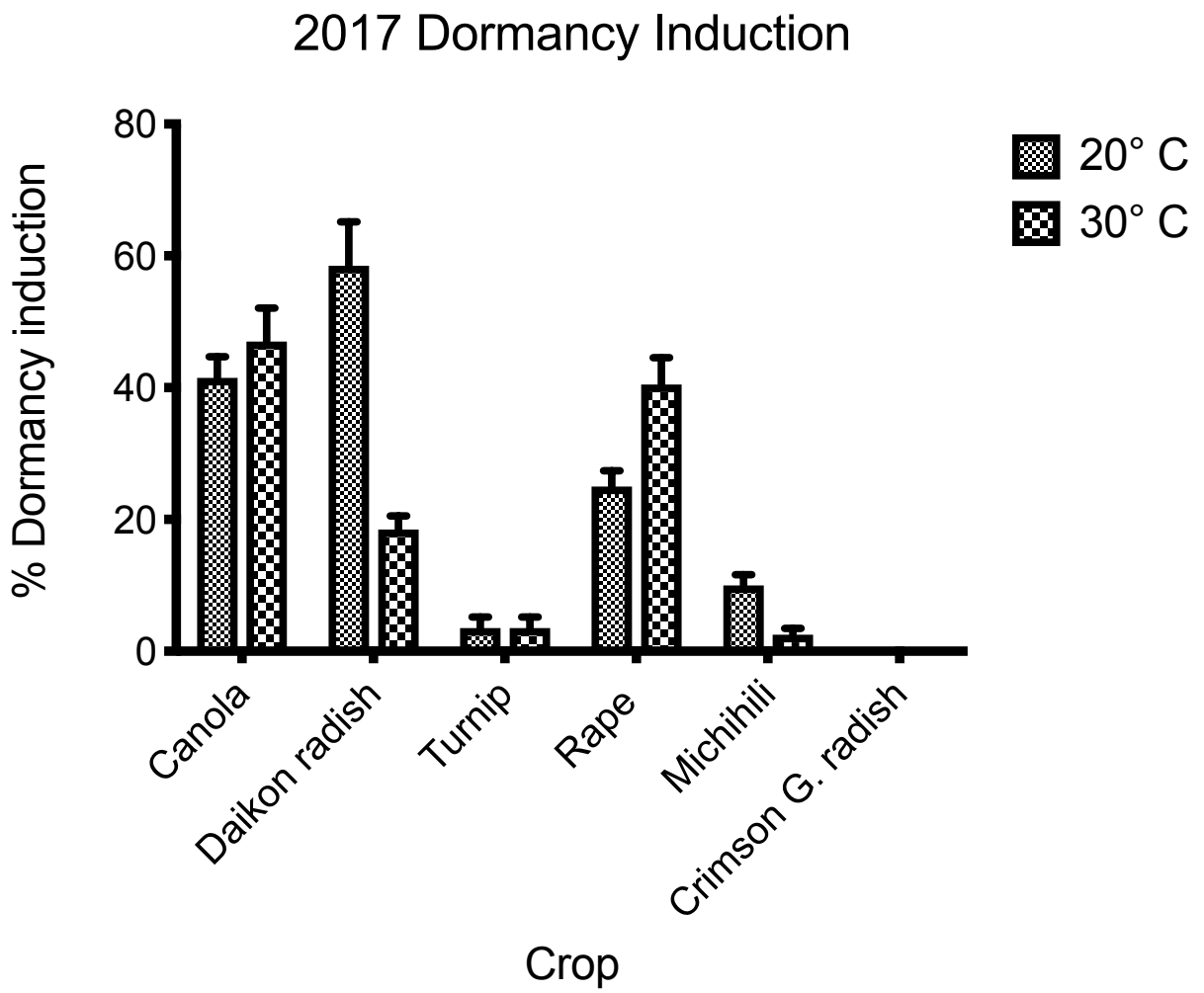


Figure 3.2 – Secondary dormancy induction of 2017 harvested seed incubated at 20 and 30 C for 28 d in PEG solution. No seeds of 'Crimson Giant (G.)' radish were induced into secondary dormancy. Bars indicate the standard error of the mean.

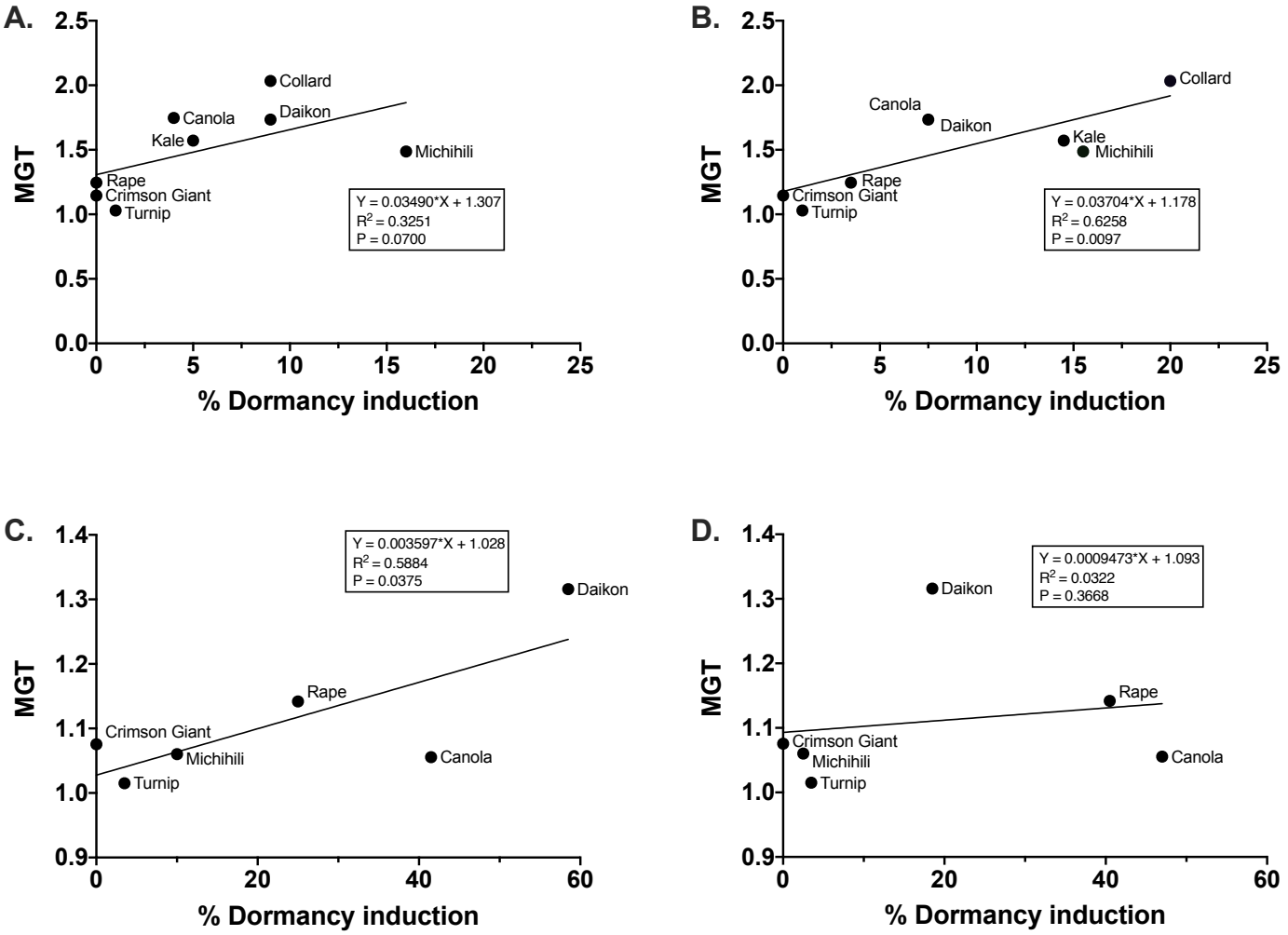


Figure 3.3 – Plots of mean germination time (MGT) and the percent of secondary dormancy induction in eight Brassicaceae crops harvested in 2016 (A and B, 20 and 30 C incubation temperatures, respectively), and six Brassicaceae crops harvested in 2017 (C and D, 20 and 30 C incubation temperatures, respectively).

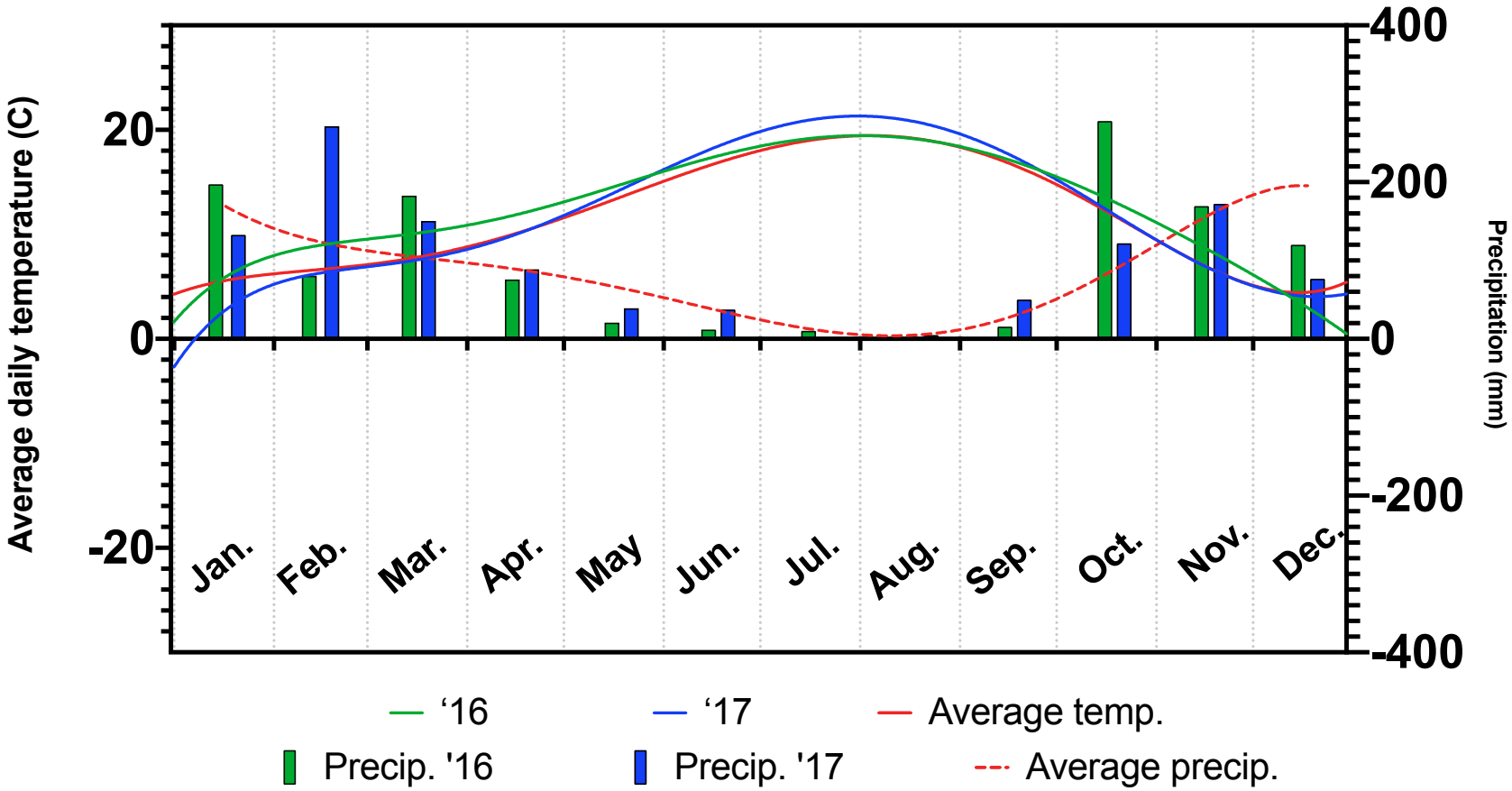


Figure 3.4 – Average daily temperatures and monthly precipitation in 2016 and 2017, 20-year average daily temperature and monthly precipitation (mm) (1992-2012) from the Hyslop Research Farm (CRVO) weather station.

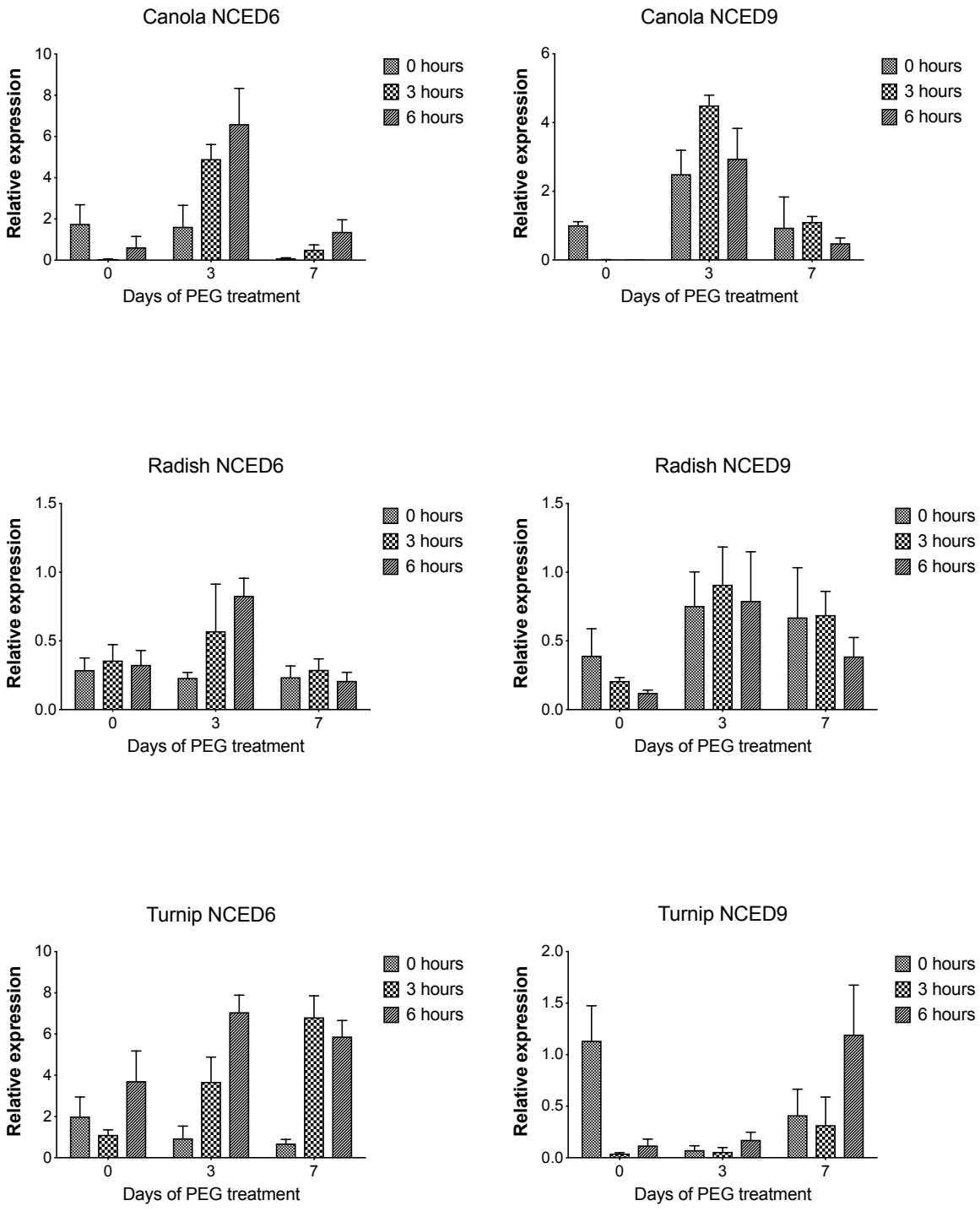


Figure 3.5 – Relative expression of 9-cis-epoxycarotenoid dioxygenase (NCED) 6 and 9 in canola, radish, and turnip after 0, 3, and 7 d of osmotic stress incubation in polyethylene glycol (PEG) solution. Samples were analyzed at 0, 3, and 6 hours of imbibition following stress treatment. Bars indicate the standard error of the mean.

Table 3.1 – Primer sequences used in real-time PCR reactions.

| Species | Gene | Gene ID | Primer sequences (5'to 3') | Amplicon length |
|---|---|---|---|---|
| *B. napus* | *BnNCED6* | BnaA07g06050D & BnaC07g07580D | F: ATCCTTGGCCGAGATGCA | 164 &164 |
| | | | R: CTCTTCGTCCCTCACAAACC | |
| | *BnNCED9* | BnaA07g39250D & BnaC06g38870D | F: GAAGCTACGGTTACGCTTCC | 168 & 161 |
| | | | R: CCTCAGGCAACAATTCTCATAGAC | |
| | *BnActin7* | BnaC09g46850D | F: GATTCCGTTGCCCTGAAGTA | 148 |
| | | | R: GAACCACCACTGAGGACGAT | |
| *B. rapa* | *BrNCED6* | Bra015002 | F: GCATCGGAGATGAAGCAAGT | 174 |
| | | | R: CAACTGATTCTCGCTCACGA | |
| | *BrNCED9* | Bra035033 & Bra008358 | F: GGAGGAGAGCCTCTGTTT | 155 & 152 |
| | | | R: GGAAGCGTAACCGTAGCTTCAA | |
| | *BrActin7* | Bra009081 | F: GATTCCGTTGCCCTGAAGTA | 148 |
| | | | R: GAACCACCACTGAGGACGAT | |
| *R. sativus* | *RsNCED6* | (No gene ID) | F: TGCGACTACCGGAGAGAG | 120 |
| | | | R: AAAGCGGGTTTGCACAATTA | |
| | *RsNCED9* | Rs577640 & Rs449870 | F: TATTTCAACGGTCATCTCTTAGCC | 140 & 140 |
| | | | R: GGGTTTCCGGGTCGAGTT | |
| | *RsActin7* | Rs027260 | F: ATCGTTTGTCGGAATGGAAGC | 148 |
| | | | R: ATGGTCGAGCCACCACTAAG | |

Table 3.2 – Germination characteristics of seed harvested in 2016 and 2017.

| | Mean number of seeds germinated day$^{-1}$ | | | | | Germination parameters[b] | | |
|---|---|---|---|---|---|---|---|---|
| Crop | 1[a] | 2 | 3 | 4 | 5 | FGP | MGT | GI |
| *2016* | | | | | | --%-- | | |
| Canola | 18.8 | 28.0 | 0.5 | 1.0 | 1.3 | 99 | 1.75 | 211 |
| Daikon radish | 12.0 | 29.3 | 0.8 | 0.0 | 0.0 | 91.75 | 1.73 | 179 |
| Turnip | 48.5 | 1.5 | 0.0 | 0.0 | 0.0 | 100 | 1.03 | 249 |
| Forage rape | 38.8 | 10.0 | 0.8 | 0.3 | 0.0 | 99.5 | 1.25 | 237 |
| Kale | 22.0 | 25.0 | 0.3 | 0.3 | 0.3 | 95.5 | 1.57 | 212 |
| Collard | 2.3 | 43.3 | 1.3 | 1.3 | 0.0 | 96 | 2.03 | 191 |
| Michihili | 26.0 | 20.0 | 0.8 | 0.5 | 0.0 | 94.5 | 1.49 | 213 |
| Crimson G. radish | 42.5 | 7.3 | 0.0 | 0.0 | 0.0 | 99.5 | 1.15 | 242 |
| *2017* | | | | | | | | |
| Canola | 47.0 | 2.3 | 0.3 | 0.0 | 0.0 | 99 | 1.056 | 245 |
| Daikon radish | 32.5 | 15.0 | 0.0 | 0.0 | 0.0 | 97.25 | 1.316 | 223 |
| Turnip | 49.3 | 0.8 | 0.0 | 0.0 | 0.0 | 100 | 1.015 | 249 |
| Forage rape | 44.8 | 3.3 | 0.3 | 0.8 | 0.3 | 98.5 | 1.142 | 239 |
| Michihili | 46.8 | 1.5 | 0.0 | 0.5 | 0.0 | 97.5 | 1.060 | 241 |
| Crimson G. radish | 46.8 | 2.5 | 0.3 | 0.3 | 0.0 | 99.5 | 1.076 | 245 |

[a] Days on which germination was recorded.
[b] FGP, final germination percentage; MGT, mean germination time; GI, germination index.

# CHAPTER 4

## The Effect of 2,4-D and Related Herbicides on *Brassica* Seed Germination and Recruitment

Gabriel D. Flick and Carol A. Mallory-Smith[1]

[1]Department of Crop and Soil Science, 107 Crop Science Building

Oregon State University, Corvallis, OR 97331

Formatted for submission to Weed Technology for publication

## Abstract

The production of Brassicaceae crops can result in the substantial input of crop seeds into the soil seed bank. These seeds are known to persist and subsequent volunteers can be damaging to the seed purity of other crops in the rotation. This research examined seed germination and root and stem growth as a result of herbicide treatment and timing. The herbicides 2,4-D, MCPA, dicamba, and dicamba plus diflufenzopyr were applied at the maximum labeled rate. The application of 2,4-D and MCPA resulted in the greatest reduction in root and stem growth in a greenhouse. When applied pre-emergence, 2,4-D and MCPA reduced canola emergence by 45% and 60%, respectively, compared to untreated controls in a greenhouse. 2,4-D applied preemergence reduced emergence only when application occurred near rainfall events under field conditions. Therefore, the need for rainfall or irrigation may limit the effectiveness of these chemicals to reduce the seed bank under field conditions.

## Introduction

The Willamette Valley of Oregon, and similar regions of Washington state produce more than 50% of the U.S. supply of *Brassica* vegetable seeds (Schreiber and Ritchie 1995). These crops are produced in small to mid-sized isolated fields, to ensure purity, and on-farm crop diversity is generally high. The Willamette Valley is a large producer of grass seed and cereal crops on fields less suited for the production of high-value vegetable seed. Rotational options in these fields are limited due to topography, lack of irrigation, and poor drainage. Grass and cereal producers are interested in including canola as a broadleaf rotational crop in these systems. However, there are concerns about potentially increased disease and insect pressure, canola seed persistence, and possible contamination of seed crops which could lead to unfavorable market responses (Loberg 2013).

Seed persistence is linked to the ability of the seed to enter secondary dormancy; a condition influenced by genetic predisposition and environmental conditions (Gruber et al. 2010). Although canola seeds have little or no primary dormancy, it has been documented that high osmotic pressure in the absence of light can influence the development of secondary dormancy (Gruber et al. 2004; Momoh et al. 2002; Pekrun et al. 1997). Canola seeds in secondary dormancy produced volunteer seedlings 5 yr after harvest in Canada (Legere and Simard 2001). In the UK, canola seeds have remained viable 11 yr after burial (Lutman et al. 2003). Reducing the number of seeds entering secondary dormancy or relieving secondary dormancy in seeds within the seed bank would reduce problems associated with long term seed persistence and volunteers in subsequent crops. Delaying tillage was

suggested as a way to reduce input of dormant seeds into the seed bank because the chances of seed predation and degradation increase (Pekrun et al. 1998). However, a recent increase in disease pressure from black leg (*Leptosphaeria maculans* (Desm.) Ces. & De Not. [syn. *Plenodomus lingam* (Tode) Höhn.]) in Oregon, prompted the recommendation of deep tillage immediately after harvest to bury infected residue (Ocamb and Mallory-Smith 2015). Unfortunately, tillage operations in subsequent years bring these buried seeds to the surface where they can germinate and affect crop purity.

Phenoxy herbicides are commonly used in grass and cereal rotations within the Willamette Valley. Hamner et al. (1946) reported the effects of 2,4-D on plant growth when applied to soil and manure. Soil application of 2,4-D prevented germination of cabbage, clover, and wheat seed or produced abnormal seedlings which failed to mature. At a rate of 2.24 kg ae $ha^{-1}$ of 2,4-D or MCPA, no normal seedlings of turnip established (Allard et al. 1946). Mustard seeds in a dormant state were found to be relatively tolerant to 2,4-D (Mitchell and Brown 1947). However as soon as the seed coat was broken, the seeds became sensitive. Seeds soaked in various concentrations of 2,4-D acid (1 ppm – 100 ppm) for 4 h followed by germination in sand exhibited notably reduced germination at 10 ppm and almost complete inhibition at 100 ppm (Hamner et al. 1946). Seed producers have noted that applications of 2,4-D at the maximum labeled rate to fallow ground after harvest of Brassicas reduced the persistence of volunteers.

The objective of this research was to determine if phenoxy herbicides that are labeled for fallow application and already used within current grass seed and cereal rotations could be utilized to reduce the persistence of *Brassica* seed.

## Materials and Methods

**Greenhouse Studies.** Experiments were conducted in a greenhouse at Oregon State University in Corvallis, OR, between July 2015 and March 2016. Canola seeds were collected at harvest from a production field in the Willamette Valley during on June 23, 2015. The seeds were hand cleaned, but not sized, and stored in the dark at room temperature (22±2 C) for 1 to 9 months before use. Growing media used were potting mix (Sungro Professional Growing Mix SS#1, Agawam, MA) and/or a Woodburn silt-loam field soil (fine-silty, mixed, mesic Aqullic Argixerolls).

To determine differences in soil media, 50 seeds were placed on the soil surface of 52 by 26 by 6 cm trays filled with either potting soil or field soil. 2,4-D (Table 1) was applied at the maximum labeled fallow application rate of 2.24 kg ae $ha^{-1}$ utilizing a spray chamber (Generation III Research Sprayer, DeVries Manufacturing, Hollandale, MN) calibrated to deliver 187 L $ha^{-1}$ at 275 kPa. Tillage was simulated immediately after application by hand mixing the top 3 cm of soil and water was applied. Trays were watered as needed to maintain soil moisture near field capacity. Total emergence was recorded 26 d after treatment. There was no difference in emergence between growing media when herbicide and tillage treatments were applied (data not shown). Therefore, potting soil was used in subsequent experiments.

*2,4-D Study.* A second pot study evaluating three different 2,4-D treatment timings in relation to watering and seed incorporation was conducted. Applications of 2,4-D with and without incorporation were made as previously described to seeds on the soil surface 14, 7, and 0 d before watering. All trays were watered after the 0 d treatment was applied and watered as needed to maintain soil moisture near field

capacity. Seedling counts were taken 28 d after initial watering. Abnormal seedlings were defined by a lack of rooting structures, swollen hypocotyls, and deformed cotyledons.

The study utilized a completely randomized design with three replications plus an untreated check and was repeated. Data were subjected to ANOVA using the PROC GLM procedure in SAS (Statistical Analysis Systems, version 9.4, SAS Institute Inc., Cary, NC). Fisher's protected LSD was used to separate means at the 0.05 level of significance.

*Herbicide Comparisons.* The four herbicides tested and application rates are provided in Table 4.1. Fifty seeds were spread on trays filled with potting soil. Herbicides were applied as described previously. Seeds were incorporated immediately after treatment and watered. Trays were watered as needed to maintain soil moisture near field capacity. Total emergence was recorded 35 d after treatment.

A completely randomized design was used and included an untreated check. Each treatment was replicated four times and the experiment was repeated. Homogeneity of variance tests were conducted using the PROC GLIMMIX procedure in SAS. Means separation at the 0.05 significance level was determined using PROC GLM and Tukey's HSD.

The same four herbicides and rates (Table 4.1) used in the previous study were applied to 500 seeds spread on plastic trays. Seeds were air-dried for 24 h and then stored in plastic germination boxes in a germination chamber (SG2-22 Hoffman Manufacturing Inc. Corvallis, OR) set to 25 C with a 12 h light cycle to reflect field conditions after harvest. At 1, 7, and 14 d after treatment 50 random seeds per treatment were placed in 11 by 11 cm clear plastic germination boxes with moistened

blue blotter paper (Anchor Paper Company, Saint Paul, MN) and returned to the germinator. Seedlings were allowed to grow for 7 d at which point root and stem lengths of 10 seedlings per box were measured.

Each treatment was replicated three times and the experiment was repeated. Separate analysis was conducted on each run using PROC GLIMMIX in SAS. Data were square root transformed at a 0.3 power to meet normality and homoscedasticity assumptions for ANOVA; however, non-transformed data are presented for clarity. Means were separated using Fisher's protected LSD at the 0.05 significance level.

**Field study.** A herbicide trial was established at the Oregon State University Hyslop Research Farm (44°38'01"N, 123°11'26"W) near Corvallis, OR. Soil in the trial area was a Woodburn silt-loam. The trial was located in a field used for seed production of turnip (*Brassica rapa* var. *rapa* L.), mustard (*Brassica juncea* L. Czern.), and Chinese cabbage (*Brassica rapa* L. ssp. *chinensis* (L.) Hanelt). Plots were 2.4 by 7.3 m, oriented perpendicular to the direction of the previous crop planting to encompass all three crops and laid out in a randomized complete block with three replications. A Taylor-Way 670 offset disk (Taylor/Pittsburgh Implement, Athens, TN) tilled soil to 15 cm on July 10, 2016, preceding treatment. Treatments included a pre-rain application of 2,4-D at 2.12 kg $ha^{-1}$ on August 28, and a post-rain application of 2,4-D on September 4, 2016, after 16 mm of rainfall. Visual evaluations of seedling emergence were made 62 and 120 d after application on a scale from 0 (no control) to 100 (complete control). Data were subjected to ANOVA using the PROC GLM procedure in SAS 9.4. Means were separated with Tukey's HSD at the 0.05 significance level.

## Results and Discussion

*2,4-D study.* 2,4-D treatments reduced seedling emergence at all three application timings. Normal seedling emergence was reduced by 98.4% and 99.4% for incorporated 2,4-D and unincorporated 2,4-D treatments, respectively. Incorporation was not a significant factor, and there was no interaction between treatment and timing. The porous nature of potting soil and the small size of the canola seeds allowed the physical impact of watering to sufficiently incorporate them into the soil surface. Absence of root structures, swollen hypocotyls, and deformed cotyledons similar to those described by Hamner et al. (1946) were observed in all 2,4-D treatments. Abnormal seedlings survived on the moist soil surface in the controlled environment of the greenhouse; however, their survival in the field is unlikely. Limited water uptake from lack of root development could limit their ability to survive drying soils or harsh rain events. Therefore, only normal seedlings were included in the analysis.

*Herbicide Comparison.* 2,4-D and MCPA treatments reduced emergence by 46 and 60%, respectively, at 35 d (Figure 4.1). Dicamba, and dicamba plus diflufenzopyr did not reduce emergence when compared to the nontreated control. As noted in the previous experiment, abnormal seedlings survived on the moist soil surface of the trays, but field survival is unlikely.

Measurements of root and stem length taken from seedlings grown in germination boxes were consistent with results from greenhouse trials. Applications of 2,4-D and MCPA resulted in seedlings with the shortest roots and stems at all timings (Table 4.2). In both greenhouse and germination box studies, 2,4-D and

MCPA had the greatest negative effect on seedling growth. However, only 2,4-D was selected for the field study.

*Field study.* After rainfall, volunteer plants emerged in the nontreated control plots of the trial established on August 28. Applications of 2,4-D before and after the rainfall event were equally effective in reducing volunteer emergence by greater than 90%. No new volunteers were recruited in treated plots 120 d after application when the trial was terminated.

Everson and Dunham (1951) reported that when seeds were treated with 2,4-D, marked reduction in radical growth occurred when seeds germinated. However, this is in contrast with reports that seeds of clover and mustard were unaffected by similar 2,4-D applications (Mitchell and Brown 1947).

The results of this study agree with the earlier work presented by Everson and Dunham (1951). In this study herbicide application directly to seeds had a negative affect on growth and establishment of canola when imbibition occurred within14 d after application.

In the field study, pre-rain 2,4-D treatments applied within 2 d of precipitation reduced seedling emergence similar to the level in the greenhouse experiments. Both applications were effective in controlling volunteer emergence either through contact with the seed, non-emerged plant structures or by direct contact with foliage. Combined, greenhouse and field studies indicate that 2,4-D whether applied directly to the seed or broadcast over tilled soil near the time of imbibition can affect the growth and establishment of volunteer canola seedlings. Two other field studies were established but not reported on because of a lack of moisture, which prevented imbibition and growth of volunteer seedlings in non-treated controls and treated plots.

There are many factors, most specifically timing in relation to rainfall events, that make the utilization of phenoxy herbicides as pre-emergent treatments challenging. Under conditions suitable for imbibition, and when rainfall or irrigation is certain, application of 2,4-D may be able to provide volunteer control. However, further investigation into the timing of application in relation to rainfall or irrigation is needed.

## Literature Cited

Allard RW, DeRose HR, Swanson CP (1946) Some effects of plant growth-regulators on seed germination and seedling development. Bot Gaz 107:575–583

Everson LE, Dunham RS (1951) The effect of 2,4-dichlorophenoxyacetic acid on certain weed and crop seeds. Technical Bulletin197. University of Minnesota. 24 p

Gruber S, Bühler A, Möhring J, Claupein W (2010) Sleepers in the soil—Vertical distribution by tillage and long-term survival of oilseed rape seeds compared with plastic pellets. Eur J Agron 33:81–88

Gruber S, Pekrun C, Claupein W (2004) Seed persistence of oilseed rape (*Brassica napus*): variation in transgenic and conventionally bred cultivars. J Agric Sci 142:29–40

Hamner CL, Moulton JE, Tukey HB (1946) Treatment of muck and manure with 2,4-dichlorophenoxyacetic acid to inhibit germination of weed seeds. Science 103:476–477

Legere A, Simard MJ (2001) Presence and persistence of volunteer canola in Canadian cropping systems. Pages 143-147 *in* Proceedings of the Brighton Crop Protection Conference Weeds. Brighton, UK: British Crop Protection Council

Loberg G (2013) Testimony in support of HB 2427. Salem, Oregon. https://olis.leg.state.or.us/liz/2013R1/Downloads/CommitteeMeetingDocument/13695. Accessed January 5, 2018

Lutman PJW, Freeman SE, Pekrun C (2003) The long-term persistence of seeds of oilseed rape (*Brassica napus*) in arable fields. J Agric Sci 141:231–240

Mitchell JW, Brown JW (1947) Relative sensitivity of dormant and germinating seeds to 2,4-D. Science 106:266–267

Momoh EJJ, Zhou WJ, Kristiansson B (2002) Variation in the development of secondary dormancy in oilseed rape genotypes under conditions of stress. Weed Res 42:446–455

Ocamb CM, Mallory-Smith C (2015) Management of black leg in Oregon on *Brassica* seed crops. Oregon State University Extension Service. http://mtvernon.wsu.edu/wp-content/uploads/2016/01/Clinic-closeup-Black-leg-management-in-Brassica-seed-crops-and-oilseed-Jan-2015-version-II.pdf. Accessed January 15, 2018

Pekrun C, Hewitt JDJ, Lutman PJW (1998) Cultural control of volunteer oilseed rape (*Brassica napus*). J Agric Sci 130:155–163

Pekrun C, Lutman PJW, Baeumer K (1997) Induction of secondary dormancy in rape seeds (*Brassica napus* L.) by prolonged imbibition under conditions of water stress or oxygen deficiency in darkness. Eur J Agron 6:245–255

Schreiber A, Ritchie L (1995) Washington Minor Crops. Richland, Washington: Washington State University. 325 p

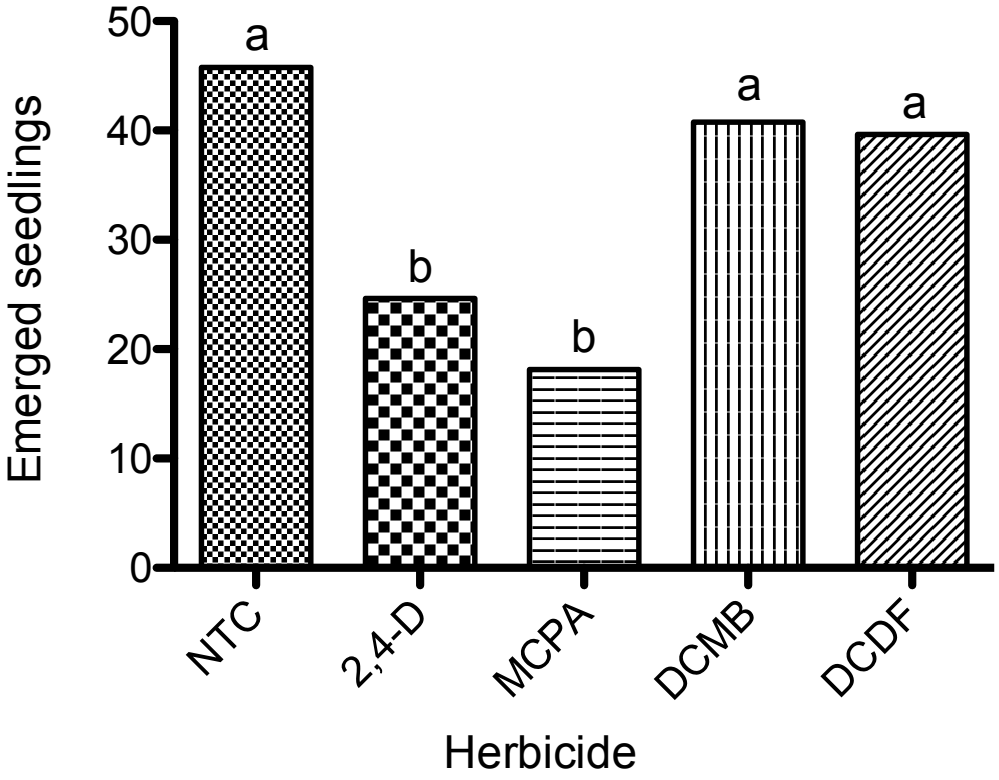


Figure 4.1 – Number of emerged seedlings from 50 seeds planted in trays in the greenhouse at 35 d after treatment. Treatment codes: nontreated control (NTC), 2,4-D (2,4-D), MCPA (MCPA), dicamba (DCMB), dicamba plus diflufenzopyr (DCDF). Bars which have the same letter are not different according to Tukey's HSD ($P \leq 0.05$).

Table 4.1 – Herbicides applied in greenhouse experiments.

| Common name | Trade name | Rate[a] | Manufacturer |
|---|---|---|---|
| | | kg ai or ae ha$^{-1}$ | |
| 2,4-D | Base Camp Amine 4 | 2.24 | Wilbur Ellis Company LLC, Fresno, CA |
| Dicamba | Clarity | 1.12 | BASF Corp., Research Triangle Park, NC |
| Dicamba + diflufenzopyr | Distinct | 0.14+ 0.056 | BASF Corp., Research Triangle Park, NC |
| MCPA | MCP Amine 4 | 3.36 | Loveland Products, Inc., Greeley, CO |

[a]Herbicide rate expressed as active ingredient or acid equivalent as appropriate.

Table 4.2 – Means of canola seedling root and stem lengths.

| Herbicide | Timing[a] | Length of structure | | | |
|---|---|---|---|---|---|
| | | Run 1 | | Run 2 | |
| | | Root | Stem | Root | Stem |
| | | cm | | | |
| Control | 1 | 9.03 b[b] | 3.69 b | 10.97 bc | 2.62 cd |
| | 7 | 11.12 a | 3.02 e | 12.41 a | 2.34 de |
| | 14 | 7.55 c | 2.89 ef | 10.75 cd | 2.50 d |
| 2,4-D | 1 | 0.26 e | 0.32 h | 0.38 g | 0.39 gh |
| | 7 | 0.34 e | 0.65 g | 0.61 g | 0.74 f |
| | 14 | 0.26 e | 0.36 h | 0.47 g | 0.62 fg |
| MCPA | 1 | 0.18 e | 0.32 h | 0.21 g | 0.30 h |
| | 7 | 0.31 e | 0.48 gh | 0.14 g | 0.19 h |
| | 14 | 0.26 e | 0.32 h | 0.29 g | 0.45 fgh |
| Dicamba | 1 | 9.05 b | 3.98 a | 9.19 ef | 2.44 de |
| | 7 | 9.05 b | 3.28 d | 8.79 f | 2.94 ab |
| | 14 | 6.32 d | 3.43 cd | 8.87 f | 2.85 bc |
| Dicamba + | 1 | 9.18 b | 3.61 bc | 10.02 de | 2.13 e |
| diflufenzopyr | 7 | 10.37 a | 2.83 ef | 11.70 ab | 3.11 ab |
| | 14 | 6.65 d | 2.76 f | 9.11 f | 3.18 a |

[a] Days between herbicide application to seed and the beginning of the 7 d germination and growth period in a growth chamber is given under timing

[b] Means within a column followed by the same letter are not different according to Fisher's protected LSD test at P = 0.05.

# CHAPTER 5

## General Conclusion

The Willamette Valley of Oregon is a region with a rich history of diverse crop production which provides producers with a broad range of cropping options but also presents challenges. Interest in growing canola as a rotational crop in grass seed and cereal rotations has met resistance from members of the seed production industry who produce a large range of other Brassicaceae seed crops. The Oregon Legislative House Bill 2427 instructed the College of Agricultural Sciences at Oregon State University to, "…use field monitoring and other research to develop information and recommendations regarding whether, and under what conditions, canola… is compatible with the growing of other [Brassicaceae] crops." As one part of the larger project, the studies presented here were conducted to gain a better understanding of the persistence and control of canola seeds and volunteers within the Valley. In order to draw comparisons, several other Brassicaceae seed crops were included in the studies.

Field surveys were conducted to determine average harvest seed losses of canola, radish, turnip, and forage rape within the Valley. Results were similar to those reported in other regions for canola, and losses of canola were not different from any of the other crops surveyed. Depth of emergence studies were designed to simulate seed burial at depths representative of common tillage practices. Canola and turnip emergence percentages were similar. Radish seeds, due to their larger size and greater energy reserves, were able to emerge from deeper depths. These results

complimented the field tillage study in which radish seedling emergence was greater than canola or turnip under deep tillage over three years. Deep tillage had the lowest number of emerged seedlings over a three-year period compared to no-tillage and shallow tillage.

An examination of the seedbank revealed that the greatest seed persistence after 38 months occurred under deep tillage treatment. There was no difference in the persistence of canola, radish, or turnip. However, tillage treatment affected seed persistence and deep tillage should be avoided in order to limit the formation of a persistent seed bank. In another study examining the viability of radish seeds buried in pods, burial depth did not affect the rate of viability decline, although overall seed persistence was greater at deeper depths. These studies which measured emergence and seed persistence at a single point in time left a large number of seeds unaccounted for, especially in the deep tillage treatment. Seeds face many vicissitudes once shed from the mother plant. Future work should examine those factors; such as predation, pathogen attack, or fatal germination, especially if they could be manipulated to reduce persistence.

Secondary dormancy induction studies were conducted on seeds harvested from fields in 2016 and 2017. Differences in the overall levels of dormancy induction were different for each year indicating that the environment may have played a role in determining the potential for dormancy induction. Weather data and dormancy patterns agreed with the currently accepted relationship between temperature and dormancy; lower temperatures and low drought stress during seed production increase the potential for dormancy. Canola and daikon radish exhibited similar secondary

dormancy induction potentials. ‘Crimson Giant’ red radish displayed no secondary dormancy potential, followed by turnip which had the lowest induction level.

There is a relationship between speed of germination and secondary dormancy induction potential. Seeds which germinate more slowly have higher secondary dormancy potential. There were trends in the relative expression in seeds of NCED genes, which are the first committed step in ABA biosynthesis, but they did not entirely predict secondary dormancy potential. Dormancy induction is a complicated process involving multiple gene families. Further work elucidating the effect of cultural practices and environmental factors on secondary dormancy potential could help producers to predict when crops might have greater dormancy levels and plan control strategies and tillage practices accordingly.

A final study examined the effects of 2,4-D and several related synthetic auxin herbicides to determine if herbicides which were already in use on Willamette Valley farms could provide pre-emergent control or help reduce seed persistence. Greenhouse studies were promising, and applications of 2,4-D and MCPA to the soil surface with and without incorporation reduced normal seedling growth by more than 98%. When applied directly to the seed, the same two herbicides also reduced root and shoot length of the subsequent seedlings. Field trials were inconclusive due to inconsistent moisture. In one trial, preemergent applications applied just before or immediately following precipitation effectively reduced seedling emergence for 120 days. When moisture either from precipitation or irrigation is certain, applications of 2,4-D could provide volunteer control, but more studies are needed. Further work should investigate timing of field applications and if 2,4-D interacts with molecular pathways involved in dormancy.

In the studies reported here, canola was not found to persist to a greater extent than any of the other Brassicaceae crops with which it was compared, nor did canola display a greater potential for secondary dormancy induction.

# Bibliography

1986 Amendments (1986). Substitute Senate Bill 5044. Code 15.65.21

Al-Mudaris MA (1998) Notes on various parameters recording the speed of seed germination. J Agric Rural Dev Trop Subtrop 99:147–154

Allard RW, DeRose HR, Swanson CP (1946) Some effects of plant growth-regulators on seed germination and seedling development. Bot Gaz 107:575–583

Amen RD (1968) A model of seed dormancy. Bot Rev 34:1–31

Anchez RA, Eyherabide G, Miguel L de (1981) The influence of irradiance and water deficit during fruit development on seed dormancy in *Datura ferox* L. Weed Res 21:127–132

Anderson N (2017) Willamette Valley Field Crops: Grass Seed. Oregon State University. https://valleyfieldcrops.oregonstate.edu/grass-seed. Accessed February 6, 2019

Anstey TH (1986) One Hundred Harvests. Ottawa, Canada: Canadian Government Publishing Centre. 432 p

AP (1986) State issues order limiting areas for rapeseed growing. Times News 5. Twin Falls, Idaho

Appelqvist LA, Ohlson R, eds. (1972) Rapeseed. Amsterdam: Elsevier. 393 p

Baker J, Preston C (2008) Canola (*Brassica napus* L.) seedbank declines rapidly in farmer-managed fields in South Australia. Aust J Agric Res 59:780-784

Baskin JM, Baskin CC (1998) Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination. London, UK: Academic Press. 666 p

Bell JM (1982) From rapeseed to canola: A brief history of research for superior meal and edible oil. Poult Sci 61:613–622

Benvenuti S, MacChia M, Miele S (2001) Quantitative analysis of emergence of seedlings from buried weed seeds with increasing soil depth. Weed Sci 49:528–535

Bewley J (1997) Seed Germination and dormancy. Plant Cell 9:1055–1066

Bewley JD, Black M (1994) Seeds: Physiology of Development and Germination. 2nd edn. New York: Plenum Press. 445 p

Bewley JD, Bradford KJ, Hilhorst HWM, Nonogaki H (2013) Seeds: Physiology of Development, Germination and Dormancy 3rd edn. New York: Springer. 392 p

Boulter GS (1983) The History and Marketing of Rapeseed Oil in Canada. Pages 62-82 *in* High and Low Erucic Acid Rapeseed Oils. New York: Academic Press

Brenchley WE, Warington K (1933) The weed seed population of arable soil: II. Influence of crop, soil and methods of cultivation upon the relative abundance of viable seeds. J Ecol 21:103-127

Brewer DH (2005) Oregon Seed Production. Silverton, OR: OSU Seed Certification Services. 74 p

Brown J, Davis JB, Erickson DA, Brown AP (1999) Effects of swathing on yield and quality of spring canola in Northern Idaho. J Prod Agric 12:33–37

Brust GE, House GJ (1988) Weed seed destruction by arthropods and rodents in low-input soybean agroecosystems. Am J Altern Agric 3:19–25

Burnside OC, Wicks GA, Fenster CR (1977) Longevity of shattercane seed in soil across Nebraska. Weed Res 17:139–143

Busch L, Gunter V, Mentele T, Tachikawa M, Tanaka K (1994) Socializing nature: technoscience and the transformation of rapeseed into canola. Crop Sci 34:607–614

Cadman CSC, Toorop PE, Hilhorst HWM, Finch-Savage WE (2006) Gene expression profiles of arabidopsis Cvi seeds during dormancy cycling indicate a common underlying dormancy control mechanism. Plant J 46:805–822

Calhoun W, Crane JM, Stamp DL (1975) Development of a low glucosinolate, high erucic acid rapeseed breeding program. J Am Oil Chem Soc 52:363–365

Calhoun W, Jolliff GD, Crane JM (1983) Registration of Indore Rapeseed. Crop Sci 23:184–185

Canola Council of Canada (2016) History of varietal development. https://www.canolacouncil.org/canola-encyclopedia/crop-development/history-of-varietal-development/. Accessed January 23, 2017

Carroll KK (1953) Erucic acid as the factor in rape oil affecting adrenal cholesterol in the rat. J Biol Chem 200:287–292

Cavalieri A, Harker KN, Hall LM, Willenborg CJ, Haile TA, Shirtliffe SJ, Gulden RH (2016) Evaluation of the causes of on-farm harvest losses in canola in the Northern Great Plains. Crop Sci 56:2005-2015

Chancellor RJ (1986) Decline of arable weed seeds during 20 years in soil under grass and the periodicity of seedling emergence after cultivation. J Appl Ecol 23:631–637

Cheam A, Lee S (2009) Inversion ploughing: effects of long-term deep burial on weed seed reserves. Department of Agriculture and Food, Western Australia. 5 p

Chen M, MacGregor DR, Dave A, Florance H, Moore K, Paszkiewicz K, Smirnoff N, Graham IA, Penfield S (2014) Maternal temperature history activates Flowering Locus T in fruits to control progeny dormancy according to time of year. Proc Natl Acad Sci 111:18787–18792

Chepil WS (1946) Germination of weed seeds: II. The influence of tillage treatments on germination. Sci Agric 26:347–357

Chiang GCK, Bartsch M, Barua D, Nakabayashi K, Debieu M, Kronholm I, Koornneef M, Soppe WJJ, Donohue K, Meaux JD (2011) DOG1 expression is predicted by the seed-maturation environment and contributes to geographical variation in germination in *Arabidopsis thaliana*. Mol Ecol 20:3336–3349

Child RD, Chauvaux N, John K, Ulvskov P, Onckelen HAV (1998) Ethylene biosynthesis in oilseed rape pods in relation to pod shatter. J Exp Bot 49:829–838

Chiwocha SDS, Abrams SR, Ambrose SJ, Cutler AJ, Loewen M, Ross ARS, Kermode AR (2003) A method for profiling classes of plant hormones and their metabolites using liquid chromatography-electrospray ionization tandem mass spectrometry: an analysis of hormone regulation of thermodormancy of lettuce (*Lactuca sativa* L.) seeds. Plant J 35:405–417

Claassen BJ (2016) Investigations of Black Leg and Light Leaf Spot on Brassicaceae Hosts in Oregon. Masters thesis. Corvallis, OR: Oregon State University. 67 p

Clements DR, Benott DL, Murphy SD, Swanton CJ (1996) Tillage effects on weed seed return and seedbank composition. Weed Sci 44:314–322

Coba K (2009) Canola advisory committee members and other interested parties. Salem, OR: Oregon Department of Agriculture. 2 p

Code G, Walsh M, Reeves T (1987) Effect and depth and duration of burial of wild radish seed on seed viability and seedling emergence. Pages 136–138 *in* Proceedings of the Weed Seed Biology Workshop. Orange, Australia

Daun JK (1983) The Introduction of Low Erucic Acid Rapeseed Varieties into Canadian Production. Pages 161-180 *in* High and Low Erucic Acid Rapeseed Oils. New York, NY: Academic Press

de Jong TJ, Tudela Isanta M, Hesse E (2013) Comparison of the crop species *Brassica napus* and wild *B. rapa*: characteristics relevant for building up a persistent seed bank in the soil. Seed Sci Res 23:169–179

Downey RK, Harvey BL (1963) Methods of breeding for oil quality in rape. Can J Plant Sci 43:271–275

Eslami SV, Gill GS, McDonald G (2010) Effect of water stress during seed development on morphometric characteristics and dormancy of wild radish (*Raphanus raphanistrum* L.) seeds. Int J Plant Prod:10

Everson LE, Dunham RS (1951) The effect of 2,4-dichlorophenoxyacetic acid on certain weed and crop seeds. Technical Bulletin197. University of Minnesota. 24 p

Ferguson BT, Chastain TG, Garbacik CJ, Chastain BT, Wysocki DJ (2016) Spring nitrogen and cultivar affect seed production in winter canola. Agron J 108:1124-1131

Footitt S, Douterelo-Soler I, Clay H, Finch-Savage WE (2011) Dormancy cycling in arabidopsis seeds is controlled by seasonally distinct hormone-signaling pathways. Proc Natl Acad Sci 108:20236–20241

Gruber S, Bühler A, Möhring J, Claupein W (2010) Sleepers in the soil—vertical distribution by tillage and long-term survival of oilseed rape seeds compared with plastic pellets. Eur J Agron 33:81–88

Gruber S, Pekrun C, Claupein W (2004) Seed persistence of oilseed rape (*Brassica napus*): variation in transgenic and conventionally bred cultivars. J Agric Sci 142:29–40

Gulden RH, Chiwocha S, Abrams S, McGregor I, Kermode A, Shirtliffe S (2004) Response to abscisic acid application and hormone profiles in spring *Brassica napus* seed in relation to secondary dormancy. Can J Bot 82:1618–1624

Gulden RH, Shirtliffe SJ, Thomas AG (2003) Harvest losses of canola (*Brassica napus*) cause large seedbank inputs. Weed Sci 51:83–86

Gulden RH, Thomas AG, Shirtliffe SJ (2004) Relative contribution of genotype, seed size and environment to secondary seed dormancy potential in Canadian spring oilseed rape (*Brassica napus*). Weed Res 44:97–106

Hails RS, Rees M, Kohn DD, Crawley MJ (1997) Burial and seed survival in *Brassica napus* subsp. *oleifera* and *Sinapis arvensis* including a comparison of transgenic and non-transgenic lines of the crop. Proc Biol Sci 264:1–7

Hamner CL, Moulton JE, Tukey HB (1946) Treatment of muck and manure with 2,4-dichlorophenoxyacetic acid to inhibit germination of weed seeds. Science 103:476–477

Hang AN, Morrison KJ, Parker R (1982) Rapeseed culture and use. Washington State University: Cooperative Extension. 3 p

HB 3382 (2015) 78th Assembly, 2015 Reg. Sess. (OR 2015). https://olis.leg.state.or.us/liz/2015R1/Downloads/MeasureDocument/HB3382. Accessed January 15, 2017

HB 2427 (2013) 77th Assembly, 2013 Reg. Sess. (OR 2013). https://olis.leg.state.or.us/liz/2013R1/Downloads/MeasureDocument/HB2427. Accessed January 15, 2017

He H, de Souza Vidigal D, Snoek LB, Schnabel S, Nijveen H, Hilhorst H, Bentsink L (2014) Interaction between parental environment and genotype affects plant and seed performance in arabidopsis. J Exp Bot 65:6603–6615

He M, Cui S, Yang X, Mu G, Chen H, Liu L (2017) Selection of suitable reference genes for abiotic stress-responsive gene expression studies in peanut by real-time quantitative PCR. Electron J Biotechnol 28:76–86

Hedge IC (1976) A Systematic and Geographical Survey of the Old World Cruciferae. Pages 1-45 *in* The Biology and Chemistry of the Cruciferae. New York, NY: Academic Press Inc.

Hoffman ML, Owen MDK, Buhler DD (1998) Effects of crop and weed management on density and vertical distribution of weed seeds in soil. Agron J 90:793–799

Hyslop GR, Schoth HA (1937) Rape Extension Bulletin 499. Federal Cooperative Extension Service. 4 p

IDAPA (2014) Rules relating to rapeseed production and establishment of rapeseed districts in the state of Idaho. Code 02.06.13. https://adminrules.idaho.gov/rules/current/02/020613.pdf. Accessed January 22, 2017

Inglis DA, du Toit L, Miller T, (2013) Production of *Brassica* Seed Crops in Washington State: A Case Study on the Complexities of Coexistence. Mt. Vernon, WA: Washington State University Extension. 19 p

Iñiguez-Luy F, Sass M, Jung G, Johns M, Nienhuis J (2006) Development of robust scar markers that distinguish the six cultivated *Brassica* species and subspecies of the U-triangle. Am Soc Hortic Sci 131:424–432

Jourdan PS, Earle ED, Mutschler MA (1988) Synthesis of male sterile, triazine-resistant *Brassica napus* by somatic hybridization between cytoplasmic male sterile *B. oleracea* and atrazine-resistant *B. campestris*. Theor Appl Genet 78:445–455

Kadkol GP, Macmillan RH, Burrow RP, Halloran GM (1984) Evaluation of *Brassica* genotypes for resistance to shatter. I. Development of a laboratory test. Euphytica 33:63–73

Karimmojeni H, Bazrafshan AH, Majidi MM, Torabian S, Rashidi B (2014) Effect of maternal nitrogen and drought stress on seed dormancy and germinability of *Amaranthus retroflexus*. Plant Species Biol 29:E1–E8

Karow R (1986) Production and Research History of Winter Rape in the Pacific Northwest. Moscow, Idaho. 2 p

Karow R (2008) ODA Bioenergy Oilseeds Project Quarterly Report. Corvallis, OR: Oregon State University. 20 p

Karow R (2010) OSU Western OR Canola Research Report. Corvallis, OR: Oregon State University. 10 p

Karow R (2012) Canola in the Willamette Valley - A State Regulation, High Value Crop, Scientific Unknowns, and Right to Farm Conundrum. Corvallis, OR: Oregon State University. 39 p

Kendall SL, Hellwege A, Marriot P, Whalley C, Graham IA, Penfield S (2011) Induction of dormancy in arabidopsis summer annuals requires parallel regulation of dog1 and hormone metabolism by low temperature and cbf transcription factors. Plant Cell 23:2568–2580

Kende H, Zeevaart J (1997) The five “classical” plant hormones. Plant Cell 9:1197–1210

Kephart KD, Murray GA, eds. (1990) Dryland Winter Rapeseed Production Guide. Moscow, ID: University of Idaho Cooperative Extension Service. 23 p

Kimber D, McGregor DI, eds. (1995) *Brassica* Oilseeds: Production and Utilization. Wallingford, UK: CAB International. 394 p

Koornneef MLJ, Brinkhorst-van der Swan DLC, Karssen CM (1982) The isolation of abscisic acid (aba) deficient mutants by selection of induced revertants in non-

germinating gibberellin sensitive lines of *Arabidopsis thaliana* (L.) Heynh. Theor Appl Genet 61:385–393

Kovach DA, Thill DC, Young FL (1988) A water-spray system for removing seed from soil. Weed Technol 2:338–341

Lefebvre V, North H, Frey A, Sotta B, Seo M, Okamoto M, Nambara E, Marion-Poll A (2006) Functional analysis of arabidopsis NCED6 and NCED9 genes indicates that ABA synthesized in the endosperm is involved in the induction of seed dormancy. Plant J 45:309–319

Legere A, Simard MJ (2001) Presence and persistence of volunteer canola in Canadian cropping systems. Pages 143-148 *in* Brighton Crop Protection Conference, Weeds. Brighton, UK: British Crop Protection Council

Leist N, Krämer S, Jonitz A, eds. (2011) ISTA Working Sheets on Tetrazolium Testing Volume 1: Agricultural, Vegetable and Horticultural Species with Supplements 2011. International Seed Testing Association (ISTA). 176 p

Loberg G (2013) Testimony in support of HB 2427. Salem, Oregon. https://olis.leg.state.or.us/liz/2013R1/Downloads/CommitteeMeetingDocument/13695. Accessed January 5, 2018

Lutman PJW (1993) The occurrence and persistence of volunteer oilseed rape (*Brassica napus*). Asp Appl Biol 35:29–36

Lutman PJW, Freeman SE, Pekrun C (2003) The long-term persistence of seeds of oilseed rape (*Brassica napus*) in arable fields. J Agric Sci 141:231–240

MacLeod J (1981) Harvesting. Pages 107–119 *in* Oilseed Rape Book. A manual for growers, farmers and advisors. Cambridge: Cambridge Agricultural Publishing

Mallory-Smith C, Berry P, Flick G, Ocamb CM, Claassen BJ, Green J (2017) Final Report - House Bill 2427. Corvallis, OR: Oregon State University. 105 p https://www.oregon.gov/ODA/shared/Documents/Publications/Administration/HB2427ReportCanola.pdf

Martinez-Andujar C, Ordiz MI, Huang Z, Nonogaki M, Beachy RN, Nonogaki H (2011) Induction of 9-cis-epoxycarotenoid dioxygenase in *Arabidopsis thaliana* seeds enhances seed dormancy. Proc Natl Acad Sci 108:17225–17229

Medd R (1992) Techniques for monitoring seedbanks of annual grass weeds on arable soils. Pages 333–335 *in* Proceedings of the First International Weed Control Congress. Melbourne, Australia: Weed Science Society of Victoria

Michel BE, Wiggins OK, Outlaw WHJ (1983) A guide to establishing water potential of aqueous two-phase solutions (polyethylene glycol plus dextran) by amendment with mannitol. Plant Physiol 72:60–65

Mitchell JW, Brown JW (1947) Relative sensitivity of dormant and germinating seeds to 2,4-D. Science 106:266–267

Mohler CL, Galford AE (1997) Weed seedling emergence and seed survival: separating the effects of seed position and soil modification by tillage. Weed Res 37:147–155

Momoh EJJ, Zhou WJ, Kristiansson B (2002) Variation in the development of secondary dormancy in oilseed rape genotypes under conditions of stress. Weed Res 42:446–455

Mortenson E (2012) Oregon specialty seed growers and activist groups file suit to stop canola planting. http://www.oregonlive.com/business/index.ssf/2012/08/oregon_specialty_seed_growers_1.html. Accessed January 23, 2019

Morton CT, Buchele WF (1960) Emergence energy of plant seedlings. Agric Eng 41:428–31, 453–55

Munir J, Dorn LA, Donohue K, Schmitt J (2001) The effect of maternal photoperiod on seasonal dormancy in *Arabidopsis thaliana* (Brassicaceae). Am J Bot 88:1240–1249

NASDA (2012) Oregon: ODA adopts canola control area rule for Willamette Valley. https://www.nasda.org/news/oregon-oda-adopts-canola-control-area-rule-for-willamette-valley. Accessed January 23, 2019

Nonogaki M, Sall K, Nambara E, Nonogaki H (2014) Amplification of ABA biosynthesis and signaling through a positive feedback mechanism in seeds. Plant J 78:527–539

Ocamb CM (2014) Disease Alert - Black leg in Brassicaceae seed fields in the Willamette Valley. Corvallis, OR: Oregon State University Extension Service. 2 p

Ocamb CM, Mallory-Smith C (2015) Management of black leg in Oregon on *Brassica* seed crops. Oregon State University Extension Service. http://mtvernon.wsu.edu/wp-content/uploads/2016/01/Clinic-closeup-Black-leg-management-in-Brassica-seed-crops-and-oilseed-Jan-2015-version-II.pdf. Accessed January 15, 2018

ODA (2019) State of Oregon Agriculture: Report from the State Board of Agriculture.

https://www.oregon.gov/oda/shared/documents/publications/administration/boardreport.pdf. Accessed March 1, 2019

OECD (2012) Consensus Document on the Biology of the *Brassica* Crops (*Brassica* spp.). Paris: OECD. 142 p

OGTR (2016) Biology of Canola and Indian mustard. Australian Government Department of Health. 94 p

Orchard TJ (1975) Estimating the parameters of plant seedling emergence. Seed Sci Technol 5:61–69

Oregon State Land-Use Planning Committee (1941) To Meet the Impacts of War and National Defense. Corvallis, OR: Oregon State Agricultural Program. 100 p

Pari L, Assirelli A, Suardi A, Civitarese V, Del Giudice A, Costa C, Santangelo E (2012) The harvest of oilseed rape (*Brassica napus* L.): The effective yield losses at on-farm scale in the Italian area. Biomass Bioenergy 46:453–458

Pekrun C, Hewitt JDJ, Lutman PJW (1998) Cultural control of volunteer oilseed rape (*Brassica napus*). J Agric Sci 130:155–163

Pekrun C, Lutman PJW, Baeumer K (1997) Induction of secondary dormancy in rape seeds (*Brassica napus* L.) by prolonged imbibition under conditions of water stress or oxygen deficiency in darkness. Eur J Agron 6:245–255

Penfield S, MacGregor DR (2017) Effects of environmental variation during seed production on seed dormancy and germination. J Exp Bot 68:819–825

Pessel D, Lecomte J, Emeriau V, Krouti M, Messean A, Gouyon PH (2001) Persistence of oilseed rape (*Brassica napus* L.) outside of cultivated fields. Theor Appl Genet 102:841–846

Peters NCB (1982) Production and dormancy of wild oat (*Avena fatua*) seed from plants grown under soil waterstress. Ann Appl Biol 100:189–196

Pons TL (1991) Induction of dark dormancy in seeds: its importance for the seed bank in the soil. Funct Ecol 5:669-675

Prakash S (1973) Artificial synthesis of *Brassica juncea* Coss. Genetica 44:249–263

Price JS, Hobson RN, Neale MA, Bruce DM (1996) Seed losses in commercial harvesting of oilseed rape. J Agric Eng Res 65:183–191

Quinn MP (2010) Potential Impacts of Canola *(Brassica napus* L.) on *Brassica* Vegetable Seed Production in the Willamette Valley of Oregon. Ph.D dissertation. Corvallis, OR: Oregon State University. 79 p

Rampton HH, Ching TM (1966) Longevity and dormancy in seeds of several cool-season grasses and legumes buried in soil. Agron J 58:220–222

Reddy LV, Metzger RJ, Ching TM (1985) Effect of temperature on seed dormancy of wheat. Crop Sci 25:455–458

Reid KE, Olsson N, Schlosser J, Peng F, Lund ST (2006) An optimized grapevine RNA isolation procedure and statistical determination of reference genes for real-time RT-PCR during berry development. BMC Plant Biol 6:27-38

Rice KJ (1989) Impacts of seed banks on grassland community structure and population dynamics. Pages 211-230 *in* MA Leck, VT Parker, RL Simpson, eds. Ecology of Soil Seed Banks. San Diego, CA: Academic Press Inc.

Roberts HA, Boddrell JE (1983) Seed survival and periodicity of seedling emergence in eight species of Cruciferae. Ann Appl Biol 103:301–304

Roberts HA, Dawkins PA (1967) Effect of cultivation on the numbers of viable weed seeds in soil. Weed Res 7:290–301

Sarla N, Raut RN (1988) Synthesis of *Brassica carinata* from *Brassica nigra* x *Brassica oleracea* hybrids obtained by ovary culture. Theor Appl Genet 76:846–849

Sawhney R, Quick WA, Hsiao AI (1985) The effect of temperature during parental vegetative growth on seed germination of wild oats (*Avena fatua* L.). Ann Bot 55:25–28

Schopfer P, Plachy C (1984) Control of seed germination by abscisic acid: ii. effect on embryo water uptake in *Brassica napus* L. Plant Physiol 76:155–160

Schreiber A, Ritchie L (1995) Washington Minor Crops. Richland, WA: Washington State University. 325 p

Schwartz SH (1997) Specific oxidative cleavage of carotenoids by VP14 of maize. Science 276:1872–1874

SeedTec (1990) Canola Management/Production Guide. Woodland, CA: SeedTec International INC. 24 p

Seo M, Hanada A, Kuwahara A, Endo A, Okamoto M, Yamauchi Y, North H, Marion-Poll A, Sun T, Koshiba T, Kamiya Y, Yamaguchi S, Nambara E (2006) Regulation of hormone metabolism in arabidopsis seeds: phytochrome regulation of abscisic acid metabolism and abscisic acid regulation of gibberellin metabolism. Plant J 48:354–366

Seo M, Nambara E, Choi G, Yamaguchi S (2009) Interaction of light and hormone signals in germinating seeds. Plant Mol Biol 69:463–472

Shahidi F (1990) Rapeseed and Canola: Global Production and Distribution. Pages 3-14 *in* Canola and Rapeseed Production, Chemistry, Nutrition and Processing Technology. New York, NY: Van Nostrand Reinhold

Soriano A, Zeiger E, Servy E, Suero A (1968) The effect of cultivation on the vertical distribution of seeds in the soil. J Appl Ecol 5:253-257

Springthorpe V, Penfield S (2015) Flowering time and seed dormancy control use external coincidence to generate life history strategy. https://elifesciences.org/articles/05557. Accessed March 1, 2019. 17 p

Taiz L, Zeiger E (2010) Plant Physiology 5th edn. Sunderland, MA: Sinauer Associates Inc. 782 p

Tan B-C, Joseph LM, Deng W-T, Liu L, Li Q-B, Cline K, McCarty DR (2003) Molecular characterization of the arabidopsis 9-cis epoxycarotenoid dioxygenase gene family. Plant J 35:44–56

Tang H, Cuevas HE, Das S, Sezen UU, Zhou C, Guo H, Goff VH, Ge Z, Clemente TE, Paterson AH (2013) Seed shattering in a wild sorghum is conferred by a locus unrelated to domestication. Proc Natl Acad Sci 110:15824–15829

Taylor GH, Bartlett A (1993) The climate of Oregon. Climate zone 2, Willamette Valley. Corvallis, OR: Oregon Climate Service, Oregon State University. 11 p

Thomas MD, Breithaupt LR, Nielsen NI, others (1944) Oregon's miscellaneous specialty crops 1936-1943. Corvallis, OR: Federal Cooperative Extension Service, Oregon State College. 15 p

Thomas T, Raper C (1975) Seed germinability as affected by the environmental temperature of the mother plant. Tob Sci 19:98–100

Thompson K, Grime JP (1979) Seasonal variation in the seed banks of herbaceous species in ten contrasting habitats. J Ecol 67:893–921

Thompson K, Grime JP (1983) A comparative study of germination responses to diurnally-fluctuating temperatures. J Appl Ecol 20:141–156

Toole EH, Brown E (1946) Final results of the Duvel buried seed experiment. J Agric Res 72:201–210

Tricault Y, Matejicek A, Darmency H (2018) Variation of seed dormancy and longevity in *Raphanus raphanistrum* L. Seed Sci Res 28:34–40

Tsuyuzaki S (1994) Rapid seed extraction from soils by a flotation method. Weed Res 34:433–436

U N (1935) Genome-analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. Jpn J Bot 7:389–452

University of Idaho, Oregon State University, Washington State University (1979) Development of oilseeds as alternative crops for the Pacific Northwest. 7 p

USDA (1996) Crambe, Industrial Rapeseed, and Tung Provide Valuable Oils. Economic Research Service, USDA. 23 p

Van Mourik TA, Stomph TJ, Murdoch AJ (2005) Why high seed densities within buried mesh bags may overestimate depletion rates of soil seed banks: Appraisal of the seed bag burial method. J Appl Ecol 42:299–305

Villiers TA, Wareing PF (1960) Interaction of growth inhibitor and a natural germination stimulator in the dormancy of *Fraxinus excelsior* L. Nature 185:112–114

Wang R, Ripley VL, Rakow G (2007) Pod shatter resistance evaluation in cultivars and breeding lines of *Brassica napus, B. juncea* and *Sinapis alba*. Plant Breed 126:588–595

Wareing PF, Saunders PF (1971) Hormones and dormancy. Annu Rev Plant Physiol 22:261–288

Warkentin H (1985) Combine attachment for harvesting radish seeds and the like. U.S. Patent. No. 4,524,780. Washington, DC: U.S. Patent and Trademark Office

Weber EA, Frick K, Gruber S, Claupein W (2010) Research and development towards a laboratory method for testing the genotypic predisposition of oilseed rape (*Brassica napus* L.) to secondary dormancy. Seed Sci Technol 38:298–310

Zhang J, Drummond FA, Liebman M, Hartke A (1997) TB163: Insect Predation of Seeds and Plant Population Dynamics. Orono, ME: Maine Agricultural and Forest Experiment Station. 32 p