AN ABSTRACT OF THE THESIS OF

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Title: Applied Nitrogen Effects on Yellow Mustard (*Sinapis alba* L.) Production in the Willamette Valley.

Abstract approved:

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Nitrogen management recommendations for yellow mustard (*Sinapis alba* L.) production in the high rainfall environment of Oregon's Willamette Valley (WV) are not available. The objectives of this investigation were: (i) to determine the effect of applied nitrogen (N) on seed yield and yield components in yellow mustard, and (ii) to ascertain the effect of applied N on dry matter partitioning and oil production characteristics in yellow mustard. Field trials were conducted over a two-year period at Corvallis, Oregon, USA on 'IdaGold' yellow mustard with five N application rates: 0, 56, 112, 168, and 224 kg N ha^{-1}.

Although rainfall differed greatly between the two years, the effect of applied N on seed yield and yield components were similar. Seed yield was increased by 15%-85% with applied N, primarily as a result of N-induced increases in seed weight and number. Yield ranged from 1,080 kg ha^{-1} with 0 kg N ha^{-1} to 2,580 kg ha^{-1} with 224 kg N ha^{-1}. Primary racemes $plant^{-1}$ was positively associated with increased N; however, secondary racemes $plant^{-1}$ was not consistently related to N. Siliques $plant^{-1}$ increased in proportion to N rate and was the result of greater numbers of siliques main $stem^{-1}$ and siliques primary $raceme^{-1}$. The relationship of applied N and siliques $plant^{-1}$ was manifested in seed yield, and as an example, the 224 kg N ha^{-1} rate yielded 95% and 172% more seeds than 0 kg N ha^{-1} in 2013 and 2014, respectively. Contributions to yield

among main stem and branches varied: the main stem raceme contributed 63-66%, primary branch racemes contributed 33-36%, and secondary branch racemes accounted for the remainder. Seeds on main stems were on average 2.4% heavier than seeds on primary branch racemes within N rates. Nevertheless, N rate differentially affected seed weight as main stem seed weight was increased by 11% with 168 and by 5% with 56 kg N ha^{-1} in 2013 and 2014, respectively. No effect of N fertilizer on primary branch seed weight was evident in 2013, but 168 and 224 kg N ha^{-1} increased seed weight in 2014.

Stand density, plant height, and above-ground biomass were determined at three developmental stages (stem elongation – BBCH 30, inflorescence emergence – BBCH 50, and harvest – BBCH 87) while leaf area index (LAI) and tissue N content were measured at BBCH 30 and BBCH 50. Applied N affected most dry matter partitioning and oil production characteristics with the exception of stand density, harvest index (HI), and seed protein concentration. Plant height, biomass, LAI, and tissue N concentration at BBCH 30 and 50 as well as crop growth rate (CGR) from BBCH 30 to 87 were related to the rate of applied N. Applied N increased plant height by 24 to 105%. Although lodging occurred in both years at the highest N rates, lodging did not negatively influence plant growth and seed yield development, nor total oil yield. As a measure of stand photosynthetic capacity, LAI was lowest across developmental stages with 0 kg N ha^{-1} in 2013, and in the 0 and 56 kg N ha^{-1} treatments in 2014. Increases in CGR by applied N ranged from 8 to 44% in 2013 and from 27 to 109% in 2014. Biomass N content at BBCH 30 was increased by all rates of applied N, but by BBCH 50, biomass N was only elevated over the control by rates ≥168 and ≥112 kg N ha^{-1} in 2013 and 2014, respectively. The rate of applied N incrementally decreased N use efficiency (NUE) with reductions ranging from 29% to 71% with only 56 kg N ha^{-1}. Seed oil concentration was generally inversely related to applied N rate; but highest oil yields were observed with 224 kg N ha^{-1}. Optimum yellow mustard plant growth, seed yield, and oil yield were found with 168-224 kg N ha^{-1} under WV conditions. The results of this study improves our understanding of yellow mustard production in response to applied N in a high rainfall environment.

Applied Nitrogen Effects on Yellow Mustard (*Sinapis alba* L.) Production in the Willamette Valley

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

Alyssa Susan DuVal, Author

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CHAPTER 1: Introduction and Literature Review

1.1. Introduction

Oregon's Willamette Valley is recognized for its vast agricultural diversity and productivity; however, in addition to environmental and economic constraints, new state governmental regulations severely limit available rotation cropping options. *Sinapis alba*, yellow or white mustard, is one oilseed crop that can assist in further diversification of Willamette Valley crop production, especially in non-irrigated fields. Although little research has been conducted on yellow mustard production in the Willamette Valley, studies completed in neighboring regions suggest that *S. alba* has the potential to emerge as a viable, competitive crop.

Agriculturalists are interested in incorporating oilseed crops into current Willamette Valley grass seed rotation practices because they provide an excellent opportunity to control common grassy weeds as well as insect and disease pests. Instead of rotating to another grass species (i.e. wheat/oats) or leaving the ground fallow, dryland farmers would have increased freedom in choosing a broadleaf crop suited for his/her available equipment and management practices. Moreover, there are numerous markets available for oilseed crops and their byproducts including biofuels, soil amendments, bio-pesticides, livestock feed, and human food products.

Despite the benefits associated with oilseed rotation cropping, some agriculturalists are reluctant to permit wide-scale *Brassica* crop production throughout the Willamette Valley. Such apprehension included potential increases in disease pressures, cross-pollination with high-valued specialty *Brassica* seed crops, as well as negative impacts on international trading. Allowing for further examination of this matter, the Oregon Department of Agriculture (ODA) issued an official administrative ruling in 2012 that established a canola (*Brassica napus*) protected district spanning ten counties, which is comprised of two zones, the exclusion and the allowable zones. *Brassica* species oilseed crops were prohibited from being grown in approximately 770,000 hectares of Oregon's Willamette Valley (which also comprises the majority of land dedicated to specialty seed production); however, of the remaining 690,000 hectares in the protected district, *Brassica* oilseed crops could have been produced on a maximum of 1,000 hectares, under strict regulation and observation. Although the ODA ruling appeared as a consensus between interest groups, the Oregon State legislature passed House Bill 2427 in August 2013, which banned all but 200 hectares of canola production within the Willamette Valley protected district cultivated for Oregon State University research purposes. Albeit yellow mustard resembles canola plants and is a member of the *Brassica* family, its taxonomic classification is in the *Sinapis* genus; thereby, under this law, agriculturalists throughout the entire Willamette Valley are able to produce yellow mustard as a multi-purpose, low-input oilseed crop.

Proper agronomic management practices are vital to obtaining optimal yields. Without production recommendations established for yellow mustard cultivated in Willamette Valley climatic conditions, these agriculturalists are limited by the lack of comprehensive documentation and guidance. For example, nitrogen fertilizer recommendations are currently available only for yellow mustard production in low- to moderate-precipitation zones in the Pacific Northwest. Farmers recognize the important relationship of proper fertilizer application rates to crop yield potential; however, high-precipitation environments add another element to consideration--sound ecosystem stewardship. Nitrogen fertilizers must be applied within a crop-specific ideal range in order promote sufficient vegetative plant growth, yet over-application might elicit stand lodging as well as nitrogen fertilizer leaching into groundwater systems.

Additionally, to date, no information has been published addressing the effect of nitrogen fertilizer on yellow mustard plant physiology—including leaf area and tissue N concentration at critical growth stages—or components of seed yield. It is well recognized that nitrogen is essential to plant growth and contributes to seed yield; however, the exact relationship of applied nitrogen fertilizers with growth and developmental capacity as well as optimal economic yield varies among plant species. To address these uncertainties, the effect of applied nitrogen fertilizer on yellow mustard seed yield, yield components, and oil concentration, as well as numerous physiological responses to nitrogen fertilizer, were evaluated on 'IdaGold' yellow mustard produced under non-irrigated Willamette Valley conditions.

1.2. Literature Review

1.2.1. Origin and Use

Mustard has been evident in human history for centuries, as marked by numerous references to mustard apparent in Christianity, Buddhism, and Islam. There are four different types of mustard—yellow/white (*Sinapis alba*), brown (*Brassica juncea*), Ethiopian (*B. carinata*), and black (*B. nigra*). Yellow mustard is likely native to the Mediterranean (Vaughan, 1977) and other European temperate regions (Western IPM, 2002; Oplinger et al., 1997; Wysocki and Corp, 2002), from where it spread and derived a number of commodities. Field crop production of *S. alba* expanded into North America prior to WWII, at which point California and Montana were major producers (Western IPM, 2002; Wysocki and Corp, 2002).

Although mustard greens were originally utilized as human and livestock feed, the ancient Greeks and Romans recognized its medicinal properties and developed mustard plasters and pastes (Western IPM, 2002; Oplinger et al., 1997) used to combat chest congestion (Scheindlin, 2004) and relive chest pains. Today, *S. alba* seeds are ground into the base for condiment mustard, dry mustard powder for seasonings and sauces (Western IPM, 2002; Oplinger et al., 1997; Vaughan, 1977), or mustard flour as a stabilizer in sausage production to inhibit E-coli growth. The oils extracted from seeds during cold pressing have also been recognized as having anti-microbial to sterilant-level properties (Ekanayake et al., 2012). Yellow mustard greens can also be incorporated into salads (USDA, 1997) or used as livestock fodder (Vaughan, 1977), as well as a moderate-biomass green manure crop (Krstić et al., 2010).

Aside from use as human and livestock feed, yellow mustard has a strong potential for utilization as a bio-pesticide. Sulfur compounds are released with the degradation of glucosinolate in yellow mustard, which can then be incorporated into alternative pest management systems targeting insects, nematodes, fungicides, and weedy plants. Yellow mustard extract helped promote the growth of rice paddies in India by acting as a fertilizer (Mishra and Sinha, 2010) and suppresses growth of competing organisms. *Sinapis alba* use as a biological control for herbicide-resistant plants is becoming increasingly recognized due to its rapid vegetative growth, glucosinolate concentrations, and competitive abilities. In fact, yellow mustard controlled weeds better than *B. juncea*, open pollinated canola (*B. napus*), and hybrid canola, due to *S. alba* early seedling emergence, rapid biomass accumulation, and efficient canopy closure. Yellow mustard consistently controlled broadleaf and grassy weeds to the point of containing less than half the weed population present in other mustard species (Beckie et al., 2008). *Sinapis alba* also has horticultural applications as demonstrated in Boydston et al. (2008), which illustrated how byproducts from mustard oil pressing applied at increasing rates were positively correlated with weed suppression in container ornamentals.

1.2.2. Plant Morphology and Physiology

Yellow mustard is an annual broadleaf crop with a strong, deep taproot (Oplinger et al., 1997; Wysocki and Corp, 2002) and pinnately compound leaves (Kozloff, 2005). *Sinapis alba* inflorescence is a raceme comprised of cruciferous flowers, each with four sepals, four white or yellow petals, and six stamens (Kozloff, 2005). The mature fruit is a silique that is 1.3-1.9 cm in length (Western IPM, 2002; Oplinger et al., 1997), is divided lengthwise into two compartments that split open at maturity, is covered with flattened hairs, and has a prominent, flattened beak at least one-quarter the length of the entire silique (Kozloff, 2005). Typically, only half of the flowers actually develop seed, with the flowers that pollinated within the first 15 days post-bud initiation yielding the majority of total seed production. Temperature during the bloom period is important because if conditions are too warm, yellow mustard flowers may be damaged or experience sudden death (Western IPM, 2002), thereby decreasing potential yield.

1.2.3. Genetics

The primary source of contention over large-scale *Brassica* oilseed production in Willamette Valley is the risk of cross-pollination within the Brassicaceae family and the subsequent influence on Oregon's high-value vegetable seed industry. *Sinapis alba* is a member of the Brassica family; however, it is a distant relative of other cultivated members of the family (Hawkins et al., 1996). Figure 1.1 depicts the phylogenetic classification of yellow mustard, brown mustard (*B. juncea)*, canola and rape (*B*. *napus*), field mustard (*B. rapa*), and cabbage (*B*. *oleracea*) (NRCS, 2012). Vaughan (1977) organized the results of previous, multi-disciplinary studies concerning species relatedness within the Brassicaceae family, into a single comprehensive document. Isoenzyme analysis revealed that *S. alba* did not have β-glucosidases in common with any *Brassica* taxa, gel electrophoresis placed *S. alba* an entire locus away from Brassicas, and serological seed protein results showed that yellow mustard is more closely related to black mustard (*B. nigra*) than any other *Brassica* species (Vaughan, 1977). The findings reported by Hawkins et al. (1996) support previous genetic studies comparing *S. alba* with other Brassicaceae family members, recognizing that *S. alba* analysis revealed a unique southern blot analysis and amplified the BN28 gene at the same base pair length as black mustard.

Yellow mustard is a cross-pollinating species (Smartt and Simmonds, 1995) that maintains a strong sporophytic self-incompatibility mechanism (Olsson, 1960). Although yellow mustard is a wind-pollinated plant, agriculturalists should not be concerned with the potential for *S. alba* to cross-pollinate with *B. oleracea* and *B. rapa* vegetables (Brown et al., 2005; Quinn, 2010). Lelivelt et al. (1993) attempted to transfer genetic resistance to the beet cyst nematode from yellow mustard, 2n=24, to *B. napus*, 2n=38, via somatic and sexual hybridization; yet, found it impossible to obtain successful reproductive hybrid offspring that produced adequate pollen count, seed quality, and seed quantity, without utilizing embryo rescue as somatic hybrids were mitotically unstable and sterile.

Further efforts at intergeneric hybridization between *S. alba* and *B. napus* were conducted by Brown et al. (1997) at the University of Idaho, where they conducted emasculation, hand pollination, ovary excision, and embryo rescue. Plantlets were generated after several cycles; however, the hybrids were sterile. Efficiency in developing fertile *S. alba* X *B. napus* hybrids is very low considering that Brown et al. (1997) only obtained two fertile hybrids out of a possible 2,200 bud pollinations. Producing intergeneric hybrids of yellow mustard and *Brassica* species would be beneficial for transferring pest tolerance or resistance to Brassicas, as well as potential cold tolerance to *Sinapis*; however, these endeavors have proven difficult and inconsistent.

1.2.4. Tissue Culture

In contrast to many of the *Brassica* species, *S. alba* has been historically recognized for its "low genetic predisposition for in vitro culture" (Klóska et al., 2012). Yet, regardless of the strong genotypic response to tissue culture (Murata and Orton, 1987), researchers have successfully micropropagated many yellow mustard cultivars through organogenesis, embryogenesis, and protoplasts. Yellow mustard enzymatically isolated root protoplasts were capable of forming 1-2mm calli at slow rates but could not differentiate to develop plantlets (Xu et al., 1982). Tissue culture of *S. alba* cultivar 'Arda' cotyledons and anthers illustrated that greater plantlet survival rates were obtained from cotyledon-derived plantlets (90%) than from anther-derived plantlets

(20% at maximum); yet, even if these plants reached reproductive maturity, they all were sterile (Jain et al., 1989).

Yellow mustard cultivars historically contain high levels of glucosinolates, especially sinalbin, in the meal and high erucic acid in seeds and oil; yet, plant breeders have developed new varieties with low glucosinolates and low erucic acid. Klóska et al. (2012) determined that both low and high concentration cultivars maintained similar shoot regeneration aptitude. Interestingly, all tissues of the high erucic acid content cultivar generated significantly more shoots than tissues of the low erucic acid cultivar. Regenerated plantlets were then transplanted into soil media and acclimated in growth rooms, after which the plants were moved to greenhouses and allowed to reach reproductive maturity. Although only a few seeds were harvested from the tissue-culture generated plants, the protocols developed "allow for the generation of sufficient plant material, in a short period of time, for direct use in [yellow] mustard breeding projects" (Klóska et al., 2012).

Tissue culture in yellow mustard has proven invaluable, as stated by Zarychta and Zenkteler (2010), "because of the [increasing] demand for such [improved *S. alba*] varieties and the very slow traditional method of propagation, it is important to develop an efficient micropropagation technique to rapidly disseminate clones once they are identified." Researchers are also able to generate doubled haploids via anther culture as well as implement embryo rescue and protoplast fusion techniques for intergeneric hybridization. Through these in vitro practices, researchers and plant breeders will be able to develop improved yellow mustard cultivars and potentially develop reliable, specialized markets for *S. alba* byproducts.

1.2.5. Agricultural Ecology

Spring planting of yellow mustard is essential, as it is a cool-season crop with a growing season of only 80-85 days (Oplinger et al., 1997; Wysocki and Corp, 2002). Fall plantings of mustard do not cope well with winter environmental conditions, often optimal harvest conditions are not available at plant maturity and insect pollinator activity is not adequate during the early bloom stage (Duncan, 1965). Yellow mustard seeds have the potential to germinate when soil temperatures are 4.4°C (Western IPM, 2002; Oplinger et al., 1997; Wysocki and Corp, 2002). Since seedlings cannot tolerate frosts below -3.3°C, *S. alba* must be planted as early as possible in the spring but late enough to avoid severe freezing (Wysocki and Corp, 2002). Waterlogged soils will result in stunted plant growth (Oplinger et al., 1997) and reduced seedling emergence. Duncan (1965) suggests planting mustard in March to early April under dryland conditions but waiting until mid-May for irrigated fields in the Willamette Valley of Oregon. Contrary to these historical recommendations, preliminary mustard studies conducted by Oregon State University from 2006-2008 illustrated that yields were significantly greater for *S. alba* planted from mid-February to early-March, 1718 kg ha^{-1} and 1922 kg ha^{-1}, respectively, compared to those obtained from April planting dates ranging from 84-110 kg ha^{-1} (Chastain, unpublished data).

Ideal soils for mustard production should be well drained and medium in texture (Western IPM, 2002; Oplinger et al., 1997; Wysocki and Corp, 2002) with a neutral pH (Western IPM, 2002; Oplinger et al., 1997), but *S. alba* is adaptable to a variety of soil types as long as soil water is adequate throughout the growing season. Yellow mustard can be planted under conventional tillage or direct seeding systems. If conventional tillage is preferred, *S. alba* should be sown into firm, moist, clean seedbeds at a depth of 1.3-2.5 cm (Brown et al., 2005; Western IPM, 2002; Oplinger et al., 1997; Wysocki and Corp, 2002) and a rate of 9-13 kg ha^{-1} (Western IPM, 2002; Davis et al., 2010; Wysocki and Corp, 2002). Brown et al. (2005) diverged from the typical seeding rate recommendations and noted that planting at 8-9 kg ha^{-1} establishes adequate stands and sufficient yields. Planting should not occur at rates greater than 11.2 kg ha^{-1} due to an increased tendency for mustard plants to compete with one another. If minimum or no-till production is desired, planting at 9-10 kg ha^{-1} (Brown et al., 2005) and no more than 2.5 cm in depth (Wysocki and Corp, 2002) should provide optimal productivity.

Stand density is another critical element of *S. alba* production as it influences all yield components, especially primary and secondary racemes $plant^{-1}$ and seed weight. Yellow mustard in the Pacific Northwest is planted with 15 to 20 cm row spacing as per University of Idaho recommendations (Brown et al., 2005; Wysocki and Corp, 2002). Hassan and Arif (2012) analyzed the effects of nine different plant-to-plant spacing in rows and concluded that 15 cm is the optimal plant spacing for yellow mustard in dryland conditions, observing an inverse relationship between plant spacing and overall seed yield. Their study also revealed that plant spacing had significant impacts on the number of primary racemes produced plant^{-1} as well as the number of seeds silique^{-1}.

Seedlings emerge roughly 5-10 days post-planting, given adequate temperature and moisture conditions, with canopy closure occurring after four weeks at a cultivar- and condition-specific height ranging from 76 to 114 cm in mid-western states (Oplinger et al., 1997) or 107 to 127 cm in the Pacific Northwest (Davis et al., 2010). Flower buds emerge one week later with flower initiation following in 5-10 days. Seed yield potential was linearly correlated with flowering duration, which was dependent upon water availability (Western IPM, 2002; Oplinger et al., 1997) but, according to Wysocki and Corp (2002), ideally spans a minimum of 15 days.

When the crop reaches full maturity, it can either be harvested by swathing when the majority of seeds turn yellowish-green and followed by combining when seed moisture is lower than 10% (Western IPM, 2002; Oplinger et al., 1997), or direct-combined at low seed moisture as a result of high seed shatter resistance (Brown et al., 2005). Seed yield in yellow mustard is highly dependent upon water availability and summer temperature conditions, especially because high temperatures can lead to flower and/or pod abortion if experienced at sensitive plant growth periods (Western IPM, 2002). Yellow mustard can out-yield spring canola in low rainfall areas, producing 55% more yield when annual precipitation is less than 305 mm (Oplinger et al., 1997; Wysocki and Corp, 2002). The national average for yellow mustard seed yield ranges from 647 to 1,111 kg ha^{-1} (NASS, 2015).

1.2.6. Nutrient Management

Nitrogen fertilizer recommendations are currently not available for yellow mustard production in the high rainfall (≥1000 mm) areas of the Willamette Valley. The University of Idaho recommends a minimum of 14 kg ha^{-1} of available N in areas with annual rainfall between 480-560 mm be provided for each 0.5 kg of expected seed yield, 13 kg ha^{-1} for every 45 kg of expected yield in areas receiving 360-460 mm, and 12 kg ha^{-1} for every 45 kg of yield in regions with ≤ 330 mm annual precipitation (Brown et al., 2005). In terms of applied N fertilizer, Oplinger et al. (1997) suggests that 112 kg N ha^{-1} will provide optimum yields, with limited plant lodging (Rakow et al., 2009). One consideration of yellow mustard is that it has the capability to extract over half of its water requirements from below 1.5 m in depth, thereby allowing it to utilize leached nitrates existing at greater depth in the soil profile (Brown et al., 2005; Hassan and Arif, 2012).

Phosphorus deficiencies are rare in Willamette Valley soils and are not likely of concern for yellow mustard production. Applications are required at a rate of 45-67 kg P_2O_5 ha^{-1} only when soils have less than 4 ppm phosphorus content (Brown et al., 2005); however, Wysocki and Corp (2002) acknowledged that an application of 22.4 kg P_2O_5 ha^{-1}, similar to the recommendations for cereals, is usually adequate. Boron applications of roughly 1.1 kg ha^{-1} for deficient soils should never be banded but instead broadcast prior to or simultaneously with planting (Brown et al., 2005; Oplinger et al., 1997).

Yellow mustard requires sulfur in order to produce ample seed glucosinolates and proteins. Applying 40 kg S ha^{-1} had the greatest influence on most seed yield components in yellow mustard, especially seed weight (Barczak et al., 2011). Brown et al. (2005) recommends that growers in the Pacific Northwest apply sulfur when soils are considered deficient at 16.8-22.4 kg S ha^{-1}.

1.2.7. Pest Management

One of the many benefits of producing yellow mustard is its low-input pest management practices. Being a competitive broadleaf crop, weed management in yellow mustard is relatively simple and infrequently needed (Brown et al., 2005). Fields can be kept clean prior to planting via broad-spectrum herbicide applications, and post-emergence weeds are controlled with selective herbicides in the spring.

Yellow mustard is tolerant or resistant to many common insects including cabbage seedpod weevil and cabbage aphid (Brown, 1998; Brown et al., 2005) but can be damaged by flea beetles and diamondback moth larvae if feeding on young yellow mustard seedlings or cotyledons (Western IPM, 2002; Oplinger et al., 1997; Wysocki and Corp, 2002). Brown et al. (2005) reported that plots without insect control produced the greatest seed yield even with minute quantities of insect damage.

Plant diseases affecting yellow mustard include downy mildew, white rust (Rakow et al., 2009), and leaf mosaic virus (Western IPM, 2002); however, fungicides are rarely required since yellow mustard is sufficiently resistant to Sclerotinia stem rot, white mold (Brown et al., 2005), and blackleg (Rakow et al., 2009). To prevent potential soil-borne disease problems, mustard should be produced in a four-year rotation and not in close proximity to other members of the Brassica family (Western IPM, 2002; Oplinger et al., 1997). Including rotations with small grain crops is highly recommended as these species do not share most diseases and can be easily managed (Wysocki and Corp, 2002; Brown, 1998).

1.2.8. Leaf Area Index

Leaf area index is an estimate of a crop's photosynthetic capacity and photoassimilate production potential. Crop management practices aim to efficiently produce the optimum leaf area for a given crop and environment. Leaf area is reported primarily as leaf area index (LAI), the ratio of the measured leaf area to ground area, and estimates solar radiation interception (Cheema et al., 2001). The optimal LAI varies with crop species but is typically approximately 3.0 for perennial ryegrass (*Lolium perenne*) (Simon and Lemaire, 1987), 3.11 for Indian mustard (*B. juncea*) (Kumar et al., 1997), 3.5 for red clover (*Trifolium pratense*) (Joggi et al., 1983), 4.0 for sugar beets (*Beta vulgaris*) (Watson, 1947), and 6.0 for winter oilseed rape (*B. napus*) (Hunková et al., 2009).

A number of environmental and agronomic factors are directly connected with optimizing leaf area indices and promoting crop yield; in fact, Watson (1947) illustrated that LAI as well as its integral over time, or leaf area duration, significantly contribute to dry matter accumulation, which in turn relate to ascertaining agronomic treatment effects on seed yields. The ability of nitrogen fertilizers to improve LAI across crop species has been of scientific interest for decades. Increases in LAI in maize (Wolfe et al., 1988) and oilseed rape (Scott et al., 1973) are strongly correlated with increases in applied N fertilizers. Allen and Morgan (1972) observed a significant relationship of increased N fertilizer rates with improved oilseed rape LAI, lasting roughly from six to ten weeks post-planting and reaching peak LAI at the inflorescence emergence growth stage. Additionally, Cheema et al. (2001) also demonstrated that high N application rates increased canola leaf area development, post-anthesis photosynthetic duration, as well as total crop assimilation, resulting in improved overall seed yield. While there is an absence of literature regarding LAI in yellow mustard, it can be anticipated that N fertilization would have a similar influence on yellow mustard leaf area as in related oilseed crops.

1.2.9. Biomass Accumulation

Nitrogen fertilizers consistently increase above-ground biomass (Allen and Morgan, 1972; Scott et al., 1973; Asare and Scarisbrick, 1995; Cheema et al., 2001). Allen and Morgan (1972) showed that increases in applied N rates produced oilseed rape plants with increases in growth rate, plant height, and dry matter accumulation. Rathke et al. (2005) supported these findings by attributing optimal *B. napus* seed yield to balancing seed protein and oil concentrations with 1) adequate CO_2 fixation and energy production necessary for plant vegetative growth, biomass accumulation, and subsequent reproductive development, as well as 2) proper agronomic nitrogen fertilizer management.

Since N is a very mobile essential nutrient, applications of N can be assimilated, readily moved to vegetative tissues, and afterwards remobilized to seeds. Nitrogen fertilizer applications increase N concentration in *B. napus* leaves and seeds (Bilsborrow et al., 1993) as well as *S. alba* stems at harvest. Comparatively speaking though, Kovács et al. (2009) determined that yellow mustard seed N and S concentrations were greater than aboveground vegetative biomass at harvest, which can be anticipated due to translocation from vegetative to reproductive organs. Higher seed N and S content is correlated with increased seed protein content.

Plant growth and productivity can also be analyzed in terms of harvest index (HI), which is the ratio of harvested seed weight to total aboveground biomass weight. Harvest index varies with management practices and climatic conditions but typically ranges between 0.25 and 0.50 for oilseed rape, meaning that seeds comprise 25-50% of total aboveground plant biomass (Rathke et al., 2005), 0.14-0.17 for canola produced in Pakistan (Cheema et al., 2001), and 0.14-0.15 for brown mustard (*B. juncea*) (Kumar et al., 1997). Harvest index is an indicator of efficient photoassimilate partitioning in plant organs: HI rises with N application up to an asymptotic value and declines with over-applications of N (Cheema et al., 2001; Rathke et al., 2005).

1.2.10. Components of Seed Yield

In the Brassica family, the following constitute seed yield components: plant density, raceme branching structure, quantity of siliques borne on each raceme, silique fertility (the number of seeds produced silique^{-1}), and seed weight. These components have the capacity to compensate for adverse conditions or losses from disease or predation in a variety of ways. For example, a reduction in the number of racemes produced plant^{-1} can be offset with increases in the quantity of siliques on each raceme. The number of siliques plant^{-1} is ultimately determined by several factors including the number of buds, racemes, and flowers produced, the capacity of the source, the supply of nutrients and water, the influence of hormonal factors, and the potential for the silique to be set given the adequacy of the previously listed factors (Diepenbrock, 2000).

In a comparison of characteristics among 11 Brassicaceae species, including *S. alba*, grown under the same management and environmental conditions, it was evident that yellow mustard had the fewest primary racemes, the greatest number of secondary racemes, the lowest amount of seeds silique^{-1}, and moderately-low seed weight as compared to other members of the Brassica family (Kumari et al., 2010). Yet, similar to oilseed rape (*B. napus*), yellow mustard seed yield was influenced significantly by planting density and silique production (Diepenbrock, 2000), and a lesser extent by seed weight (Barczak et al., 2011). Zając et al. (2011) found that silique production on yellow mustard primary and secondary racemes combined for a 1:1 ratio with the quantity of main stem siliques. Additionally, seeds borne on moderate-sized yellow mustard siliques accounted for 78.7% of the total plant yield.

Nitrogen fertilizers can have a significant influence on seed yield as well as seed yield components as illustrated with *B. napus*. Applications of N triggered an increase in raceme quantity, silique production, and subsequent total seeds, while not statistically influencing seeds produced pod^{-1}, average seed weight, nor the weight of individual siliques (Allen and Morgan, 1972; Bilsborrow et al., 1993). Asare and Scarisbrick (1995) attributed seed yield increases of oilseed rape primarily to terminal racemes producing the greatest quantity of siliques plant^{-1} and secondly to increased seed weight, yet agreed that applied N did not influence the number of seeds pod^{-1}. Applications of N also increased seed yield of individual *S. alba* plants from 8.7 g seed pot-^{1} with 0.5 g N pot^{-1} to 24.8 g seed pot^{-1} from an application of 2 g N pot^{-1} in a study conducted by Kovács et al. (2009).

1.2.11. Seed Oil and Protein

Oilseed crops, especially those with inherently high seed oil content, are primarily produced with the end goal of maximizing oil yield, while others balance the importance of oil quantity and quality as well as seed protein content. Erucic acid, a monounsaturated fatty acid (Salunkhe and Desai, 1986; FSANZ, 2003), and glucosinolates, sulfur-containing secondary plant products (Mullin and Sahasrabudhe, 1977; Halkier and Du, 1997; Du and Halkier, 1998; Fahey et al., 2001), are common characteristics of *Brassicaceae* family seeds and vegetative tissues, respectively. Depending on quality, extracted oils can be utilized as cooking/edible oil, an ingredient in common products (margarine, condiments, soaps/detergents, cosmetics, etc.), industrial fuel and lubricants, or as a biofuel source. A strong market also exists for seed meals obtained as a byproduct of oil extraction processes since these meals are high in proteins; however, residual concentrations of glucosinolates are of concern to nutritionists (Asare and Scarisbrick, 1995). Seed meals low in glucosinolate content are often utilized in livestock feed rations, whereas meals high in glucosinolates can be applied to soil as bio-pesticide agents.

Brassica napus is one such crop that produces high seed oil concentrations, averaging between 43 and 48%, as well as maintains a strong presence in many livestock ration formulations due to its high protein content and low glucosinolate levels (Cheema et al., 2001; Rathke et al., 2005). Conversely, yellow mustard seeds and vegetation have high glucosinolate content, which thereby limits its utilization in monogastric and ruminant livestock feeds, while containing an average of 23-28% seed oil content (Kovács et al., 2009).

A consistent negative correlation of seed oil and seed protein concentrations has been extensively documented (Röbbelen et al., 1989; Asare and Scarisbrick, 1995; Rathke et al., 2005) and is appropriate considering differences in fatty acid and amino acid biosynthesis from carbohydrates. Rathke et al. (2005) explained that since the carbohydrate content of proteins is lower than that of oils, increased N supply intensifies the synthesis of proteins at the expense of fatty acid synthesis and thus, reduces the oil content for the seed. Nitrogen fertilizer applications have been shown to increase seed protein concentration in oilseed rape (Bilsborrow et al., 1993) and yellow mustard (Kovács et al., 2006), elevate *B. napus* seed glucosinolate content (Bilsborrow et al., 1993; Asare and Scarisbrick, 1995), as well as improve the quantities of many essential and nonessential amino acids in *S. alba* (Kovács et al., 2009).

Although higher N fertilizer rates decrease individual seed oil content compared to no applied N, the overall oil yield is greater due to increased seed yields.

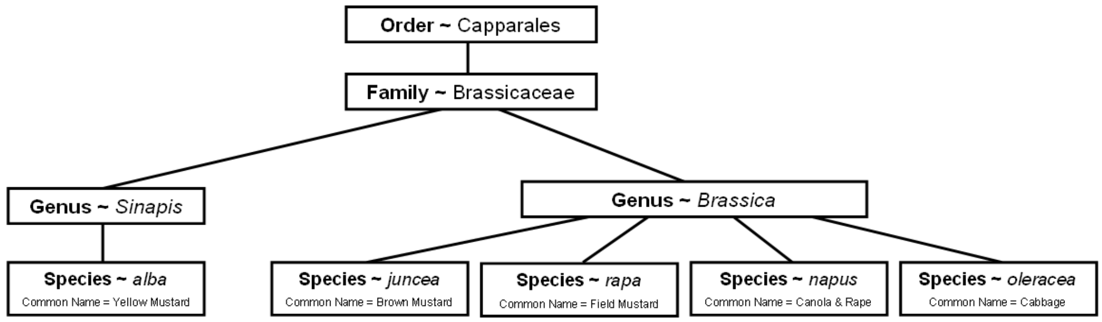

Figure 1.1. Comparison of phylogenetic classification of five members of the Brassicaceae family.

CHAPTER 2: Applied nitrogen effects on seed yield and yield components in yellow mustard (*Sinapis alba* L.).

ABSTRACT

Nitrogen management recommendations for yellow mustard (*Sinapis alba* L.) production in the high rainfall environment of Oregon's Willamette Valley (WV) are not available. The objective of this study was to determine the effect of applied nitrogen (N) on seed yield and yield components in "IdaGold" yellow mustard in the WV. Field trials were conducted over a two-year period at Corvallis, Oregon, USA with five N application rates: 0, 56, 112, 168, and 224 kg N ha^{-1}. Although rainfall differed significantly between the two years, the effect of applied N on seed yield and yield components were similar. Seed yield was increased by 15%-85% with applied N, primarily as a result of N-induced increases in seed weight and number. Yields ranged from 1,080 kg ha^{-1} with 0 kg N ha^{-1} to 2,580 kg ha^{-1} with 224 kg N ha^{-1}. Primary racemes $plant^{-1}$ were positively associated with increased N; however, secondary racemes $plant^{-1}$ were not consistently related to N. Siliques $plant^{-1}$ increased in proportion to N rate and was the result of increased siliques on main stem and primary racemes. Seed yield contributions varied among the main stem and branches: the main stem raceme contributed 63-66%, primary branch racemes contributed 33-36%, and secondary branch racemes accounted for the remaining few percent. Seeds on main stems were 1.7% - 3.3% heavier than seeds on primary raceme within N rates. Nevertheless, N rate differentially affected seed weight as main stem seed weight was increased with 112 kg

N ha^{-1} in 2013 and 56 kg N ha^{-1} in 2014. No effect of N fertilizer on primary branch seed weight was evident in 2013, but 168 and 224 kg N ha^{-1} increased seed weight in 2014. This work will serve as the basis of nitrogen management recommendations for yellow mustard in Oregon's WV.

Abbreviations: BBCH, Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie; Willamette Valley, WV

Keywords: seed yield, seed weight, seed number, racemes, siliques

2.1. Introduction

Mustard seed production has a long history and is an important crop in many countries, including Canada, Denmark, Nepal, Russia, and the United States. According to FAO statistics, Canada is the world's leading mustard producer, having produced over 154,000 tonnes of seed in 2013 (FAO, 2014). Agriculturalists recognize the tremendous versatility that yellow mustard can offer in terms of cropping system and management operations as well as marketability.

The United States produced 16,659 tonnes of total mustard—including both *Brassica juncea* and *S. alba*—in 2013, at an average yield of roughly 1 tonne ha^{-1} (NASS, 2015). Yellow mustard seed yields range from 896-1,680 kg ha^{-1} (Oplinger et al., 1997; Wysocki and Corp, 2002). However, the cultivar 'IdaGold' typically yields a national average of 1,482 kg ha^{-1} (University of Idaho, 2004) but has been shown to attain yields upwards of 2,392 kg ha^{-1} and 2,224 kg ha^{-1} at higher rainfall locations in the Pacific Northwest, USA (Davis et al., 2010).

Although not considered a major national producer of mustard, Oregon—specifically the north central counties—produced a total of 907 tonnes of mustard seed in 2011 (OAIN, 2012). Given recent Oregon State legislative actions in prohibiting the production of *Brassica* genus oilseed crops in the WV, yellow mustard presents an intriguing alternative. Stemming from this newly realized potential, comes the need for recommendations specific to yellow mustard production under WV climatic conditions, especially regarding optimal N fertilizer application rates.

A number of factors including plant density, environmental conditions, plant reproductive capacity, and harvest efficiency influence potential seed yield, which can be determined through an analysis of seed yield components. Typically, seed yield components and their relation to seed yield in Brassica family crops are expressed in the following manner (Allen and Morgan, 1972; Zając et al., 2011):

$$Seed\ Yield\ = \frac{Plants}{Unit\ Area} \times \frac{Branches}{Plant} \times \frac{Siliques}{Branch} \times \frac{Seeds}{Silique} \times \frac{Weight}{Seed}$$

To gain a deeper understanding of these seed yield components, plant measurements can be divided further according to raceme branching structure (i.e. main stem and primary/secondary/tertiary racemes). Asare and Scarisbrick (1995) determined that *B. napus* silique production on the main stem raceme was greater than other racemes, and that the quantity of siliques borne on each raceme tended to decrease from the uppermost to the lowermost racemes within the canopy. In a study of comparative morphology in winter canola and yellow mustard, Zając et al. (2011) observed that differences in raceme branching structure and raceme length between the two species significantly influenced the quantity of seeds produced. *Brassica napus* produced heavier seeds on both main stem and primary racemes compared to *S. alba*; however, keeping in mind that winter canola received twice the amount of N fertilizer versus yellow mustard, such yield differences might be anticipated.

McKenzie et al. (2006) and Kovács et al. (2009) showed that application of N in yellow mustard increased seed yield. Although the influence of applied N on yellow mustard seed yield components have not been previously studied, N generally increases plant photosynthetic capacity (Diepenbrock, 2000; Rathke et al., 2006) thereby providing for greater resource allocation to seed production. Nitrogen fertilizers have been shown to increase *B. napus* raceme and total silique production (Allen and Morgan, 1972; Bilsborrow et al., 1993; Cheema et al., 2001), as well as seed weight (Asare and Scarisbrick, 1995) and overall seed yield. The quantity of canola seed produced silique^{-1} in response to applied N has been inconsistent.

Yellow mustard and canola seed yields are highly dependent upon water availability and summer temperature conditions. High temperatures and drought conditions can lead to flower and/or pod abortion (Western IPM, 2002) if experienced at sensitive plant growth periods as well as hastened maturation (Angelini et al., 1997), as is also the case for many other crops including camelina (*Camelina sativa*), and soybeans (*Glycine max*) (Angelini et al., 1997; Liu et al., 2003). The unpredictability of precipitation during the late winter and spring months in the WV influence timing of field operations and subsequent plant growth and development. Drought conditions can be mitigated in some areas by using supplemental irrigation, which is also beneficial to improve the growth of late-planted mustard; yet is not available in many locations throughout the WV.

Considering the immediate need for nitrogen management recommendations for yellow mustard in the WV, the objectives of this study were to (i) determine the effects of applied N on yellow mustard seed yield, and (ii) ascertain the influence of N on the components of seed yield in yellow mustard.

2.2. Materials and Methods

2.2.1. Overview

Field trials were conducted during two years (2013 and 2014) at Oregon State University's Hyslop Crop Science Research Farm (44° 38' 17.6" N, 123° 11' 44.1" W) near Corvallis, Oregon, to characterize the effects of nitrogen on seed yield and seed yield components in yellow mustard. The experimental design was a randomized complete block design with four replications and five N fertilizer treatments. For ease of sampling and to minimize border effects, plots were designed to be 4.9 m x 15 m. Half of each plot (2.4 m X 15 m) was dedicated for collecting in-season vegetative measurements and yield component samples, while the other half (2.4 m X 15 m) was reserved for harvest and seed yield analyses. The soil at the site is a Woodburn silt loam (fine-silty, mixed, superactive, mesic Aquultic Argixeroll). The cultivar 'IdaGold' was utilized in this study because it is the most widely-grown open-pollinated cultivar of yellow mustard in the Pacific Northwest and is resistant to lodging, seed shatter losses, and a number of insect pests and diseases.

To ensure that the planting site was not deficient in nutrients, soil analysis was conducted according to standardized practices at the Central Analytical Laboratory for a depth of 0-20 cm. The pre-plant soil available N was 19.2 and 64.6 kg N ha^{-1} in 2013 and 2014, respectively. Soil pH was typical for the cropping region and was acceptable (5.9 in 2013 and 5.6 in 2014) for yellow mustard production. A broadcast application of 112 kg ha^{-1} potassium magnesium sulfate (0-0-22-11-22) was made prior to planting in each year to remove sulfur deficiency as a potential confounding variable. Trifluralin (α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) was applied at 5.6 kg ha^{-1} three weeks before planting to control weeds during establishment and early canopy development. No additional herbicides were applied.

A double-disk drill was utilized for planting yellow mustard at 20 kg seed ha^{-1} in a 15 cm row spacing on 11 March 2013 and 13 March 2014. An application of sodium borate foliar spray was made at 1.96 kg B ha^{-1} four days post-plant in 2013 and 26 days after planting in 2014. Post-plant pesticides were not applied in either growing season as insect and disease incidence and severity did not reach action thresholds.

Nitrogen was applied as dry granular urea (46-0-0) on 4 April 2013 and 18 April 2014 by using a tractor-mounted Gandy orbit-air fertilizer spreader. Fertilizer application dates coincided with appearance of the first few true leaves (BBCH stages 11-13). Application rates were 0, 56, 112, 168, and 224 kg N ha^{-1}. Plant growth and development for each N treatment were tracked weekly with photographic records commencing 36 days post-plant (14 days following N treatment applications) and 39 days post-plant (3 days after treatment application) in 2013 and 2014, respectively.

2.2.2. Seed Yield Components and Yield

Yellow mustard reached BBCH stage 87 (70% of siliques ripe) roughly 1,734 (9 July 2013) and 1,703 (7 July 2014) growing degree days after 1 March with base temperature = 0° C. At this time, two adjacent 0.1 m^2 quadrat aboveground biomass samples were obtained from each plot. These samples were first weighed and subsequently dried in an air-forced chamber at 70°C for 48 hours, following which plant biomass and stand density were determined. Additionally, ten random, yet representative plants were subsampled from each quadrat observation and utilized to ascertain plant height and components of seed yield (racemes plant^{-1}, siliques plant^{-1}, seeds silique^{-1}, and seed weight per raceme type).

Plots were swathed with a modified John Deere windrower and left to dry for seven to twelve days. The center 1.8 m x 15m swath from each plot was then harvested and bagged with a plot combine on 23 July 2013 and 28 July 2014. The seed obtained was cleaned with a Clipper model M2B and used to calculate total seed yield, determine seed weight, and as a source to measure seed number m^{-2}. Two 1,000 seed samples from each plot were counted by an electric seed counter (The Old Mill Company, Savage, MD) and weighed. Seed number m^{-2} was calculated for each plot by dividing the clean seed yield harvested from that plot by the individual seed weight.

2.2.3. Statistical Analysis

Statistical analyses were conducted with SAS 9.9 by using PROC GLM. Analysis of variance (ANOVA) was conducted on plot means obtained for all data to allow for interpretation without considering subsampling. Bartlett's χ^2 tests revealed that error variances were not homogenous across years; therefore, each year's results were analyzed separately. Treatment means were separated by Fisher's protected LSD values at the 5% level of significance. Regression analysis was used to elucidate the relationship between seed yield components and seed yield.

2.3. Results and Discussion

2.3.1. Growing Season Environment

Precipitation during the growing season (March to July) for yellow mustard was 159 mm greater in 2014 than in 2013 (Figure 2.1). The total rainfall for the growing season was 96 mm higher than the long-term mean in 2014 and in 2013, total rainfall for the growing season was 63 mm less than the long-term mean. Moreover, the winter months preceding planting in March were much wetter in 2014 than in 2013. Conditions in 2013 were ideal for field preparations and seed germination in contrast to the unusually high rainfall in February and early March 2014, which combined with site soil characteristics to produce saturated soils prior to planting. Mid-season precipitation in May 2013 prompted a surge in yellow mustard vegetative growth, especially for plants in the control plots, which subsequently experienced significant growth and development gains. Contrarily, vegetative accumulation was severely downgraded in 2014 when precipitation amounts decreased from April to May.

Similar temperatures were experienced during both growing seasons; however, additional growing degree days accumulated in March 2013 compared to March 2014 (Figure 2.2) may have contributed to subsequent plant growth and development variations as well as the plant's ability to compensate for latter environmental conditions. Increased presence of fasciation was observed in the 2014 season (10% incidence) compared to 2013 (3% incidence), but did not appear to negatively influence plant growth, development, nor seed yield in either year.

2.3.2. Seed Yield, Seed Weight, and Seed Number

The analysis of variance revealed that seed yield, seed weight, and seed number were consistently affected by applied N in both years (Table 2.1). Applied N increased seed yield in yellow mustard in a similar manner in each year and yield was incrementally increased with increasing rate of applied N (Table 2.2). Kovács et al. (2009) found that applied N increased seed yield of yellow mustard under greenhouse conditions but no other studies have reported the effect of applied N on seed yield of yellow mustard under field conditions. All applied N rates significantly increased seed yield over the 0 kg N ha^{-1} control in both years. The 56 kg N ha^{-1} rate increased seed yield by 15% in 2013 and by 32% increase in 2014. Applications of 224 kg N ha^{-1} resulted in 68% and 85% greater seed yield than the 0 kg N ha^{-1} control in 2013 and 2014, respectively. Although precipitation during the growing season was markedly different between the two years, yellow mustard seed yield responses to applied N were somewhat similar. The crop did not apparently benefit from the high precipitation in 2014 as seed yields were lower in 2014 than in 2013 when precipitation was lower than average for the region.

A linear relationship between rate of applied N and seed yield in yellow mustard was found in both years despite differences in growing season precipitation (Figure 2.3). Average yellow mustard seed yields were roughly 500 kg ha^{-1} less in 2014 than 2013, with the exception of the 112 and 168 kg N ha^{-1} rate treatments. Bilsborrow et al.

(1993) observed that 85% of maximum winter canola (*B. napus*) seed yield was attained by applying 150 kg N ha^{-1} and the maximum yield was reached with 250 kg N ha^{-1}.

Seed weight was increased by applied N with the 224 kg N ha^{-1} rate in 2013 and by rates ≥ 168 kg N ha^{-1} in 2014 (Table 2.2). This result is in contrast to the findings of Allen and Morgan (1972) and Bilsborrow et al. (1993) in oilseed rape where applied N had no effect on seed weight. Asare and Scarisbrick (1995) found significant differences in seed weight due to applied N in half of their oilseed rape experiments, suggesting that a longer photosynthetic duration allowed the weight differences to manifest, which may also be true for the current mustard experiment.

Seed number m^{-2} in yellow mustard was increased with all rates of applied N in both years (Table 2.2). The greatest number of seeds m^{-2} were observed with 224 kg N ha^{-1} in 2013 and with rates ≥ 112 kg N ha^{-1} in 2014. Applied N also increased seeds m^{-2} in winter oilseed rape (Wang et al., 2014) and in winter canola (Ferguson, 2015). Seed yield in yellow mustard was most strongly affected by seeds m^{-2} rather than seed weight because variation in seed weight across applied N rates was much less than observed for seeds m^{-2} (Table 2.2). As a result, there was a strong linear relationship between seeds m^{-2} and seed yield in yellow mustard evident in this study (Figure 2.4). A similar linear relationship between seeds m^{-2} and seed yield was observed in winter canola (Ferguson, 2015). Although seed yield was lower in 2014, the total quantity of seeds produced in the 224 kg N ha^{-1} treatment far surpassed all other treatments, being 27% greater than the 168 kg N ha^{-1} treatment and 172% greater than the control. Nevertheless, seed weight was somewhat greater in 2013 than in 2014, thereby making a contribution to the greater seed yield observed in 2013.

2.3.3. Components of Seed Yield

All seed yield components measured in yellow mustard were affected by applied N in both years with the exceptions of seed weight on 1° branches in 2013 and seeds silique^{-1} on 1° branches in 2014 (Table 2.1). Each main stem produced one raceme regardless of N rate (data not shown). The number of racemes plant^{-1} was increased by applied N (Tables 2.3 and 2.4). The number of 1° branch racemes was increased by applied N with the 224 kg N ha^{-1} in 2013 and with rates ≥ 112 kg N ha^{-1} in 2014 (Table 2.3). Since the appearance of 2° branch racemes was both infrequent and inconsistent, the results of these branches were omitted here.

Some disagreement in the literature exists with regard to the influence of applied N on raceme production in the Brassicaceae. Wang et al. (2014) found that applied N increased raceme production in oilseed rape, but Asare and Scarisbrick (1995) found no relationship between raceme production and applied N in oilseed rape, while variable results on raceme production with applied N in winter canola have been reported by Ferguson (2015). The effect of applied N on raceme production in yellow mustard has not been previously reported. Zając et al. (2011) found that winter oilseed rape and yellow mustard produced a similar number of total racemes plant^{-1}, ranging from 4-7, which was slightly greater than the number found in this study (Table 2.4).

The differences in the total number of racemes plant^{-1} among applied N treatments were mostly attributed to racemes plant^{-1} produced on 1° branches.

The number of siliques on the main stem and 1° branch racemes were increased by applied N in both years (Table 2.3). The greatest number of siliques were produced with 224 kg N ha^{-1}. The number of siliques was significantly increased by applied N at rates ≥ 168 kg N ha^{-1} for the main stem and 1° branch racemes in 2013 and in 1° branch racemes in 2014, and by rates ≥ 112 kg N ha^{-1} for main stem siliques in 2014. Siliques borne on the main stem and 1° branch racemes accounted for roughly 99% of the total silique production in both years. Main stem siliques accounted for 61% and 65% of the total siliques plant^{-1} in 2013 and 2014, respectively (Tables 2.3 and 2.4). Total production of siliques by the plant was greater in 2013 than in 2014 and is thought to be a contributor to the seed yield differences observed between the two years (Table 2.4). Nitrogen has been reported to increase the number of siliques in oilseed rape and in winter canola (Scott et al., 1973; Wang et al., 2014; Ferguson, 2015), but no previous studies on the effect of N on siliques in yellow mustard have been published to date. Siliques were not regularly produced on 2° branches and were too few in number to obtain statistically valid results so that data is not shown here.

The number of seeds silique^{-1} produced on the main stem of yellow mustard was consistently increased by applied N in both years (Table 2.3). Applied N increased the number of seeds silique^{-1} produced on 1° branch racemes in 2013 but not in 2014. The total number of seeds silique^{-1} was increased by applied N in both years (Table 2.4).

These results are in contrast with the reported effects of applied N in other Brassica family oilseed crops where the number of seeds silique^{-1} was not affected by N application (Asare and Scarisbrick, 1995; Ferguson, 2015).

The weight of seed produced on the main stem was affected by applied N in both years and on seed from 1° branch racemes in 2014, but N had no influence on seed weight of seed produced on 1° branch racemes in 2013 (Table 2.1). The weight of seed produced on the main stem and 1° branch racemes was incrementally increased with the rate of applied N in yellow mustard (Figure 2.5). Applied N either reduced or had no effect on seed weight produced on main stem and 1° branch racemes in winter canola (Ferguson, 2015). The origin of small seeds in oilseed rape within a given plant tended to be from lower silique and raceme locations (Diepenbrock, 2000). Overall, seed weight was greater in 2013 than in 2014 (Figure 2.5).

Diepenbrock (2000) considered stand density, siliques plant^{-1}, seeds silique^{-1}, and seed weight to be the primary seed yield components in winter oilseed rape. Siliques plant^{-1} was strongly influenced by applied N in yellow mustard (Table 2.3). Allen and Morgan (1972) found that applied N fertilizer promoted increased oilseed rape seed yields primarily through greater silique production and subsequent seeds plant^{-1}. All N application rates resulted in yellow mustard main stem racemes producing significantly more seeds plant^{-1} in 2013 when compared to the control. However, in 2014, the 56 kg N ha^{-1} application rate did not yield enough main stem seeds plant^{-1} to exceed seed production by 0 kg N ha^{-1}. The main stem raceme accounted for 63% to 66% of the total seeds plant^{-1} in yellow mustard while the primary racemes accounted for another 33% to 36% of the seeds plant^{-1}. Since seed number was the most important characteristic in determining seed yield responses to applied N in yellow mustard (Figure 2.4), the increased number of seeds most likely was a result of N-induced increases in siliques plant^{-1} from main stem and 1° branch racemes.

2.4. Conclusions

Yellow mustard responded to applied N with increased seed yield in the wet environment of Oregon's WV. While seed yield was maximum at the 224 kg N ha^{-1} rate in each year, economic considerations will likely encourage potential growers of yellow mustard to consider a more moderate rate such as 168 kg N ha^{-1}. Seed number m^{-2} was the most influential factor in determining seed yield in yellow mustard, so N management should be aimed at increasing seed number. Yellow mustard exhibited some of the same seed yield component responses to applied N as other Brassica family oilseed crops, but others were unique or unusual. Siliques produced on main stem and 1° branch racemes was the most important seed yield component for explaining the relationship of applied N to seed yield, and made major contributions to seed number m^{-2} – the primary determinant of seed yield. Seed weight was increased by applied N and thus also made a contribution to seed yield. Seeds silique^{-1} was increased by applied N, a phenomenon not previously reported in the Brassicaceae. Yellow mustard has the potential to be a valuable oilseed/condiment rotation crop for the grass seed crop dominated cropping systems in the region, and this work can serve as the basis for nitrogen management recommendations in WV yellow mustard production.

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Table 2.1. Analysis of variance for nitrogen effects on seed yield, seed weight, seed number and components of seed yield.

	Year	
Characteristics	2013	2014
Seed Yield	***	***
Seed Weight	**	***
Seed Number m^{-2}	***	***
Racemes $Plant^{-1}$		
1° Racemes	*	**
Total	**	**
Siliques $Plant^{-1}$		
Main stem	***	***
1° Racemes	***	***
Total	***	***
Seeds $Silique^{-1}$		
Main stem	***	**
1° Racemes	***	ns
Total	***	**
Seed Weight		
Main stem	**	***
1° Racemes	ns†	***

*$P \leq 0.05$.
**$P \leq 0.01$.
***$P \leq 0.001$.
†Not significant.

Table 2.2. Effect of applied N on seed yield, seed weight, and seed number in yellow mustard.

Year	Nitrogen	Seed Yield	Seed Weight	Seed number
	kg ha^{-1}	kg ha^{-1}	mg	m^{-2} (x 10^3)
2013	0	1,532 d†	6.923 bc	22.17 c
	56	1,747 c	6.825 c	25.60 b
	112	1,770 bc	6.835 bc	25.93 b
	168	1,938 b	7.042 ab	27.54 b
	224	2,579 a	7.192 a	35.90 a
2014	0	1,080 d	5.937 c	18.16 c
	56	1,426 c	5.978 c	23.85 b
	112	1,782 b	5.993 c	29.72 a
	168	1,953 a	6.198 b	31.53 a
	224	2,010 a	6.484 a	31.02 a

†Means within each column and year are not statistically significant by Fisher's LSD (P=0.05) if followed by the same letter.

Table 2.3. Effect of applied N rate on yellow mustard seed yield components plant^{-1}.

		Racemes plant^{-1}	Siliques plant^{-1}		Seeds silique^{-1}	
Year	Nitrogen	1° racemes	Main stem	1° racemes	Main stem	1° racemes
	kg ha^{-1}					
2013	0	2.0 b†	36.3 c	20.3 c	3.7 b	3.4 c
	56	2.2 b	38.7 c	19.5 c	4.4 a	4.0 b
	112	2.4 ab	40.7 bc	26.0 bc	4.5 a	4.1 ab
	168	2.5 ab	45.3 ab	32.2 ab	4.5 a	4.1 ab
	224	2.8 a	47.7 a	38.6 a	4.6 a	4.5 a
2014	0	1.3 b	20.9 d	7.7 c	3.9 b	3.8 a
	56	1.8 ab	23.5 cd	12.1 bc	4.3 a	4.0 a
	112	2.1 a	28.8 bc	15.1 bc	4.5 a	4.1 a
	168	2.2 a	32.5 ab	21.8 ab	4.5 a	4.2 a
	224	2.4 a	36.5 a	28.5 a	4.7 a	4.8 a

†Means presented within each column and year are not statistically significant by Fisher's LSD (*P*=0.05) if followed by the same letter.

Table 2.4. Effect of applied N rate on yellow mustard total seed yield components.

Year	Nitrogen	Total racemes plant^{-1}	Total siliques plant^{-1}	Total seeds silique^{-1}
	kg ha^{-1}			
2013	0	3.2 b†	56.9 c	3.6 c
	56	3.3 b	58.3 c	4.3 b
	112	3.6 ab	67.9 bc	4.3 ab
	168	3.8 ab	78.4 ab	4.3 ab
	224	4.3 a	87.8 a	4.6 a
2014	0	2.5 c	28.8 d	3.9 b
	56	2.9 bc	35.6 cd	4.2 ab
	112	3.3 ab	44.1 bc	4.3 ab
	168	3.5 ab	54.7 ab	4.4 a
	224	3.9 a	65.8 a	4.7 a

†Means presented within each column and year are not statistically significant by Fisher's LSD (*P*=0.05) if followed by the same letter.

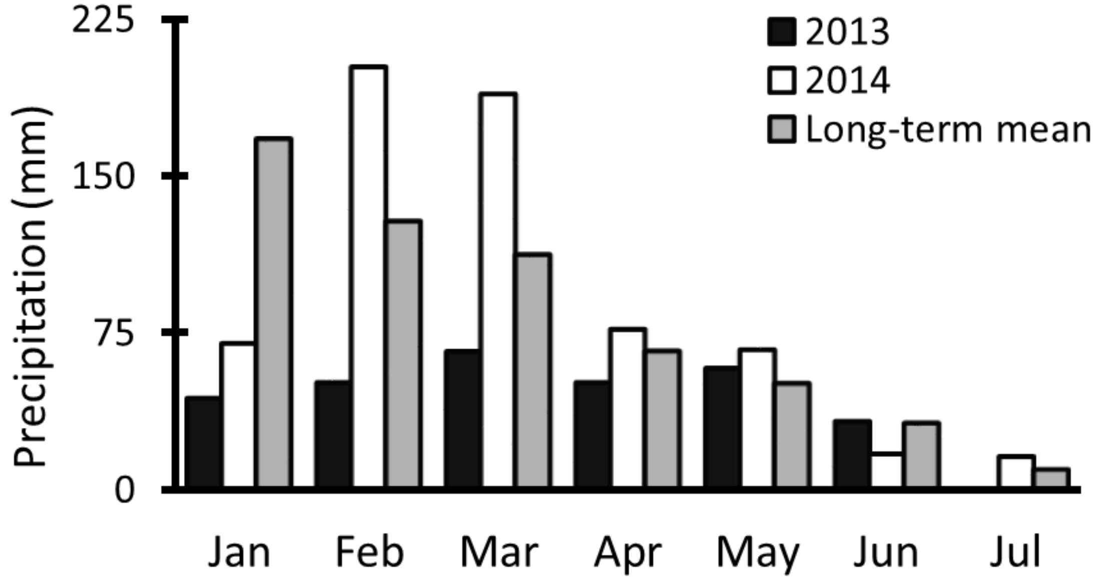

Figure 2.1. Monthly precipitation during the study period and long-term monthly mean precipitation for Corvallis, Oregon.

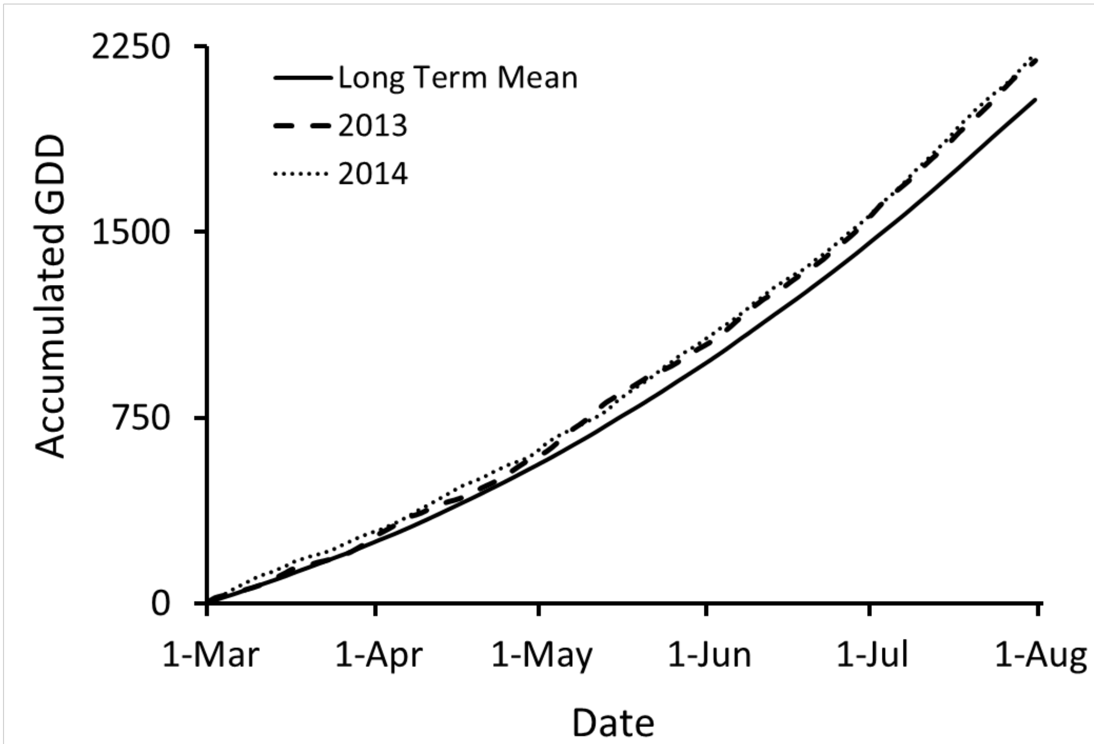

Figure 2.2. Accumulated growing degree days (GDD) from 1 March to 31 July during the two-year study period compared with long-term mean accumulated GDD for Corvallis, Oregon. Base temperature = 0° C.

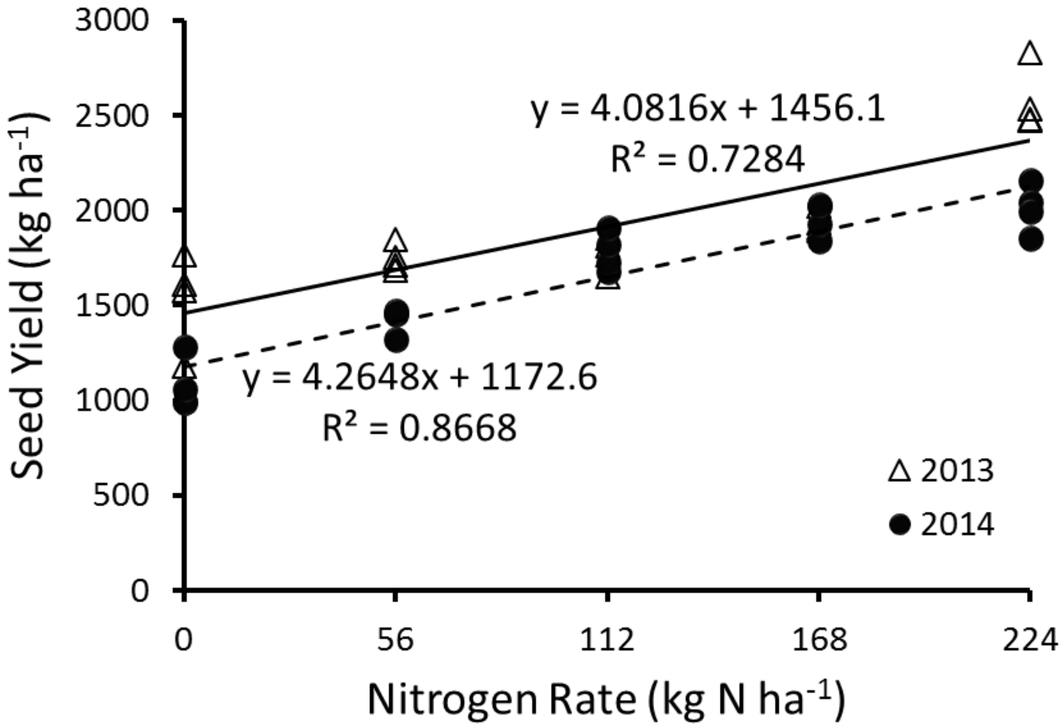

Figure 2.3. Influence of applied nitrogen rate on yellow mustard seed yield in 2013 and 2014.

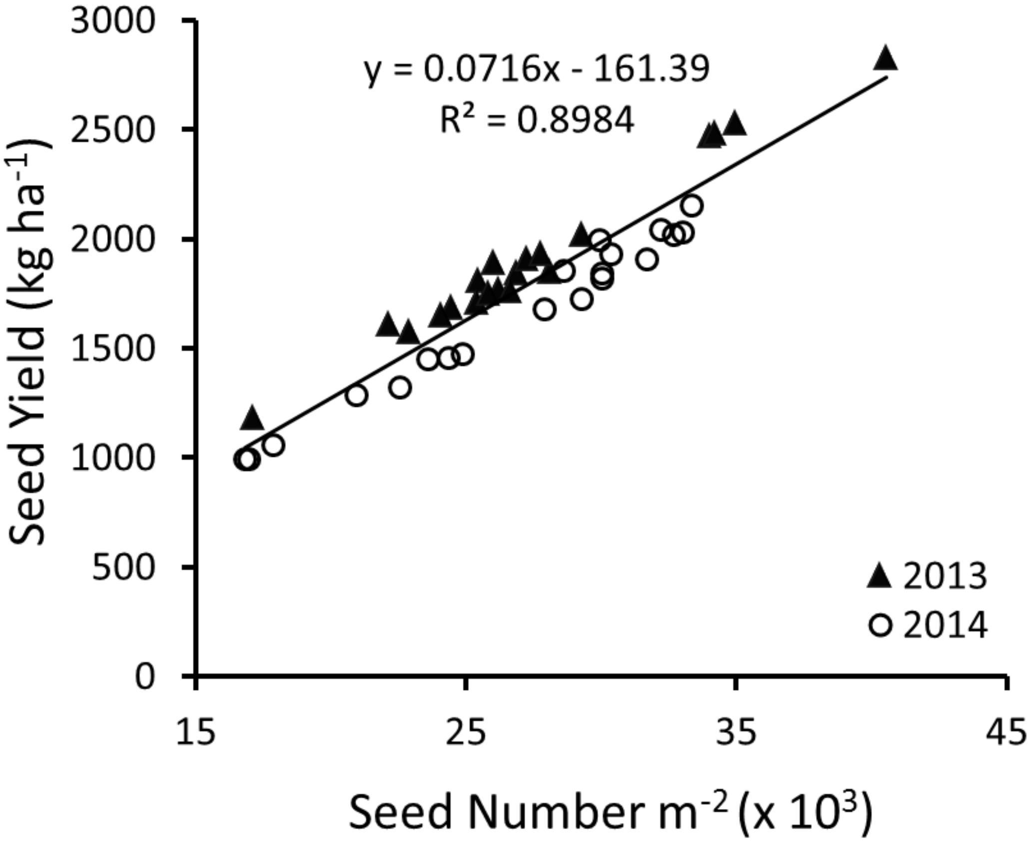

Figure 2.4. Relationship of seed yield and seed number m^{-2} in yellow mustard over a two-year period.

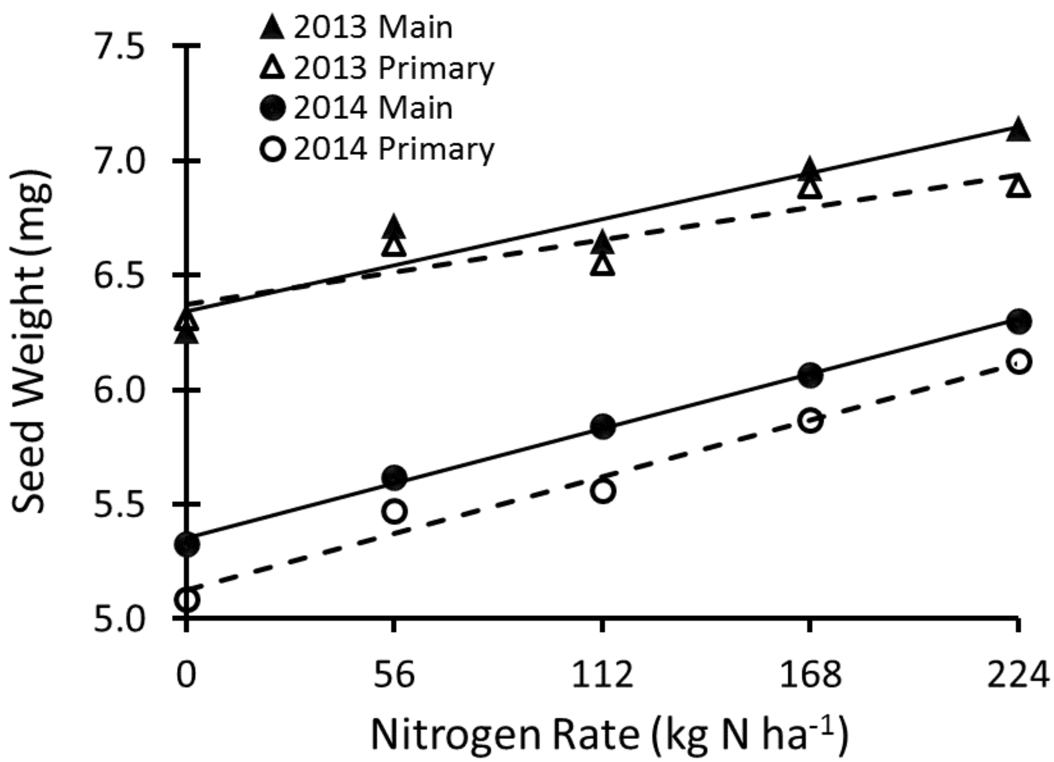

Figure 2.5. Effect of nitrogen rate on yellow mustard main stem and primary seed weight for 2013 and 2014. Equations for the fitted lines are: 2013 Main, y = 0.0036x + 6.3411, R^2 = 0.8986; 2013 Primary, y = 0.0025x + 6.3733, R^2 = 0.8358; 2014 Main, y = 0.0043x + 5.3538, R^2 = 0.9972; 2014 Primary, y = 0.0044x + 5.1252, R^2 = 0.9765.

CHAPTER 3: Applied nitrogen effects on dry matter partitioning and oil production characteristics in yellow mustard (*Sinapis alba* L.).

ABSTRACT

No information is available for production of yellow mustard (*Sinapis alba* L.) in high rainfall environments such as Oregon's Willamette Valley (WV). The objective of this study was to ascertain the effect of applied nitrogen (N) on dry matter partitioning and oil production characteristics in yellow mustard. Field trials were conducted over a two-year period at Corvallis, Oregon on 'IdaGold' yellow mustard with five N rates (0, 56, 112, 168, and 224 kg N ha^{-1}). Stand density, plant height, and above-ground biomass were determined at three developmental stages (stem elongation – BBCH 30, inflorescence emergence – BBCH 50, and harvest – BBCH 87) while leaf area index (LAI) and tissue N content was measured at BBCH 30 and BBCH 50. Applied N affected most dry matter partitioning and oil production characteristics with the exception of stand density, harvest index (HI), and seed protein concentration. Plant height, biomass, LAI, and tissue N concentration at BBCH 30 and 50 as well as crop growth rate (CGR) from BBCH 30 to 87 were related to the rate of applied N. Applied N increased plant height by 24 to 105%. Although lodging occurred in both years at the highest N rates, lodging did not negatively influence plant growth and seed yield development, nor total oil yield. As a measure of stand photosynthetic capacity, LAI was lowest across developmental stages with 0 kg N ha^{-1} in 2013, and in the 0 and 56 kg N ha^{-1} treatments in 2014. Increases in CGR by applied N ranged from 8 to 44% in 2013 and from 27 to

109% in 2014. Biomass N content at BBCH 30 was increased by all rates of applied N, but by BBCH 50, N content in biomass was only elevated over the control by rates ≥ 168 and ≥ 112 kg N ha^{-1} in 2013 and 2014, respectively. The rate of applied N incrementally decreased N use efficiency (NUE) with reductions ranging from 29% to 71% with only 56 kg N ha^{-1}. Seed oil concentration was generally inversely related to applied N rate; but highest oil yields were observed with 224 kg N ha^{-1}. The results of this study improves our understanding of dry matter partitioning and oil production characteristics in yellow mustard in response to applied N in a high rainfall environment.

Abbreviations: BBCH, Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie; crop growth rate, CGR; HI, harvest index; leaf area index, LAI; NUE, nitrogen use efficiency; Willamette Valley, WV

Keywords: stand density, biomass, tissue N content, harvest index, oil content, protein content

3.1. Introduction

Yellow mustard is a potential multipurpose rotation crop for grass seed cropping systems in the WV of western Oregon; however, no information is available on the production of yellow mustard in the high rainfall environment that is found in the region. DuVal et al. (2015a) – Chapter 2 found that applied N increased seed yield of yellow mustard in the WV through its effect on increasing seed number and to a lesser extent, seed weight. The nature of these seed yield and yield component responses to applied N as well as seed oil production potential needs to be further investigated.

The USA is one of the principal consumers of condiment mustard on a global scale and imports the majority of seed utilized in domestic mustard processing from Canada. In fact, the USA accounted for 43% of Canada's mustard seed exports in 2007 (Statistics Canada, 2008). An examination of yellow mustard as a biodiesel feedstock crop at the University of Idaho has demonstrated that excellent fuel economy and vehicle operation has been attained in a test vehicle while running on yellow mustard seed oil sourced biodiesel (Peterson and Thompson, 2005). These findings have helped to spur interest in yellow mustard oil as a biofuel feedstock and the crop as a potential candidate for Oregon's WV where legislation restricts the production of more commonly grown feedstock crops such as canola.

Oil yield is the mathematical product of fractional seed oil concentration and seed yield per unit area. The seed oil concentration is influenced by genetic makeup, environmental conditions (especially precipitation and temperature) (Rathke et al., 2006), and cropping system management. Seed oil concentrations in Brassicaceae crops range from 32-38% in camelina (*Camelina sativa*) (Wysocki et al., 2013) to 45-50% for canola/oilseed rape (*Brassica napus*) (Wittkop et al., 2009). Yellow mustard seeds typically contain between 23% and 28% oil (Kovács et al., 2009) and 29-37% protein (Salunkhe and Desai, 1986; Kovács et al., 2006), although Bell et al. (2000) observed a maximum of 44% protein. Protein concentration in *S. alba* seeds is of lesser concern to agriculturalists when compared to *B. napus*, which contains 17-22% protein (Asare and Scarisbrick, 1995; Rathke et al., 2005), since yellow mustard seed meal (a byproduct of seed oil extraction) is rarely utilized in livestock rations due to high levels of glucosinolates. Researchers have repeatedly reported a negative correlation of seed protein and oil concentrations across oilseed crops (Röbbelen et al. (eds.), 1989; Asare and Scarisbrick, 1995) that can be amplified by N fertilizer management (Bilsborrow at al., 1993; Asare and Scarisbrick, 1995; Kovács et al., 2009). Rathke et al. (2005) found that applications of 240 kg N ha^{-1} decreased *B. napus* seed oil concentration by 7-8% and increased protein content by 14-18%.

Dry matter production (biomass) is typically increased by applied N in Brassicaceae family oilseed crops (Asare and Scarisbrick, 1995; Patel et al., 1996; Wang et al., 2014; Ferguson, 2015). The efficiency of dry matter partitioning to seed yield in relation to this increased biomass is measured by the harvest index (HI). Reported values for HI in the Brassica family range from 0.11 to 0.50 (Kumar et al., 1997; Rathke et al., 2005; Svečnjak and Rengel 2006b; Ferguson, 2015), but no information is available on HI in yellow mustard.

Development of an efficient canopy for capture of light energy and carbon is essential for economic crop production. Applied N encourages rapid canopy development (Cheema et al., 2001), thereby increasing total leaf area and leaf area index (LAI) up to an optimal value. LAI is an estimate of total stand photosynthetic ability and often reaches a species-specific optimal value of 4-6 for oilseeds (Kumar et al., 1997; Hunková et al., 2009) when the plant stand achieves maximum photosynthetic capacity (Patel et al., 1996). Wolfe et al. (1988) demonstrated that N fertilizer alone accounted for a 40-50% increase in maize leaf area in 1982 and that N deficiency significantly limited leaf area expansion. Applied N causes proportional increases in LAI in oilseed rape (Allen and Morgan, 1972; Scott et al., 1973; Cheema et al., 2001) and in brown mustard (*B. juncea*) (Kumar et al., 1997). The effect of N on yellow mustard leaf area is not known.

Plant biomass accumulation is highly correlated with both the quantity of photosynthesis occurring and LAI (Watson, 1947; Kumar et al., 1997). Plant height and above ground biomass are important contributing factors to crop lodging rates, which can be exacerbated by excessive N fertilizer applications (Conley et al., 2004). Reduced light interception (Trethewey, 2009) within lodged plant stands leads to depressed photosynthetic capacity and photoassimilate accumulation, which subsequently negatively influences aboveground biomass accumulation, pollination, and development of reproductive tissues. Decreased seed yield resulting from lodging has been consistently observed in oilseed rape (*B. napus*) (Scott et al., 1973; Rathke et al., 2006).

Roughly 90% of the total aboveground N concentration is located in the seeds at harvest (Diepenbrock, 2000). Oilseed rape has a high N demand due to the elevated N content in leaves, stems, siliques, and seeds (Rathke et al., 2005; Svečnjak and Rengel, 2006b). Bilsborrow et al. (1993) demonstrated that applications of N fertilizer increased N concentrations in oilseed rape vegetation; the results of which were supported by Svečnjak and Rengel (2006b) and Wang et al. (2014) and were also observed in *S. alba* biomass (Kovács et al., 2009).

Nitrogen use efficiency (NUE) is the ratio of seed yield to total N (combined soil-available N and applied N fertilizer). However, it is typical for increases of N fertilizer application rate to result in decreased NUE in camelina (Wysocki et al., 2013) and oilseed rape and winter canola (Rathke et al., 2006; Svečnjak and Rengel, 2006a; Wang et al., 2014; Ferguson, 2015).

The objective of this study was to ascertain the effect of applied nitrogen on dry matter partitioning and oil production characteristics in yellow mustard under the conditions of Oregon's WV.

3.2. Materials and Methods

3.2.1. Overview

'IdaGold' yellow mustard was planted near Corvallis, Oregon, at Oregon State University's Hyslop Crop Science Research Farm (44° 38' 17.6" N, 123° 11' 44.1" W), in 2013 and 2014 to ascertain the effects of N fertilizer on dry matter partitioning and oil production characteristics. The soil at the site is a Woodburn silt loam (fine-silty, mixed, superactive, mesic Aquultic Argixeroll). A pre-plant soil analysis was conducted (0-20 cm) to determine soil residual N content as well as recognize the presence of any inherent nutrient deficiencies, which were corrected by applications of potassium magnesium sulfate (0-0-22-11-22) pre-plant as well as foliar sodium borate. The pre-plant soil available N was 19.2 kg N ha^{-1} in 2013, with a pH of 5.9; whereas, in 2014, soil pH was 5.6 and had a pre-plant available N content of 64.6 kg N ha^{-1}.

The experimental design was a randomized complete block with four replications and five N fertilizer treatments. Plots were designed to measure 4.9 m x 15 m. Half of each plot (2.4 m X 15 m) was dedicated for collecting destructive, in-season crop vegetative measurements, while the other half (2.4 m X 15 m) was reserved for harvest and seed yield analysis.

The BBCH (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) growth scale for canola (Meier, 2001) was utilized to determine proper treatment application, growth observation, and harvest timings. When the first few true yellow mustard leaves unfolded on 4 April 2013 and 18 April 2014, corresponding to BBCH stages 11-13, N fertilizer was broadcast applied as dry granular urea (46-0-0) at five rates (0, 56, 112, 168, and 224 kg N ha^{-1}) with a tractor-mounted Gandy orbit-air fertilizer spreader. Detailed descriptions of crop management, experimental design, and procedures were described in DuVal et al. (2015a) – Chapter 2.

3.2.2. Vegetative Measurements

Measurements were conducted when 90% of the plots reached the desired BBCH developmental stage (stem elongation, BBCH 30, and inflorescence emergence, BBCH 50) (Meier et al., 2009). Two adjacent 0.1 m^2 quadrat samples of aboveground biomass were randomly collected from each plot. Immediately following collection, samples were weighed and plant number determined. Ten plants were randomly selected from each observation and measured for height and total leaf area. One-side leaf area was measured with a LI-3100C leaf area meter (LI-COR, Lincoln, NE) and afterwards the leaf area index (LAI) was calculated utilizing the following formula:

$$\mathrm{LAI} = \frac{\text{Leaf Area } (\mathrm{m}^2)}{\text{Ground Area } (\mathrm{m}^2)}$$

All biomass was returned to the individual observation sample bags, dried in an air-forced chamber at 70°C for 48 hours, and subsequently weighed. The two observations per plot were then combined for later analysis. After the completion of all leaf area measurements, the dried biomass samples were ground in preparation for tissue analysis. Total carbon (structural and non-structural) and total nitrogen content in prepared above-ground plant tissues were determined by using a LECO CNS-2000 combustion analyzer (LECO, St. Joseph, MI).

The final biomass collection occurred at BBCH 87 when approximately 70% of siliques were ripe (9 July 2013 and 7 July 2014) and was utilized to determine final plant height and dry biomass accumulation immediately prior to harvest. Two adjacent 0.1 m^2 quadrat aboveground biomass samples per plot were removed, weighed, dried for 48 hours at 70°C in an air-forced chamber, and then re-weighed. Ten random and representative plants were subsampled from each quadrat observation and plant height quantified.

3.2.3. Seed Protein and Oil Concentrations

Shortly after the final biomass sampling date, plots were swathed with a modified John Deere windrower and left to air-dry for seven to twelve days. On 23 July 2013 and 28 July 2014, a plot combine was utilized to harvest the center 1.8 m x 15m swath from each plot. The harvested seed obtained from the center swaths were then cleaned with a Clipper model M2B seed cleaner and utilized to calculate HI. The cleaned seed was further subsampled and later used for ascertaining seed oil and protein concentrations by utilizing pulsed nuclear magnetic resonance (NMR) spectroscopy. Total oil yield was calculated as the product of total seed yield and fractional seed oil concentration.

3.2.4. Calculated Values and Statistical Analysis

Yellow mustard crop growth rate (GCR), HI, and NUE were calculated in the following manner:

Crop Growth Rate: $\mathrm{CGR} = \frac{\Delta\ \mathrm{Dry\ Weight\ (g)}}{\Delta\ \mathrm{Time}}$

Harvest Index: $\mathrm{HI} = \frac{\mathrm{Seed\ Yield}}{\mathrm{Aboveground\ Dry\ Weight}}$

Nitrogen Use Efficiency: $\mathrm{NUE} = \frac{\mathrm{Seed\ Yield}}{\mathrm{N_{Soil\ Supply}} + \mathrm{N_{Fertilzer}}}$

Analysis of variance (ANOVA) was conducted with the SAS 9.9 PROC GLM and interpreted on a plot means basis. For consistency across subject matter, applied N effects on plant height, biomass accumulation, LAI, HI, N partitioning, NUE, seed oil and protein concentration, as well as oil yield were not combined across years and instead were analyzed separately for both 2013 and 2014. Measurements were analyzed for each individual growth stage. Treatment means were separated by Fisher's protected LSD values at the 5% level of significance.

3.3. Results and Discussion

3.3.1. Stand Density

Plant stand density is related to canopy density and structure, light attenuation and photosynthetic capacity, as well as overall seed yields. Brandt (1992) reported that the best range of seeding rates for yellow mustard in trials over a 6-year period was between 8 and 12 kg ha^{-1}. Nevertheless, seed yield was not lessened even by high seeding rate up to 16 kg ha^{-1} and was only reduced at 4 kg ha^{-1}. Conversely, Hassan and Arif (2012) suggested that narrow row spacing was related to low seed yield in yellow mustard. Stand density in this study did not induce noticeable negative effects on yellow mustard plant growth or performance, nor was plant performance responses to applied N differentially influenced by stand density during the two years. Stand density was not affected by applied N in either year and at any of the sampled developmental stages (Tables 3.1 and 3.2).

3.3.2. Plant Height and Lodging

Plant height was affected by applied N at all of the sampled developmental stages and in both years (Table 3.1). The greatest increase in plant height was experienced between the stem elongation (BBCH 30) and inflorescence emergence (BBCH 50) developmental stages in both years (Figure 3.1). Applied N caused increases in plant height across all three developmental stages and both years. The change in plant height from BBCH 30 to BBCH 50 was 303% and 606% in 2013 and 2014, respectively. When measured at harvest (BBCH 87), the average plant height ranged between 94 and 95 cm and was only 7% greater than at BBCH 50 in 2013 but was 45% greater than at BBCH 50 in 2014. These results indicate that yellow mustard reaches nearly full mature height during flowering when produced under the dry conditions of 2013, but experiences more growth under wet conditions as in 2014. Zając et al. (2011) observed average yellow mustard plant height at 133 cm at harvest while Hassan and Arif (2012) reported that yellow mustard was 132-146 cm tall at maturity. 'IdaGold' yellow mustard plant height ranged from 107 cm to 160 cm across multiple study locations and production environments (Brown et al., 1998; Davis et al., 2003; Davis et al., 2011). The combination of wet weather and boron toxicity symptoms early in the 2014 growing season hindered plant development—enough so that height at BBCH 30 was only roughly half the height at the same stage as in 2013.

Lodging is a potential impediment for growing Brassica family oilseed crops in the wet environment of western Oregon (Ferguson, 2015). No information is available in the literature on lodging effects on seed yield in yellow mustard, but in other Brassica family crops lodging is more severe at increased rate of applied N. Lodging in yellow mustard was present only in the two highest N rates (168 and 224 kg N ha^{-1}) in 2013, and appeared at a lesser extent in 2014 (data not shown). In both years, lodging was not considered to be severe and there were no adverse impacts on seed yield or other measures of yellow mustard performance.

3.3.3. Leaf Area Index

Leaf area index (LAI) responded to applied N in both years and this effect was observed at the BBCH 30 and BBCH 50 developmental stages (Table 3.1). All applied N rates produced LAI values that were greater than the 0 kg N ha^{-1} rate in 2013 (Table 3.2). Only applied rates ≥ 112 kg N ha^{-1} produced LAI values greater than the 0 kg N ha^{-1} rate in 2014. The increase in average LAI from BBCH 30 to BBCH 50 was 18% in 2013 and 116% in 2014. Leaf area index is one indicator of the potential photosynthetic capacity of a crop canopy and has been shown to increase with applied N in other Brassica family crops (Allen and Morgan, 1972; Scott et al., 1973; Wolfe et al., 1988; Kumar et al., 1997; Diepenbrock, 2000; Cheema et al., 2001; Rathke et al., 2006), but no reports are available on applied N effects on LAI in yellow mustard, until now. The optimum LAI values for other oilseed crops are reported to range from 4.0 to 6.0 (Kumar et al., 1997; Hunková et al., 2009).

3.3.4. Biomass Accumulation, CGR, and HI

Applied N affected aboveground biomass accumulation in yellow mustard in both years and at all three developmental stages (Table 3.1). Biomass was increased by applied N across all stages of development in both years (Figure 3.2). Nitrogen fertilizers promote aboveground biomass accumulation in *B. napus* (Scott et al., 1973; Rathke et al., 2006; Svečnjak and Rengel, 2006b; Wang et al., 2014); in fact, Cheema et al. (2001) reported that 90 kg N ha^{-1} supported significantly greater oilseed rape biomass accumulation. Yellow mustard control plots experienced growth at all measurements, ranging from 208% (BBCH 30 to 50) to 318% (BBCH 50 to 87) in 2013 and 211% to 233% in 2014; however, the control consistently accumulated the least biomass when compared to treatments receiving applied N. An application of 56 kg N ha^{-1} in 2013 yielded significantly greater biomass accumulation compared to the control at BBCH 30 and BBCH 50. At BBCH 87, only the 168 and 224 kg N ha^{-1} treatments amassed significantly more biomass than the control, which was 33% and 34% less than that attained by the two highest N application rates. During the 2014 growing season, 224 kg N ha^{-1} was required to generate significantly greater aboveground biomass at BBCH 30 when compared to the control. 56 kg N ha^{-1} was sufficient for differences at BBCH 50, but at BBCH 87, ≥ 112 kg N ha^{-1} was needed to produce significantly more biomass than the control in 2014. Overall, applications of N fertilizer stimulated aboveground biomass accumulation increases ranging from 16% to 154% and from 27% to 150% in 2013 and 2014, respectively.

Crop growth rate in yellow mustard was influenced by applied N in both years (Table 3.1). Elevated CGRs were noted with applied N rates ≥ 168 kg N ha^{-1} in 2013 and by rates ≥ 112 kg N ha^{-1} in 2014 (Table 3.3). In 2013, CGR was somewhat greater than that observed in 2014, possibly as a result of more favorable growing conditions in 2013, but also as a result of some short-lived B toxicity. Allen and Morgan (1972) reported a positive correlation between available N and CGR in oilseed rape, stating that CGR peaked during silique development and subsequently declined. When produced under ideal agronomic and environmental conditions, oilseed rape is capable of CGR of over

25 g d^{-1} m^{-2} (Diepenbrock, 2000). Yellow mustard tested in this study had CGRs that ranged from under 10 to just over 21 g d^{-1} m^{-2} depending on applied N rate (Table 3.3).

Harvest index generally provides a measure of how applied N might impact partitioning to seed in relation to total aboveground biomass production; however, HI in yellow mustard was not affected by applied N in either year (Tables 3.1 and 3.3). Ferguson (2015) found that high N rates reduced HI in winter canola in western Oregon. Reported values for HI in the Brassica family range from 0.11 to 0.50 (Kumar et al., 1997; Rathke et al., 2005; Svečnjak and Rengel 2006b; Ferguson, 2015). Values for HI in yellow mustard ranged from 0.12 to 0.18 in this study.

3.3.5. Tissue N Content and NUE

The N content of tissues at BBCH 30 and BBCH 50 were affected by applied N in both years (Table 3.1). The concentration of N in plant tissues increased incrementally in a linear fashion across applied N rates at both BBCH 30 and BBCH 50 (Figure 3.3). The slope of the BBCH 50 regression line was less than the slope at BBCH 30 suggesting that more utilization of tissue N was taking place in the high N rates than at the lower N rates to support reproductive development. Hocking et al. (1997) determined that more than 50% of the total N content of mature *B. napus* plants was accumulated prior to flowering and that approximately 55% of the seed N concentration was mobilized from plant vegetative tissues. From seedling growth through early rosette development (Hocking et al., 1997), tissue N will accumulate, reach a maximum value, and subsequently, inherently decline as the growing season progresses (Wang et al., 2014).

Nitrogen use efficiency (NUE) in yellow mustard was influenced by applied N in both years (Table 3.1). All applied N rates reduced NUE in yellow mustard in both years (Table 3.3). An inverse relationship of NUE and N fertilizer application rates has been consistently documented in oilseed crops (Hocking et al., 1997; Svečnjak and Rengel, 2006a; Wysocki et al., 2013; Wang et al., 2014) and results from changes in N uptake throughout plant growth and development (Rathke et al., 2006). Svečnjak and Rengel (2006a) recognized that high NUE oilseed rape cultivars were directly associated with low aboveground tissue N concentrations, which is also the case for yellow mustard in this study and is illustrated in Table 3.3. The average NUE of yellow mustard in this study, 27.5 and 10.8 in 2013 and 2014 respectively, is within the range of NUE values obtained for oilseed rape (12.0 to 27.0) (Hocking et al., 1997; Svečnjak and Rengel, 2006a; Wang et al., 2014) as well as Willamette Valley produced camelina (*Camelina sativa*) (8.7 to 27.3) (Wysocki et al., 2013).

When measured at harvest, seed N content is greater than that of the mature vegetation in oilseed rape (Diepenbrock, 2000; Svečnjak and Rengel, 2006b) as well as yellow mustard (Kovács et al., 2009); such nutrient translocation can be often be magnified by increased N fertilizer applications. Greater application rates of N significantly increase N concentrations of plant vegetative tissues at all growth stages (Bilsborrow et al., 1993; Hocking et al., 1997; Svečnjak and Rengel, 2006b; Kovács et al., 2009; Wang et al., 2014). Svečnjak and Rengel (2006a) observed a 46% decline in *B. napus* N concentration from high to low N fertilizer application rates. As illustrated in

Figure 3.3, an application of 56 kg N ha^{-1} promoted increased tissue N concentration above the control by 35.4-40.4% at BBCH 30 and 1.4-14.7% at BBCH 50.

3.3.6. Seed Protein, Oil Content, and Oil Yield

Seed protein content did not respond to applied N in either year (Table 3.1). Seed protein content did not vary much from 24% regardless of applied N rate or year (Table 3.4). Although of less concern in yellow mustard production due to restricted nutritional value, seed protein content is a major consideration in many other oilseed crops, especially *B. napus*. Rathke et al. (2006) recognized that environmental conditions contribute to variations in oilseed rape seed characteristics, including seed oil and protein concentration as well as seed quality, citing that droughts in particular reduce seed oil content and increase seed glucosinolates. This negative relationship of seed oil and protein concentration is well documented across oilseed crop species (Piper and Boote, 1999; Rathke et al., 2006). Nitrogen fertilizers contribute to this negative correlation; since N is a key element of proteins, increased application rates of N fertilizers result in subsequent elevated seed protein content. Bilsborrow et al. (1993) examined the effect of N fertilizers on *B. napus* and determined that N fertilizer, particularly applications of 75-210 kg N ha^{-1}, increased average seed protein concentration by approximately 12%. Seed protein content of oilseed rape ranged from 17.7% to 21.6% for plants receiving low and high N fertilizer rates, respectively (Rathke et al., 2005). Kovács et al. (2006) found that seed protein concentration in yellow mustard was positively influenced by increasing N fertilizer, noting a minimum protein content of 29.2% in low-N environments and a maximum of 37% in treatments receiving the highest N fertilizer rate.

The concentration of oil in the seed was affected by applied N in both years (Table 3.1). Inconsistent and variable responses of seed oil concentration were observed with applied N in yellow mustard (Table 3.4). Yellow mustard seeds are reported to average 26.8% oil (Salunkhe and Desai, 1986), which is slightly less than observed in this study (27.3% and 27.5% in 2013 and 2014, respectively). Generally, N fertilizer causes reductions in seed oil content in *S. alba* (Kovács et al., 2009) and *B. napus* (Allen and Morgan, 1972; Cheema et al., 2001; Rathke et al., 2005; Rathke et al., 2006), although Hocking et al. (1997) did not observe a significant effect of N on oilseed rape seed oil concentration.

Oil yield in yellow mustard was influenced by applied N in both years (Table 3.1). All applied N rates increased oil yield over the untreated control in both years (Table 3.4). Greatest oil yields were noted with the 224 kg N ha^{-1} rate in 2013 and in rates ≥ 168 kg N ha^{-1} in 2014. When supplemented with up to 100 kg N ha^{-1}, *B. napus* can achieve optimal oil yields after which application rate oil yield declines significantly (Cheema et al., 2001). Optimal oil yields for oilseed rape average 1,790 kg ha^{-1} (Cheema et al., 2001; Rathke et al., 2005), but have been as low as 702 kg ha^{-1} (Cheema et al., 2001) during poor climatic and agronomic growing conditions. Oil yields were greatest in 2013 and resulted from a more favorable growing environment when compared to 2014, which averaged 13.3% less than 2013.

3.4. Conclusions

Applied N had several important effects on dry matter partitioning and oil production characteristics in yellow mustard. Several characteristics were likely influential in determining the nature of seed yield responses to applied N in yellow mustard. The depletion of tissue N concentration levels as the plant progressed into reproductive development (BBCH 50) at high levels of applied N was one of these characteristics. While elevated N content levels indicate successful uptake of applied N, this later season depletion of N provided evidence of the utilization of N in the support of flowering and presumably, subsequent seed production. Characteristics of the canopy were increased by applied N including biomass, CGR, plant height, and LAI, indicating that the plant had a greater potential photosynthetic capacity (source) available to support greater numbers of seed produced as well as increased seed weight. The different growing season environments observed between the two years was manifested in several ways including varying degrees of reduction found in 2014 in characteristics such as CGR, LAI, and early season plant height and biomass. However, few differences were readily apparent in other characteristics of dry matter partitioning and oil production.

Other characteristics were not influenced by applied N in yellow mustard including stand density, HI, and seed protein and as a result, it was likely that they were not influential in seed yield responses to applied N. Applied N effects on seed oil concentration were generally small and variable, and so were not able to offset the gains in seed yield attained by increasing the applied N rate. Consequently, oil yield in yellow mustard was significantly improved by the application of N fertilizer. The results of this investigation improves our understanding of the application of N in yellow mustard grown in the high rainfall environment of Oregon's WV.

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Table 3.1. Analysis of variance for nitrogen effects on stand density, vegetative characteristics, nitrogen use efficiency, seed oil and protein concentration, and total oil yield in yellow mustard.

	Year	
Characteristics	2013	2014
Stand density		
BBCH 30	ns†	ns
BBCH 50	ns	ns
BBCH 87	ns	ns
Plant Height		
BBCH 30	***	*
BBCH 50	***	***
BBCH 87	***	***
Biomass Accumulation		
BBCH 30	***	*
BBCH 50	***	***
BBCH 87	**	***
Leaf Area Index		
BBCH 30	***	***
BBCH 50	***	***
Crop Growth Rate	*	**
Harvest Index	ns	ns
Nitrogen Use Efficiency	***	***
Tissue N Content		
BBCH 30	***	***
BBCH 50	***	***
Seed Protein Content	ns	ns
Seed Oil Concentration	***	***
Oil Yield	***	***

*$P \leq 0.05$.
**$P \leq 0.01$.
***$P \leq 0.001$.
†Not significant.

Table 3.2. Nitrogen fertilizer effects on yellow mustard stand density and leaf area index (LAI).

		Stand density			Leaf Area Index	
Year	Nitrogen	BBCH 30	BBCH 50	BBCH 87	BBCH 30	BBCH 50
	kg ha^{-1}		plants m^{-2}			
2013	0	354 a†	308 a	312 a	1.36 b	1.79 c
	56	309 a	304 a	301 a	3.12 a	3.01 b
	112	299 a	283 a	301 a	3.28 a	2.90 b
	168	289 a	272 a	296 a	3.60 a	4.94 a
	224	288 a	257 a	265 a	3.67 a	5.09 a
2014	0	397 a	354 a	305 a	0.80 c	1.30 d
	56	371 a	346 a	284 a	1.32 bc	2.15 cd
	112	357 a	344 a	283 a	1.74 ab	3.25 ab
	168	351 a	322 a	256 a	2.05 a	4.27 b
	224	291 a	300 a	241 a	2.36 a	6.94 a

†Means within columns and years followed by the same letter are not significantly different by Fisher's protected LSD values (*P* = 0.05).

Table 3.3. Nitrogen rate effects on crop growth rate (CGR), harvest index (HI), and nitrogen use efficiency (NUE) in yellow mustard.

Year	Nitrogen	Crop Growth Rate	Harvest Index	N Use Efficiency
	kg ha^{-1}	g d^{-1} m^{-2}		
2013	0	14.81 b†	0.16 a	79.86 a
	56	15.92 ab	0.15 a	23.24 b
	112	17.65 ab	0.12 a	13.50 c
	168	21.04 a	0.12 a	10.35 c
	224	21.27 a	0.15 a	10.61 c
2014	0	9.69 c	0.18 a	16.71 a
	56	12.29 bc	0.18 a	11.82 b
	112	17.45 ab	0.16 a	10.09 c
	168	20.23 a	0.16 a	8.40 d
	224	19.64 a	0.17 a	6.97 e

†Means within years followed by the same letter are not significantly different by Fisher's protected LSD values (P = 0.05).

Table 3.4. Nitrogen rate effects on seed protein and oil concentration, and oil yield in yellow mustard.

Year	Nitrogen	Seed Protein Concentration	Seed Oil Concentration	Oil Yield
	kg ha^{-1}	%	%	kg ha^{-1}
2013	0	24.17 a†	26.31 b	403.84 c
	56	23.92 a	28.43 a	496.75 b
	112	23.98 a	28.41 a	502.97 b
	168	24.21 a	26.99 b	523.04 b
	224	24.43 a	26.32 b	678.48 a
2014	0	24.34 a	28.13 ab	301.02 d
	56	24.19 a	28.71 a	409.47 c
	112	24.44 a	27.55 b	491.15 b
	168	24.63 a	26.80 c	523.98 a
	224	24.93 a	26.36 c	530.20 a

†Means within columns and years followed by the same letter are not significantly different by Fisher's protected LSD values (P = 0.05).

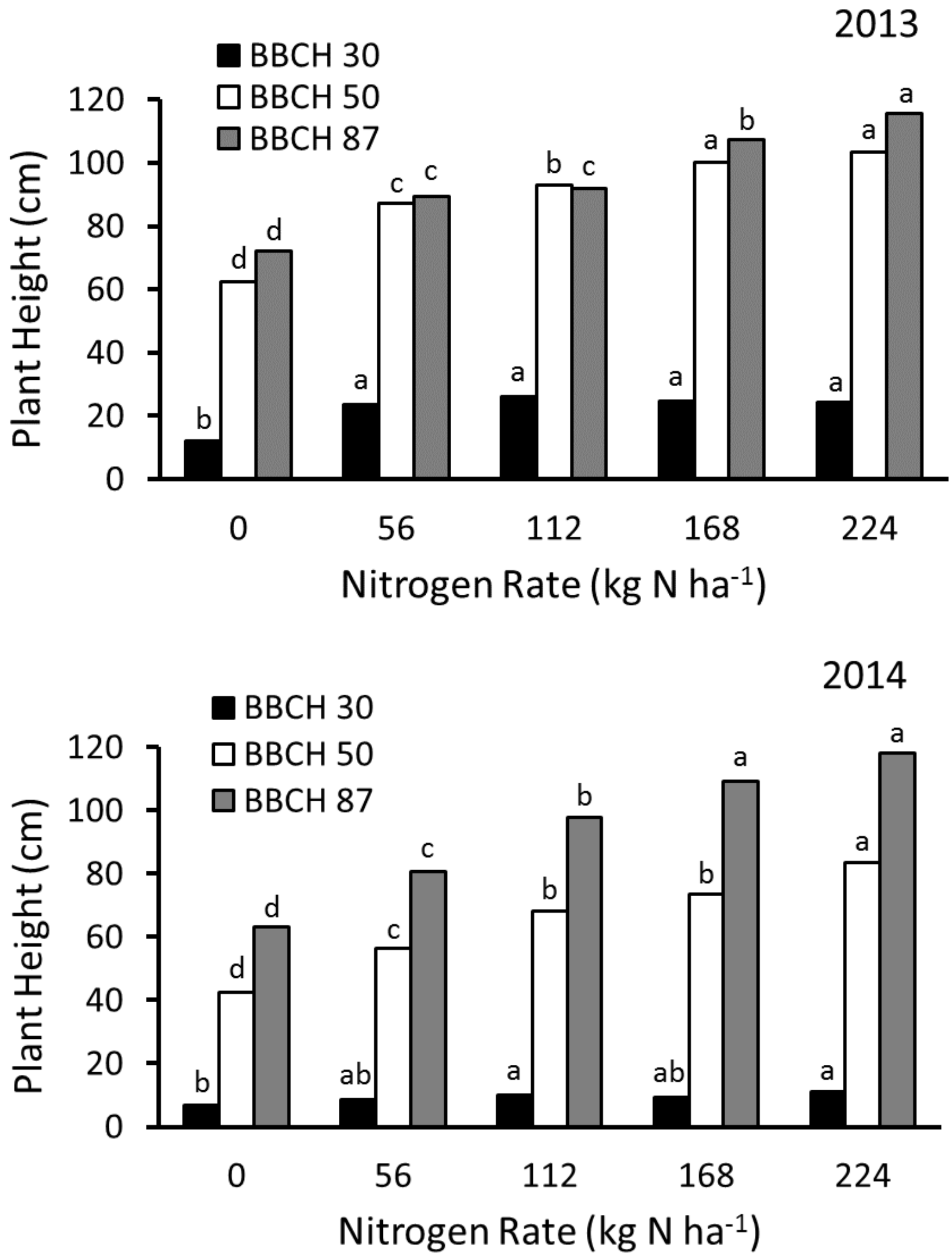
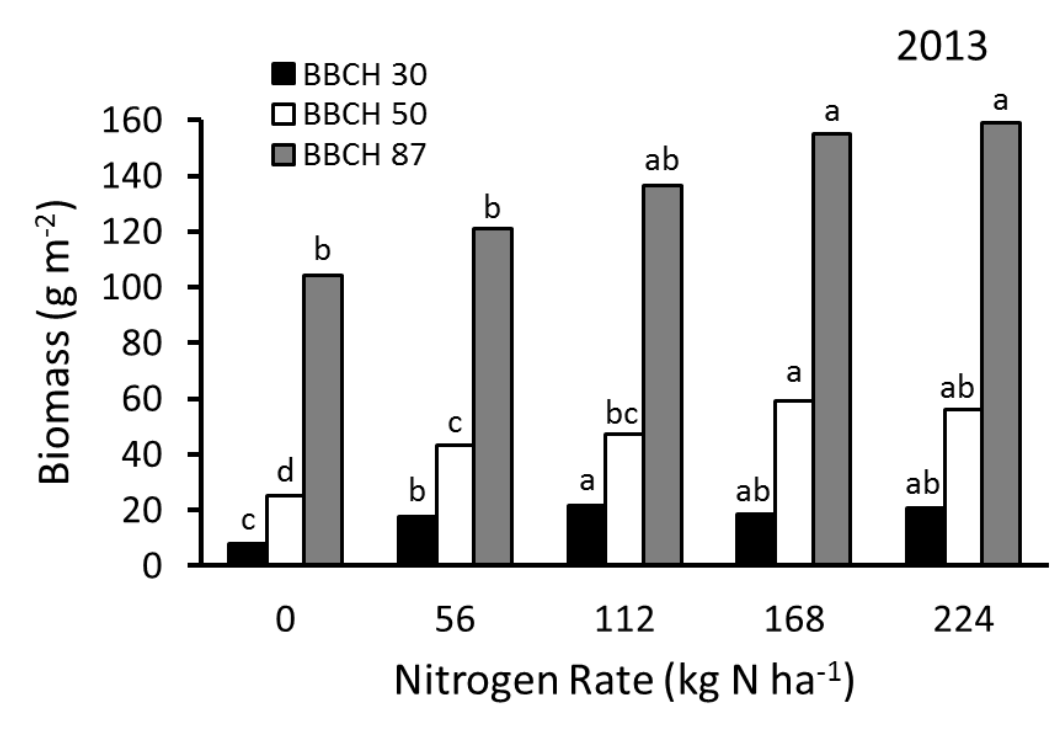


Figure 3.1. Nitrogen rate effects on yellow mustard plant height at stem elongation (BBCH 30), inflorescence emergence (BBCH 50), and harvest (BBCH 87). Means within development stages followed by the same letter are not significantly different by Fisher's protected LSD values (*P* = 0.05).



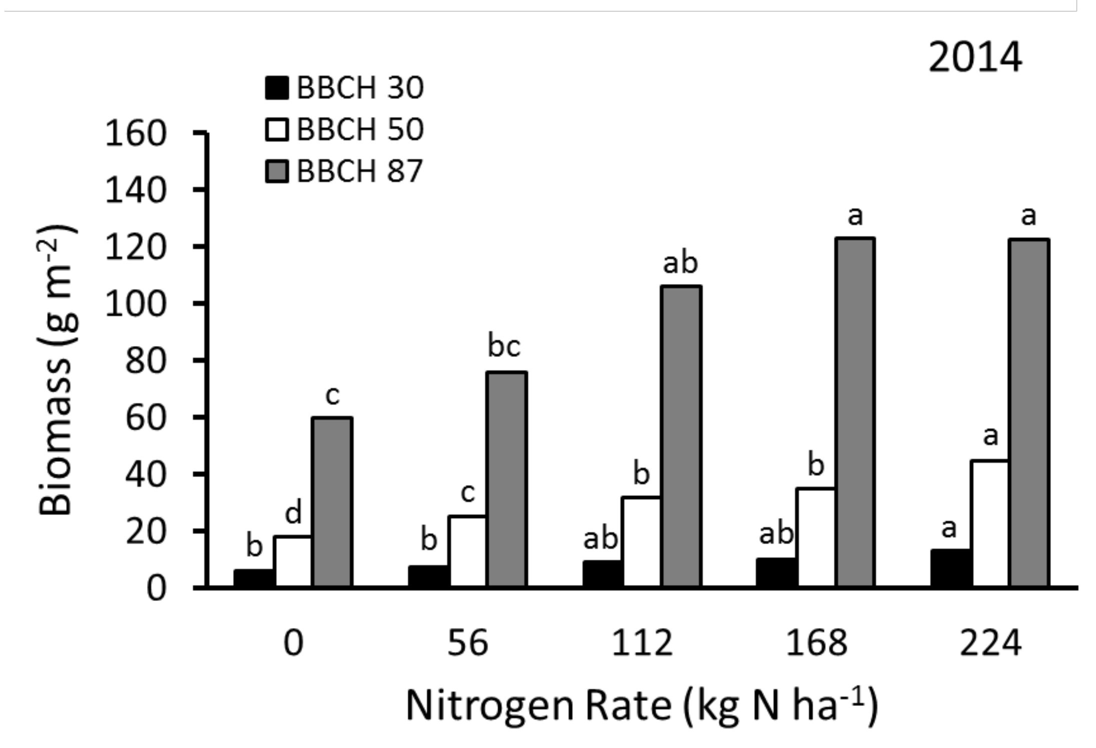


Figure 3.2. Nitrogen rate effects on yellow mustard aboveground biomass at stem elongation (BBCH 30), inflorescence emergence (BBCH 50), and harvest (BBCH 87). Means within development stages followed by the same letter are not significantly different by Fisher's protected LSD values (*P* = 0.05).

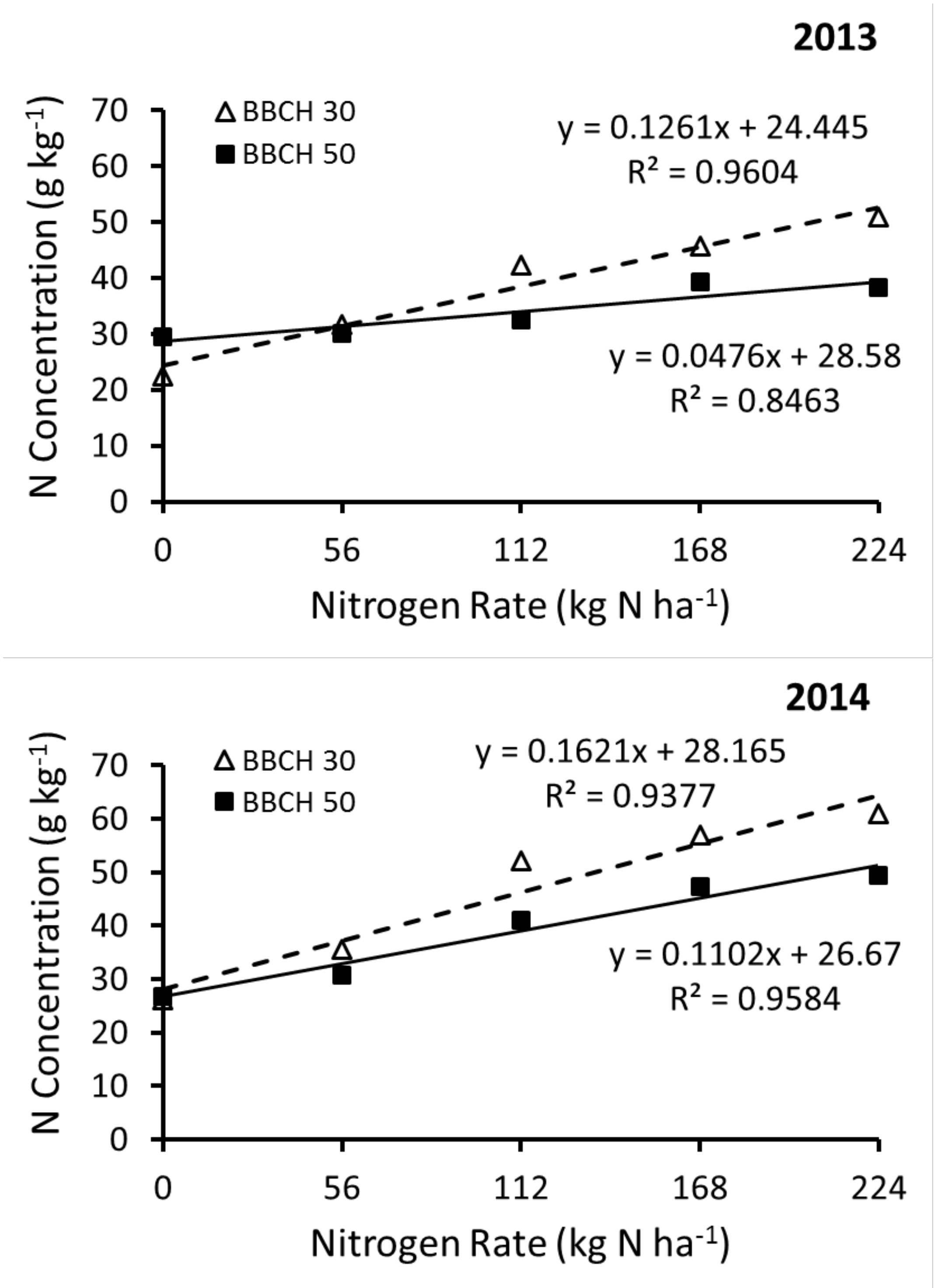


Figure 3.3. Relationship of applied N and tissue N concentration at stem elongation (BBCH 30) and inflorescence emergence (BBCH 50) in yellow mustard.

CHAPTER 4: Conclusions and Recommendations

Considering the significant controversy over widespread *Brassica napus* (canola) production and potential contamination with high-valued specialty *Brassica* seed crops, yellow mustard is a viable, albeit somewhat lower yielding alternative. Yellow mustard is exempt from the Oregon House Bill 2427, which passed in 2013 and established oilseed *Brassica* production restrictions for Willamette Valley agriculturalists. Yellow mustard is a short-term, multi-purpose, limited input oilseed crop that is ideal for incorporation into Willamette Valley agriculture, especially as a rotation crop within grass seed production systems. This study was initiated to provide background information needed to establish N fertilizer recommendations for agriculturalists producing yellow mustard under non-irrigated Willamette Valley conditions. Throughout the two-year study, the effects of applied N on 'IdaGold' yellow mustard growth and development characteristics (including plant height, aboveground biomass accumulation, LAI, and tissue N concentration), seed yield components, as well as seed and oil yields were ascertained. Although seasonal weather conditions and applied B toxicity differentially influenced yellow mustard growth in 2013 and 2014, the effects of N fertilizer on seed yield were evident at varying degrees of significance.

Nitrogen fertilizer significantly increased plant height, aboveground biomass accumulation, LAI, and tissue N concentration, but did not influence yellow mustard HI (DuVal et al., 2015b – Chapter 3). The greatest vegetative growth in terms of plant height and biomass generally occurred between stem elongation and inflorescence emergence, with continued (but lesser) growth from inflorescence emergence to maturity. The control LAI was significantly inferior compared to all N treatments, where N applications increased LAI by 62-194%. Vegetative tissue N concentration displayed a similar positive correlation with N fertilizer; conversely, NUE was negatively correlated with N, where an application of 56 kg N ha^{-1} caused a 30-71% decline in NUE compared to the control.

Supported by the aforementioned increases in aboveground biomass accumulation and LAI, N fertilizer significantly enhanced yellow mustard total seed yield, with the 224 kg N ha^{-1} treatment generating the maximal yield across years. Plots not receiving supplemental N consistently exhibited inferior performance compared to those receiving the greatest applied N rates, especially concerning seed yield (DuVal et al., 2015a – Chapter 2). Yield was influenced predominantly by silique production on main stem and primary racemes as well as seed weight—all of which displayed a positive correlation with N fertilizer rate. Overall, the total raceme, silique, and seed production $plant^{-1}$ components of yield significantly increased with applied N, while thousand seed weight and N fertilizer were positively related solely in 2014.

Contrary to findings across oilseed species, N fertilizer did not significantly influence seed protein content. Although, in accordance with oilseed standards, N fertilizer and seed oil concentration were negatively correlated. Applied N in actuality amplified yellow mustard oil yield due to significant increases in total seed yield. This study illustrates that 168-224 kg N ha^{-1} support the greatest seed and oil yields and is thereby the optimal N fertilizer application rate for ‘IdaGold’ yellow mustard produced under non-irrigated Willamette Valley growing conditions. Further agronomic research, aided by this N fertilizer determination, will form the basis of a comprehensive guide tailored to yellow mustard production in Oregon’s Willamette Valley.

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