Evaluation of *Medicago sativa* spp. *falcata* for Sustainable Forage Production in Michigan

By

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ABSTRACT

EVALUATION OF *MEDICAGO SATIVA* SPP. *FALCATA* FOR SUSTAINABLE FORAGE
PRODUCTION IN MICHIGAN
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Michigan forage producers would benefit from a perennial forage crop that maintained
yield and forage quality later into the growing season than conventional alfalfa (*Medicago sativa*
spp. *sativa* L.). Yellow-blossom alfalfa (*M. sativa* ssp. *falcata* Arcangeli.) is a perennial legume
with indeterminate vegetative growth characteristics that has not been evaluated for forage dry
matter yield (DMY) in Michigan. The objectives of this study were to: i) evaluate forage DMY,
quality (crude protein [CP], acid detergent fiber [ADF] and neutral detergent fiber [NDF]), and
stand persistence of cv. 'SD201', *falcata*, under three, two, and one harvest per year compared to
sativa (cv. WL346LH) and birdsfoot trefoil (*Lotus corniculatus* L.)(BFT)(cv. Norcen); ii)
determine if differing levels of taproot starch, sugar, protein, and amino N reserves are the cause
for slower post-harvest regrowth of *falcata* compared to *sativa*; and iii) evaluate forage DMY,
quality, and pest resistance of *falcata, sativa, varia* cultivars and birdsfoot trefoil under a two-
harvest per year schedule.

The *falcata* cv. SD201 produced greater DMY in the seeding and first production year in
two harvests per year than the *sativa* cv. WL346LH or the BFT cv. Norcen under three, two or
one harvest per year. The CP concentration in forage biomass of *falcata* harvested twice per year
was lower and ADF and NDF concentration was greater than WL346LH or BFT harvested three
times per year. Crude protein, ADF, and NDF of *falcata* were similar to WL346LH and lower
than that of BFT within a harvest frequency. Crude protein and total digestible nutrient yield was
greater for falcata harvested twice than sativa harvested three times. Harvest frequency had no

effect on stand density, and *falcata* persisted at plant densities similar to that of *sativa* and greater than that of BFT.

Taproot sugar concentration was similar between species but differed between years possibly due to moisture availability. Greater taproot amino N, protein, and starch concentrations were found in WL346LH compared to *falcata* when defoliated in mid to late-June and for 30 days thereafter. Lower concentrations of all three components may explain the slow regrowth of *falcata* following defoliation.

Forage DMY and pest interaction characteristics of SD201 are unique from currently marketed *M. sativa*, *falcata*, and *varia* cultivars. Greater DMY should be expected from SD201 than select *M. sativa*, *falcata*, and *varia* cultivars when one or two harvests are taken per year and the first harvest is after 600 growing degree days (GDD) base 5 °C in the first production year. Visual evidence of less alfalfa weevil [*Hypera postica* (Gyllenhal)] and potato leafhopper [*Empoasca fabae* (Harris)] damage of SD201 foliage than *M. sativa* and *varia* may suggest resistance or non-preference for *falcata*.

M. sativa spp. *falcata*, like SD201, shows promise as a summer stockpiled forage crop which would allow greater harvest timing flexibility for forage producers who utilize fair to low quality hay. Additional benefits if *falcata* grown for forage may include: a reduction in insecticide requirements, prolonged cover for nesting birds, and reduced soil compaction from fewer passes across the field per year.

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LIST OF ABBREVIATIONS

ABA – Abscisic Acid

ADF- Acid Detergent Fiber

AWD- Alfalfa Weevil Damage

BFT- Birdsfoot Trefoil

CP - Crude Protein

CPY- Crude Protein Yield

Dry Matter - DM

DMI – Dry Matter Intake

DMY – Dry Matter Yield

GDD- Growing Degree Days

HF – Harvest Frequency

IT – Insecticide Treated

NDF - Neutral Detergent Fiber

NIRS- Near-infrared Reflectance Spectrophotometry

PLH – Potato Leafhopper

PLY – Potato Leafhopper Yellowing

TDNY- Total Digestible Nutrients

TSP – Total Soluble Protein

UT - Untreated

VSP - Vegetable Storage Proteins

w/w – weight to weight ratio

BACKGROUND

Cow-calf farming operations in the North Central region operate on narrow profit margins and good forage management greatly affects profitability. Forage production represents the majority of the expenses in these operations and timeliness of haymaking is a crucial factor affecting forage quality. Alfalfa (*Medicago sativa* L.) is preferred over other forages due to high feed value and good yields. Most of the alfalfa grown in the U.S. is comprised primarily of *M. sativa* and cultivars selected for use in the North Central region, were developed for 3 to 5 harvests per year. High forage quality and yields are obtained from *M. sativa* when first harvest occurs at 399 Growing Degree Days (GDD) base 5° C (Brink and Marten, 1989; Leep, 2004) and approximately every 30 days until September. If *M. sativa* cultivars are not harvested around 399 GDD, the forage quality diminishes rapidly (Anderson et al., 1973). Typically, the target harvest period for *M. sativa* is late-May. Conditions in Michigan at this time are less than ideal for curing hay. If first harvest could be delayed two to three weeks, producers could take advantage of longer days, higher temperatures and a lower probability of precipitation.

Medicago. sativa spp. *falcata* is also known as: falcata, sickle medic, Siberian alfalfa, sholteek, and yellow-blossom alfalfa, may be suitable as a hay species for one or two-cut systems in Michigan. While *falcata* is considered a subspecies of *M. sativa* L., it is often grouped with other perennials, due to its yellow flower and non-coiled (sickle-shaped) pods. *Falcata* can be either diploid or tetraploid and varies in biochemical and morphological traits (Quiros and Bauchan, 1988). Taxonomists describe the diploid form as multiple species or subspecies divisions including: *altissima, borealis, difalcata, erecta, glandulosa, romanica,* and *tenderensis.* While both diploid and tetraploid forms are sympatric in cold regions, tetraploid forms of *falcata* occur more frequently (Lesins and Lesins, 1964). It is also interesting to note that while well-

adapted to colder climates, *falcata* specific isozymes were found in cultivars thought to be purely *sativa* from African, Flemish, and Chilean origins (Quiros and Bauchan, 1988). Niels Hansen, a South Dakota Botanist, collected *falcata* seed from Siberia, Russia in the early 1900's and introduced the species to ranchers in South Dakota in 1915 (Brashier, 1999). These plants flourished, and in 1990 it came to the attention of area forage agronomists that some ranchers were selling seed of naturalized *falcata* which originated from seed distributed by Hansen. The naturalized *falcata* alfalfa survived droughts, extreme cold temperatures, and grazing pressure from livestock. It possessed a high tolerance to Potato leafhopper (PLH) feeding, and maintained good forage quality and yields even under one or two cutting per year systems (Boe et al., 1998). This germplasm possesses desirable characteristics which include increased winter-hardiness (Brashier, 1999) and grazing tolerance (Bittman and McCartney, 1994) over *M. sativa*. *Falcata* is characterized by a more prostrate growth habit with broad subsurface crowns and a more extensive root system than *M. sativa* (Heinrichs, 1973) which may contribute to greater winter-hardiness. Germplasm from naturalized *falcata* on the SD rangeland, several USDA *falcata* PIs, and *falcata* genotypes of 'Anik' and 'Kuban' were collected by Arvid Boe, Forage Breeder, South Dakota State Univ., Brookings, SD. Boe made selections from crosses of these genotypes and one accession (SD201) is more drought tolerant, maintains forage quality longer and has greater tolerance of Potato leafhopper (*Empoasca fabae*)(PLH) than Yellowhead and select *M. sativa* cultivars (Bortnem et al., 1993; Boe et al., 1994; Bortnem et al., 1994). The characteristics of SD201 may be desirable for delayed harvests cutting schedules in Michigan. Further evaluation of *falcata* as a forage crop in Michigan is warranted.

For any new forage crop to be widely accepted the production of above-ground biomass must be close to that of the existing forage crops or it must fill a niche forage need. Producers

must justify the change in forage management systems with some gain in profitability for the new crop to become widely accepted. Yield is the common parameter of interest for producers evaluating a change in production, and much of the breeding efforts for alfalfa have reflected this interest. Many of the early cultivar selections of Mediterranean origin were made of those possessing a fast rate of regrowth following harvest and/or those with multiple pest resistance, but these selections lacked winter-hardiness. In an effort to increase winter-hardiness, breeders incorporated alfalfa germplasm from northern regions which included *falcata.* The value of *M. falcata* was also observed as a forage crop for stockpiling in the Northern Plains where forage yield and quality was found to be equivalent to that of *sativa* (Bortnem et al., 1993; Boe et al., 1994).

While yield is generally considered the most important aspect in cultivar selection, the forage quality of the crop ultimately determines animal production. The primary role of alfalfa in most animal diet rations is that of fiber. Hemicellulose, cellulose, and lignin comprise the fiber portion of the plant, and these components fed to ruminants are critical for animal health, because ruminants require roughage in the diets to maximize production and maintain rumen. High levels of carbohydrates in the diet without fiber can cause acidosis and intake of fibrous plants by ruminants increases the production of saliva which buffers the rumen pH. Microflora essential for fiber degradation in the rumen require ample fiber to survive and proper amounts to flourish (Latham et al., 1972). While grasses are an important source of fiber, intake of alfalfa is greater than grasses because alfalfa has a higher percentage of cell solubles (non-structural) that are readily absorbed in the digestive system (VanSoest, 1982). However, the cell walls of alfalfa are highly lignified and less digestible than those of grasses. The neutral detergent fiber (NDF) fraction is generally associated with dry matter intake (DMI) due to the lower digestibility of this

component that triggers satiety or ruminant sense of fullness. Crude protein (CP) content of alfalfa is high relative to other forages and is defined as: total N x 6.25. The total N portion that comprises CP is primarily that of proteins that occur in the cell solubles (albumins and globulins) and cell walls (prolamines and histones) and non-protein nitrogen (peptides, free amino acids, amides and ammonia) (NRC, 2001). The leaf of alfalfa contains 2 to 3 times the CP content as stems (Mowat et al., 1965) and the leaf tissue does not lignify as much as the stem tissue as the plant matures. Most alfalfa plants with yellow or variegated flowers (*falcata* parentage) are characterized by higher leaf percentage than other cultivars; resulting in high-quality features (Bortnem et al., 1994; Boe et al., 1998). While *falcata* alfalfa has been shown to have lower forage quality levels than *sativa* in a three or four cut system (Riday and Brummer, 2002), we expect that a higher leaf:stem ratio of *falcata* in delayed cut systems will result in higher forage quality levels than *sativa*, because Canadian researchers have shown *falcata* can be stockpiled until mid-July without significant loss of forage quality (Baron et al., 2005). Finer stems and PLH-resistance may also be contributing factors to greater forage value of *falcata* in delayed cut systems (Juiler et al., 1995; Bliss, 2007).

Persistence of a perennial forage crop is another key factor determining profitability, because establishment costs are high for alfalfa. Producers expect alfalfa stands to persist beyond four years. A Saskatchewan study demonstrated that *falcata* alfalfa was more persistent than select grazing-type *sativa* alfalfa varieties (Bittman and McCartney, 1994). The Great Lakes region, however, has higher humidity and lower mean temperatures in the summer than does the Northern Plains, so there is a greater potential for disease problems. Aphanomyces root rot (*Aphanomyces euteiches*) and anthracnose (*Colletotrichum trifolii*) are common seedling diseases in cool, poorly drained soils of the northeast. They occur most often in older *sativa*

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varieties that lack resistance. These diseases and Phytophthora root rot (*Phymatotrichopsis megaspera*), Verticillium wilt (*Verticillium albo-atrum* or *V. dahliae*), Bacterial wilt (*Clavibacter michiganese*) are potential problems for *falcata* that may affect plant persistence in the region.

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CHAPTER 1

YIELD, QUALITY, AND PERSISTENCE OF *MEDICAGO SATIVA* SPP. *FALCATA*, *M. SATIVA* ALFALFAS AND BIRDSFOOT TREFOIL UNDER THREE HARVEST FREQUENCIES

ABSTRACT

Medicago sativa spp. *falcata* alfalfa possesses indeterminate growth characteristics which may result in prolonged quality and equivalent yield to that of conventional alfalfa (*Medicago sativa* spp. *sativa* L.) when harvest is delayed. The objectives of this study were to evaluate dry matter yield (DMY), quality (crude protein [CP], acid detergent fiber [ADF], neutral detergent fiber [NDF]) and stand persistence of *falcata* 'SD201' under three harvest frequencies (one, two, and three per year) compared to conventional *sativa* 'WL346LH' and birdsfoot trefoil (*Lotus corniculatus* L.) 'Norcen' at East Lansing (EL) and Lake City (LC), MI. One or two harvests per year of *falcata* usually produced equivalent or greater DMY of three harvests of *sativa*. Norcen DMY was typically lower than *sativa* or *falcata* within each harvest frequency. The CP and NDF concentration within a harvest frequency did not differ. However, CP concentration of one or two harvests per year of *falcata* was lower than three harvests per year of *sativa* or Norcen, except at LC in 2008 when two-harvest *falcata* was not different than three-harvest *sativa*. The ADF concentrations of *falcata* were usually equal or less than *sativa* in two or three harvests per year. Harvest frequency had no effect on plant density during the four years of this study and by the end of the study; stand densities were similar for alfalfa cultivars while BFT stand densities were lower. *Falcata* has DMY potential as a crop for delayed harvest systems but high NDF concentrations may limit dry matter intake.

INTRODUCTION

Forages fuel a multi-billion dollar animal industry in the United States with annual values of beef cattle/sheep production valued at $49.6 billion and dairy production at $23.5 billion (USDA-NASS, 2008). Alfalfa is a very good forage species due to high feed values, high yields, and multi-year production capabilities. However, cutting, curing, baling, and handling alfalfa require large amounts of time and energy. Harvest operations are typically 30% of production costs and the cost of mowing/conditioning, raking and baling, based upon average custom rates in Michigan, is approximately $74/ha (Stein, 2009). Optimal quality and maximum yield of current *M. sativa* varieties is obtained when first harvest occurs at 399 Growing Degree Days (GDD) base 5° C (Weir et al., 1960; Allen, 1996; Leep, 2004) and approximately every 30 days thereafter (Brink and Marten, 1989; Sheaffer et al., 1997). Four cuttings of alfalfa per year in Michigan is a common practice and this harvest schedule produces high yield and excellent quality of current conventional alfalfa cultivars with the assumption that forage is being fed to high-producing lactating dairy cattle. A large percentage of the forage produced in Michigan, however, is utilized by livestock on maintenance diets. The dietary requirements of dry dairy cows and beef cows are lower than high-producing, lactating dairy cows. Producers feeding livestock on maintenance diets would benefit from a forage species that could be harvested later in the season without great losses in forage quality. Early-summer stockpiling would increase flexibility of harvest schedules and reduce production costs by reducing the total number of harvests per year. Typical timing of first harvest *sativa* occurs in the North Central region when conditions are less than optimal for curing hay, due to high humidity, low temperatures and short day lengths. In Michigan, the potential evapotranspiration rates less precipitation is

approximately two times greater in late June than in late May due to greater day-length and higher temperatures. Furthermore, the moisture content of the soil surface is lower in late-June than in late-May, reducing the potential for compaction during harvest. The superior forage quality characteristics of *sativa* over other forage species declines with delayed harvests (Ingalls, 1964; Sheaffer et al., 2000) but a forage crop with a less determinate growth habit may retain forage quality.

Birdsfoot trefoil (*Lotus corniculatus* L.) (BFT) is a leguminous perennial with an indeterminate growth habit that produces good animal gains (Marten et al., 1987). A good forage crop for acidic soils and poorly drained sites, BFT will produce 2/3 the dry matter yield (DMY) of *M. sativa* on well-drained soils (Beuselinck and Grant, 1995). Additionally, BFT is a legume that does not cause bloat and is a good grazing alternative with equivalent animal gains per area to alfalfa in the North-Central USA (Marten et al., 1987), however, it can be difficult to establish (Seaney and Henson, 1970; Smith et al., 1986) and stand loss is a problem due to root diseases (English, 1999).

Falcata alfalfa, also known as: sickle medic, sholteek, and yellow-blossom alfalfa, may also be suitable as a hay species for one or two-cut systems. *Falcata* is considered indigenous to Siberia (Oakley and Graver, 1917), but diploid forms may occur in northern Europe (Lesins and Lesins, 1964). *Falcata* is more winter-hardy than *M. sativa* (Heinrichs, 1973) which may be due to a more shallow and fibrous root system (Berdahl et al., 1989). A study comparing *M. sativa*, subspecies *sativa*, *caerulea*, *falcata* and *varia* in south-eastern Australia found *M. falcata* entries to be least productive and persistent (Li et al., 2010). While some *falcata* germplasm has distinct

agronomic limitations, including slow regrowth following harvest, low seed production, and decumbent growth (Riday and Brummer, 2002), the indeterminate characteristic may contribute to greater yields and prolonged forage quality when first harvest is delayed. Many conventional alfalfa cultivars possess some *falcata* germplasm and crossing between the two species occurs frequently (Small and Brookes, 1984). Riday and Brummer (2002) in the US and Mackie et al. (2005) in Australia have recognized value of *falcata* in crosses with sativa for increased DMY due to heterosis. Alfalfa cultivars possessing high levels of *falcata* germplasm are recommended in the Northern Great Plains for increased in forage yield, quality (Berdahl et al., 1989) and persistence (Bittman and McCartney, 1994). The falcata cultivar 'Yellowhead' space-planted into an existing grass pasture increased DMY by as much as 185% compared to grass pasture with no legume in North Dakota (Hendrickson et al., 2008). Boe et al. (1994) compared DMY, CP, and *in vitro* dry matter digestibility of *falcata* and *sativa* cultivars in a space-planted trial under a delayed harvest system and determined that *falcata* resulted in greater DMY than the *M. sativa* cultivars. Observations in this study also revealed less evidence of potato leafhopper (*Empoasca fabae* Harris) (PLH) feeding damage in *falcata* than *sativa* cultivars (Bortnem et al., 1993). Selections from the *falcata* PI's were found to be more drought tolerant, maintain forage quality longer and exhibit less PLH feeding damage than current *sativa* cultivars (Bortnem et al., 1993; Boe et al., 1994) and 'SD201' was selected from these PI's for summer stockpiled forage (Boe et al., 1998).

Several important environmental benefits may result from a reduced/delayed-harvest system including: prolonged cover for nesting game birds, waterfowl, and song birds (Bortnem et al., 1993; Boe et al., 1998), additional sources for nectar and pollen for honey bees (*Apis* species), increased ground cover due to fewer defoliation events, and reduced soil compaction

from fewer equipment passes over the field. If these secondary benefits are realized, they alone may justify the use of *falcata* in production fields of North America.

The ability to obtain the DMY equivalent of three or four cuttings of *sativa* in one or two cuttings of *falcata* would be very desirable for producers. A first harvest delayed until late-June would take advantage of higher temperatures; resulting in more rapid drying and decreasing curing time. Less curing time would reduce the probability for losses due to rain. However, for producers to adopt this technology, they will need answers to several key questions regarding the value and impact of this species on forage production, including: DMY potential, forage quality, and stand persistence when fewer than three harvests are taken per year in Michigan. The objectives of this study were to evaluate DMY, forage quality and stand persistence of *falcata* under one, two and three-harvests per year compared to a current high-yielding *sativa* alfalfa and BFT.

MATERIALS AND METHODS

Site Description

Two trials with 'SD201' a tetraploid *M. sativa* spp. *falcata,* seed obtained from Arvid Boe at South Dakota State University, 'Norcen' birdsfoot trefoil, an upright, public variety, and 'WL346LH', a high-yielding, sixth generation leafhopper-resistant, commercially-available *sativa* alfalfa (characterized as having: a flower color (Syn 2) is 84% purple, 14% variegated, 1% cream and 1% yellow with a trace of white) from WL Research of Madison, WI were established (17 May, 2005) in East Lansing (EL) (42.4 N, 84.3 W) on a Capac soil (fine loamy, mixed, mesic Hapladauf) and in Lake City (27 May 2005) (44.2 N, 84.1 W) on a Nester soil (fine sandy

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loam, mixed Typic Eutroboralfs) in spring 2005. A soil test (pH, phosphorus, potassium, magnesium, calcium, and cation exchange capacity) at each location was conducted using a composite sample from 20 cores (20-25cm depth) randomly located within the trial area. No lime was required at either location according to soil tests. Fertility was maintained by annual applications of 102 kg ha^{-1} of P_2O_5, 306 kg ha^{-1} of K_2O and 3.9 kg ha^{-1}of B following the July harvest. Conventional tillage (moldboard plow followed by secondary tillage, followed by cultipacking) was used to prepare the seedbed for the planting. Prior to seeding, *sativa* and *falcata* seed was inoculated with *Sinorhizobium meliloti* and birdsfoot trefoil with *Rhizobium loti* (Urbana Labs, Urbana, IL). A 0.9 m-wide Carter self-propelled nursery seeder (Carter Manufacturing, Brookston, IN) was used to plant the 7.6-m-long plots. The following seeding rates were used: 17.9 kg ha^{-1} for *sativa* and *falcata* and 11.2 kg ha^{-1} for BFT as per seeding recommendations of Blumenthal and McGraw (1999). Three harvest frequencies were used: three harvests with the first in early June, the second in mid-July and the third in early-Sept.; two harvests with the first in late-June and the second in late-August; and one harvest in mid-July. Actual harvest dates for each planned harvest frequency varied by year and location due to weather conditions (Table 1.1).

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Table 1.1. Harvest dates and growing degree day accumulation (GDD base 5°C since Mar.1 for first harvest or since previous harvest for second and third harvests) for the three planned harvest frequencies for each year (2006-2008) at East Lansing and Lake City, MI.

	East Lansing			Lake City		
			Three-harvest			
2006	9-Jun (586)	19-Jul (618)	5-Sep (770)	8-Jun (478)	24-Jul (630)	6-Sep (577)
2007	11-Jun (671)	25-Jul (675)	12-Sep (789)	7-Jun (538)	20-Jul (633)	5-Sep (744)
2008	11-Jun (554)	17-Jul (545)	9-Sep (848)	17-Jun (549)	22-Jul (495)	14-Oct (941)
			Two-harvest			
2006	20-Jun (738)	21-Aug (1026)	-	22-Jun (648)	22-Aug (849)	-
2007	20-Jun (823)	29-Aug (1113)	-	28-Jun (859)	5-Sep (1056)	-
2008	26-Jun (753)	2-Sep (1094)	-	24-Jun (627)	18-Sep (1169)	-
			One-harvest			
2006	7-Jul (990)	-	-	10-Jul (891)	-	-
2007	11-Jul (1152)	-	-	20-Jul (1170)	-	-
2008	14-Jul (1032)	-	-	22-Jul (1044)	-	-

East Lansing: Michigan Automated Weather Network, www.agweather.geo.msu.edu/mawn/
Lake City: Weather Underground, www.wunderground.com

Experimental Design

Trials were arranged in a split-plot design with harvest frequency as the main plot and plant species as the sub plot. Each plot consisted of two separate planter passes with a 0.3 m gap, so that destructive sampling (plants excavated for counts) could be obtained from one-half of the plot, while DMY was collected from the other half of the plot. Sethoxydim herbicide (0.28 kg a.i. ha^{-1}) + crop oil concentrate (0.95 L ha^{-1}) was applied in the seeding year on 16 Jun 2005 at EL and 17-Jul-2005 at LC to control annual grass weeds, and imazethapyr herbicide (0.07 kg a.i. ha^{-1}) + non-ionic surfactant (0.25 v/v) + AMS (1.9 kg ha^{-1}) was applied on 8 May 2006 at EL and 9-May 2006 at LC to control annual grass and broadleaf weeds. Cyfluthrin insecticide (0.028 kg a.i.ha^{-1}) was applied on 7 Jul 2005 at EL and 14 Jul 2005 at LC. In subsequent years, control of AW and PLH was by dimethoate (0.28 kg a.i.ha^{-1}) applied once in mid-May to all

treatments, and approximately 14 d after the first and second harvest of the two- and three-cut harvest frequencies except in EL in 2007 when the first application was not made until 19-Jul.

Yield and Yield Parameters

Above ground biomass was collected with a rotary flail harvester (Carter Manufacturing Co. Inc., Brookston, IN) set to harvest 0.9 by 7.6 m plots at a cutting height of 10 cm. Dry matter content of harvested biomass was determined by collecting a subsample which was weighed wet and then dried at 60° C for 96 h in a forced-air oven and weighed again. Forage yield was adjusted to a dry matter basis (DMY= plot yield x percentage dry matter). To explain DMY differences a 0.09 m^2 area from each plot was clipped at approx. 10 cm from the soil surface at EL in 2008 and shoots were counted. Mass per shoot was obtained by counting approximately 50 shoots, drying them at 60° C for at least 72 h and weighing (shoot $mass^{-1}$=total mass/no. of shoots). Leaf-to-stem ratios were also determined on a weight-to-weight basis on the shoot mass samples. Leaves were separated from approx. 50 shoots. Components were dried for a minimum of 72 h at 60° C and then weighed (Leaf-to-stem ratio= dry leaf weight/dry stem weight). Shoots $plant^{-1}$ were estimated at EL in 2008 as well, by dividing the shoots m^{-2} by the average spring and fall plant count m^{-2}.

Tissue Analysis

Samples were captured from biomass harvested at each harvest and dried at 60° C for a minimum of 72 h. Dried samples were ground to pass through a 1-mm screen in a Christy-Turner Lab Mill (Ipswich, Suffolk, UK) and a minimum subsample of 30 g was retained for nutritive analyses. Each sample was scanned with a FOSS 6500 near-infrared spectrophotometer (NIRS)(FOSS NIRSystems, Inc., Eden Prairie, MN) with wavelengths between 800 and 2500 nm

and reflected wavelengths were recorded using the WinISI v. 1.5 software. Crude protein (CP), ADF, and NDF were predicted from equations developed by regression of chemically derived and spectral data using a modified partial least squares regression method (Shenk and Westerhaus, 1991). Two randomly selected subsets were compiled to calibrate and validate the NIRS prediction of dry matter (DM), CP, NDF, and ADF (Table 1.2). Dry matter content was determined by drying 0.5 g of sample in ceramic crucibles at 100°C for 24 h. Samples were then ignited in a muffle furnace at 500°C for 6 h to determine ash content. Total nitrogen was determined by the Hach modified Kjeldahl procedure (Hach et al., 1985; Watkins et al., 1987), and CP was estimated by multiplying total N by 6.25. The Van Soest and Robertson method (1985) for fiber determination was used for NDF and ADF determination with the addition of 1 ml of alpha-amylase to the neutral detergent solution at the onset of boiling. The weighted average (WA) for each nutritive constituent was calculated as (using CP as example):

$WACP = (CP_i \times DMY_i)/\Sigma DMY_i$ where WACP represents the WA of CP (g kg^{-1} DM), CP_i represents the CP concentration of harvest i, and DMYi represents dry matter yield of the harvest i.

Nutrient yield is expressed as crude protein yield (CPY) Mg ha^{-1} (CP g kg^{-1} x DMY Mg ha^{-1}) and total digestible nutrient yield (TDNY) Mg ha^{-1} (TDN g kg^{-1} x DMY Mg ha^{-1}). The formula used for TDN (g kg^{-1}) was: 82.38-(0.7515 x (NDF g kg^{-1} -3.41) / 1.1298. Values are reported as weighted averages.

Table 1.2. Near-infrared reflectance spectroscopy calibration and validation statistics for crude protein, acid detergent fiber, and neutral detergent fiber for legume stands harvested over three years (2006-8) in Lake City and East Lansing, MI.

Constituent	n	Mean	SEC†	SECV‡	r^2 §	1-VR¶
			----------------- g kg^{-1} -----------------			
Crude protein	73	141	4.2	4.9	0.95	0.91
Acid detergent fiber	54	314	11.0	10.9	0.97	0.94
Neutral detergent fiber	47	487	6.2	12.7	0.99	0.97

†SEC, standard error of calibration.
‡SECV, standard error of cross validation
§r^2, coefficient of determination for calibration
¶ 1-VR, 1 minus the variance ratio in cross validation during modified partial least squares regression

Persistence

Stand persistence was determined in the spring and fall of each production year by digging and counting plants in two randomly placed 0.093 m^2 quadrants of each treatment from the portion of the plot designated for destructive sampling. Plant counts were totaled for both quadrats, so that a total of 0.186 m^2 was sampled per plot at each sampling event. Shoots per plant was estimated for the first harvest at EL in 2008 by: shoot m^{-2} / average number of plants m^{-2} in the spring and fall of 2008.

Data Analysis

All data were tested for normality and unequal variances using PROC UNIVARIATE based on the Shapiro-Wilk statistic, observations of the stem and leaf plot and the AIC statistic. Analysis of variance was performed on harvest frequency (main plots) and species (subplots) as well as interactions with the PROC MIXED procedure software version SAS 8.2 (SAS Institute, 2003) using the Kenward-Rogers method for determining degrees of freedom. Years were considered fixed effects. Main effects and interactions were considered significant when $P <$ 0.05. Means were calculated and presented as least square means. The SAS macro 'PDmix80' was used to generate an letter separations based upon contrasts (Saxton, 1998). Regression analysis via PROC REG was used to test the relationships between yield and stem mass or plant counts.

RESULTS AND DISCUSSION

Forage yield

The main effects of species and harvest frequency were highly significant (Table 1.3). Year x harvest frequency or year x species interactions had greater significance than location x harvest frequency or location x species, but due to less dense stands of BFT at LC, yield data will be presented by year and location for species within harvest frequencies.

Table 1.3. Results from ANOVA for dry matter yield (DMY), crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), crude protein yield (CPY), total digestible nutrients yield (TDNY) and stand density of *falcata*, *sativa*, and BFT under three harvest frequencies (one, two and three harvests per year) over two locations (East Lansing and Lake City, MI) and three years (2006-2008).

Source	DMY	CP	ADF	NDF	CPY	TDNY	Density
Species (S)	***	***	***	***	***	***	** *
Harvest Freq. (HF)	***	***	***	***	***	***	ns
Location (L)	***	ns†	***	*	***	***	ns
Year (Y)	***	***	ns	***	***	***	***
S x HF	***	ns	*	ns	***	***	ns
S x Y	***	*	**	**	***	***	***
HF x Y	***	***	***	***	***	***	ns
S x L	ns	***	***	ns	ns	*	***
HF x L	*	ns	*	**	***	ns	*
HF x S x L	ns	ns	*	ns	ns	ns	*
HF x S x Y	ns	ns	*	**	ns	ns	*
S x HF x L x Y	ns	ns	**	**	ns	ns	ns

*Significant F tests at the P< 0.05 level
**Significant F test at the P<0.01 level
***Significant F test at the P<0.001 level
†ns = not significant

Yields of species, within a harvest frequency, were similar over the years at EL and there were no year x species or year x harvest frequency interactions, but they remain separate to make comparisons between years to LC. The EL location was harvested in 2005, but harvest frequency treatments had not been imposed. The dry matter yields by species at EL on 24 Aug 2005 were 7.23, 6.63, and 5.46 Mg ha^{-1} for *falcata*, *sativa* and BFT, respectively. The greatest DMY in the production years were obtained at EL (Fig 1.1), likely due to greater (> 43mm) rainfall May-Aug 2006-2008 (Fig. 1.2) and greater average temperatures at EL (Table 1.4), and more droughty soils at LC. The greatest yields for both locations occurred in the first production year. Top-production of alfalfa in the first full production year (second year) is well documented in three, four and five harvests per year (Vaughn et al., 1990; Kallenbach et al., 2002).
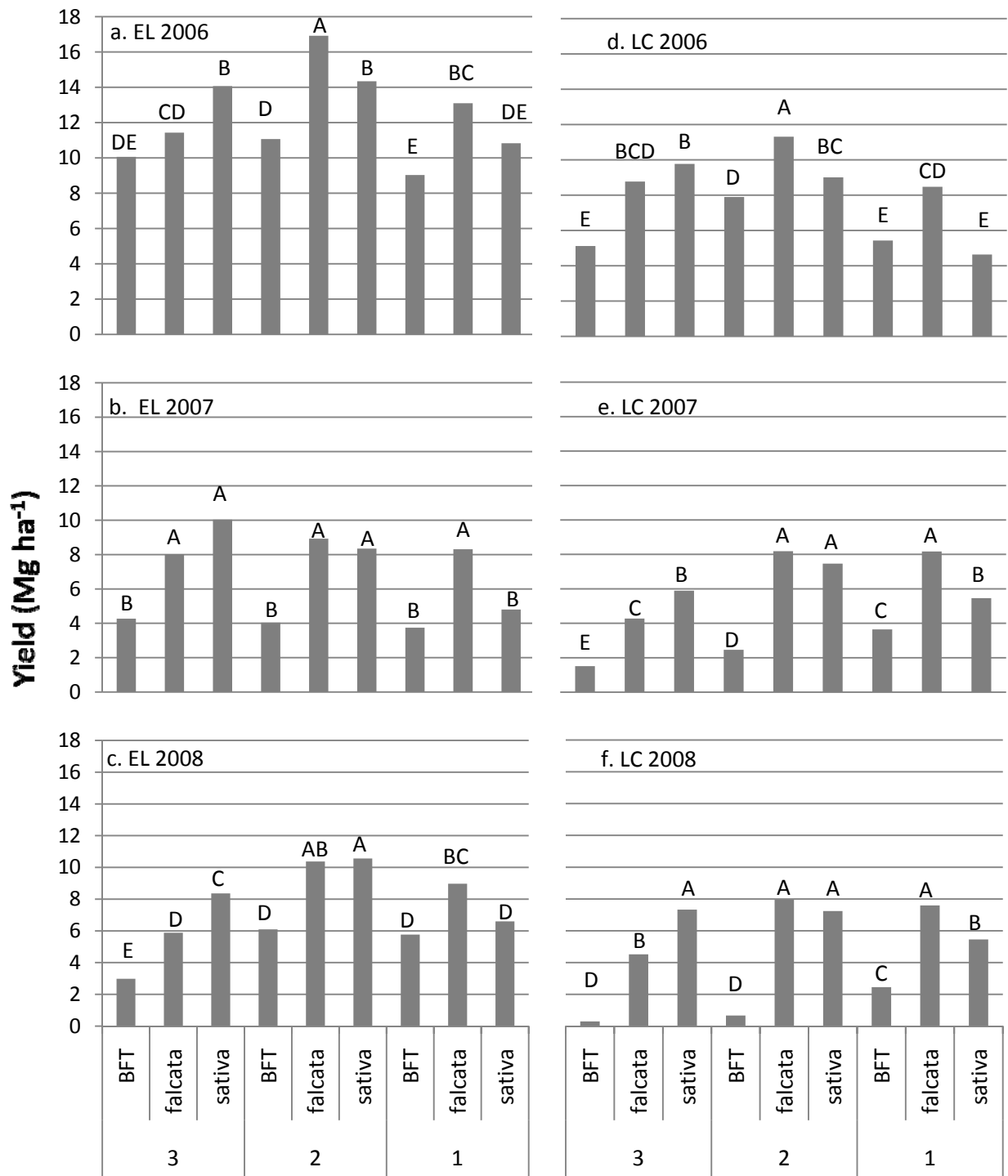
Figure 1.1. Total dry matter yield of *falcata*, *sativa*, and BFT under three harvest frequencies (three, two and one per year) for three production years (2006-2008) for East Lansing (EL) and Lake City (LC), MI. Means with the same letter are not significantly different at the 0.05 level of probability.

Table 1.4. Monthly and 30-yr. avg. air temperatures at East Lansing and Lake City, MI during the growing seasons 2005-2008. Temperatures are averages of daily maximums and minimums observed for each month.

Month	East Lansing					Lake City				
	2005	2006	2007	2008	30-yr avg.	2005	2006	2007	2008	30-yr avg.
	°C					°C				
APR	8.2	10.5	7.6	10.3	7.6	7.4	7.6	4.2	7.4	5.2
MAY	12.6	15.0	16.1	12.7	14.1	10.1	12.9	13.2	10.3	12.0
JUN	22.6	19.7	20.9	20.6	19.2	20.7	17.2	18.7	17.9	16.9
JUL	22.7	23.2	21.1	21.9	21.4	20.4	20.8	19.4	19.6	19.4
AUG	22.7	21.8	22.2	20.9	20.3	19.1	19.1	19.7	18.6	18.2
SEP	19.3	15.5	18.4	18.4	16.1	16.3	13.3	15.1	14.8	13.6
OCT	11.7	8.9	13.5	9.9	9.7	9.4	6.0	11.4	7.1	7.6

Source: Michigan Automated Weather Network http://www.agweather.geo.msu.edu/mawn/
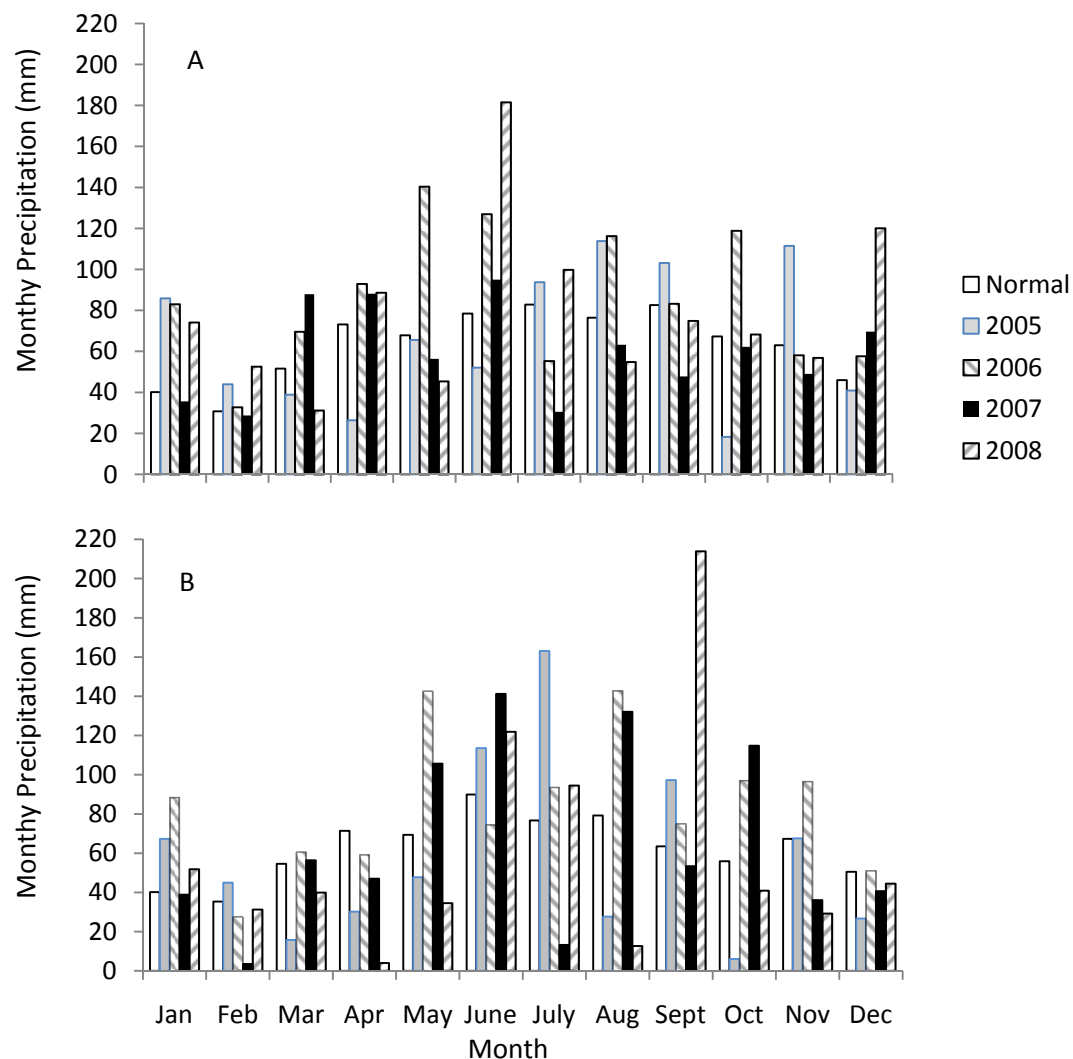
Figure 1.2. Monthly rainfall totals and the thirty (30) yr average for Lake City (A) and East Lansing (B), MI from 2005-2008. Source: Michigan Automated Weather Network

Two harvests per year of each cultivar tested produced as much or more DMY as three harvests per year. Two harvests per year of *falcata* produced greater DMY than three harvests of *falcata* in all but one year (2007 at EL). For both locations in 2006, two harvests per year of *falcata* produced greater yields than any other treatment with 16.9 and 11.3 Mg ha^{-1} in EL and LC, respectively. The next closest treatment was 2.6 Mg ha^{-1} less at EL and 1.5 Mg ha^{-1} at LC. On average, DMY of three harvests per year of *falcata* was 67% of the two harvest *falcata* treatment. A single harvest of *falcata* produced greater DMY than a single harvest of *sativa* or BFT in every year. Across years and locations, a single harvest per year of *falcata* produced 84% of the total DMY of the *falcata* two-harvest treatment and 25% greater yield than three harvests. Two harvests per year of *sativa* typically produced as much DMY as three harvests per year and exceeded three harvests at EL in 2008 and LC in 2007. However, a single harvest per year of *sativa* usually produced less DMY than two or three harvests per year which may be due to the determinate growth characteristics of *sativa* and leaf senescence. The leaf-to-stem ratios obtained in 2008 support the notion of reduction in leaf mass with increasing shoot age (Fig. 1.3).

These findings concur with those of Boe et al. (1994) , that *M. falcata*-type alfalfa produces greater DMY than *sativa*-type alfalfas when a single delayed harvest is taken. Our study, however, reveals that greater DMY can be obtained from two harvests per year of *falcata* than one harvest per year. Others have tested cultivars with a lower percentage of falcata and found similar results, including: Kust and Smith (1961) who found greater yields of 'Vernal', which contains higher levels of *falcata* (Barnes et al., 1977), in fewer harvests and Hendrickson et al. (2008) who found that a *falcata* cv.,'Yellowhead', and Vernal, a conventional cv with lesser amounts of *falcata* germplasm, produced more total biomass than *sativa* when clipping

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was delayed until flowering. Our findings also suggest that the total annual DMY of *falcata* can be reduced when harvest occurs early in the growing season (early-June). This is evident in lower DMY when *falcata* was harvested three times per year as compared to two harvests per year. There was very little above-ground biomass production of *falcata* in any of the harvest frequencies when a harvest occurred after Jul. (data not shown). Alfalfa with higher levels of *falcata* germplasm may not be storing the necessary root reserves for regrowth following defoliation as suggested by Barber et al. (1996), and instead, expends energy in continued growth of above-ground biomass.

The average DMY of the highest yielding BFT treatment (two-harvest) was only 55% of two harvests of *falcata*. BFT DMY was greatest in both locations in 2006 and declined in subsequent years. Total DMY was lower in LC each year due to thinner stands and lower temperatures than EL. BFT harvested once per year produced greater DMY than two or three harvests in 2007 and 2008 in LC. In 2006, at EL and LC two harvests of BFT produced greater DMY than one harvest. There were no differences in DMY due to harvest frequency at EL in 2007 and in 2008 two or three harvest per year of BFT produced similar DMY. In every year, BFT produced the least DMY among species in two or three harvests per year at both locations. One harvest per year of BFT produced equivalent DMY to one harvest per year of *sativa* in every year at EL and in 2006 at LC. While the cutting height was approximately 10 cm, a greater DMY from BFT in the two or three harvest frequency may have been obtained if cutting heights had been increased to 15.2 cm as suggested were in previous studies (Smith and Soberalske, 1975; Alison and Hoveland, 1989). This may have allowed for greater leaf area to support regrowth.

The BFT DMY results concur with the findings of Beuselinck and Grant (1995), that BFT produces about 2/3 the DMY of alfalfa on well-drained soils. This work should not discount the value of BFT on acidic and poorly drained soils.

Figure 1.3. Average leaf-to-stem ratio (w/w) for *falcata* and *sativa* under three harvest frequencies at East Lansing, MI in 2008. Means with the same letter are not significantly different at the 0.05 level of probability.
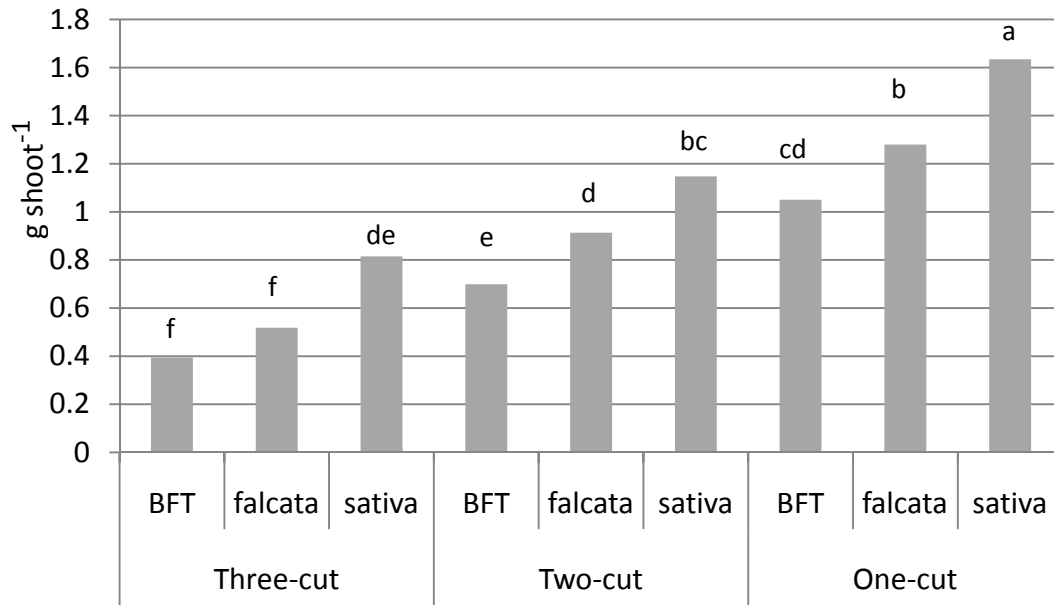
Figure 1.4. Average shoot mass of birdsfoot trefoil (BFT), *falcata*, and *sativa* under three harvest frequencies in 2008 at East Lansing, MI. Means with the same letter are not significantly different at the 0.05 level of probability.
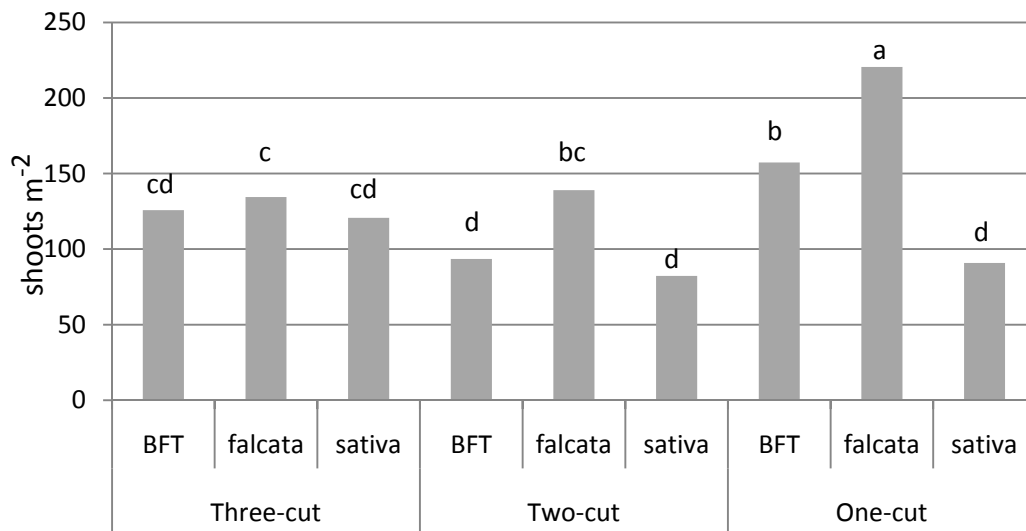
Figure 1.5. Average shoot density over harvests of birdsfoot trefoil (BFT), *falcata*, and *sativa* alfalfa under three harvest frequencies in 2008 at East Lansing, MI. Means with the same letter are not significantly different at the 0.05 level of probability.

Although there were year x species and year x harvest frequency interactions, it is important to compare multi-year total DMY production when comparing perennial forages. Two-harvests per year of *falcata* produced the greatest three-year total DMY of any species under any harvest regime at LC, but it was not significantly greater than two harvests of *sativa* at EL (Table 1.5). BFT total DMY production was lowest within each harvest frequency for both locations. Two harvests per year of *falcata* produced 11% and 19% greater yields than *sativa* harvested three times per year, at EL and LC, respectively. The total three-yr DMY from a single harvest per year of SD201 was not lower yielding than three harvests per year of *sativa*. The three harvest regime limited the total DMY of *falcata* to only 65 and 70% the production of two-harvest *falcata*, at LC and EL, respectively. The total DMY production of *falcata* suggests that it may be a viable option for producers desiring a forage species that may be harvested fewer times per year in Michigan if forage quality levels meet the needs for livestock. A better understanding of the physical characteristics of *sativa* and *falcata* in these harvest frequencies will help us explain differences in DMY.

Table 1.5. Three-yr. (2006-2008) total dry matter yield of three forage species (birdsfoot trefoil (BFT), *falcata*, and *sativa* under three harvest frequencies (HF) (three, two and one harvest per year) at East Lansing (EL) and Lake City (LC), MI. Letter designations (uppercase for EL, lowercase for LC) are for mean comparison within a year at the 0.05 level of probability.

HF	Species	EL	LC
		----Mg ha^{-1} ----	
	BFT	17.31 E	6.93 e
Three	falcata	25.33 C	17.57 c
	sativa	32.47 B	22.99 b
	BFT	21.20 D	11.02 d
Two	falcata	36.21 A	27.45 a
	sativa	33.25 AB	23.71 b
	BFT	18.54 DE	11.53 d
One	falcata	30.38 B	24.24 b
	sativa	22.23 CD	15.55 c
	Mean	26.30	17.90

Yield parameters

A comparison of yield parameters (shoot mass, shoot density, and shoots per plant) may provide an explanation for differences between *sativa* and *falcata*, and are presented for 2008 at EL. The average mass shoot^{-1} increased for all species as the number of harvests per year decreased (Fig. 1.4). The average mass shoot^{-1} for the single harvest of cultivars was twice that of the mass shoot^{-1} for the three-harvest treatment within each species. *Sativa* had the greatest mass shoot^{-1} for each harvest frequency and BFT had the least. Although a single harvest per year of *falcata* produced greater DMY than a single harvest of *sativa* at EL in 2008, it is not reflected in shoot mass, since *sativa* had 27% greater mass. However, there was no gain in shoots m^{-2} in *sativa* with fewer harvests, while *falcata* shoots m^{-2} increased with decreasing harvest frequency (Fig.1.5). The greatest shoot density of *falcata* was in one-harvest per year which had 81 more shoots m^{-2} than two-harvests per year and 97 more shoots m^{-2} than three harvests per year. Positive linear relationships were also observed between plants m^{-2} and DMY in 2006 and 2007 (Table 1.6). These relationships were highly significant in all but 2008. As in Berg et al. (2005) shoots m^{-2} were not closely associated with yields in 2008 (Table 1.6). Although plants m^{-2} and shoots m^{-2} were not taken simultaneously, based on the average of spring and fall plant counts, the shoots plant^{-1} data showed *falcata* and BFT were numerically greater than *sativa* (Fig.1.6) in each harvest frequency. Shoots plant^{-1} of *falcata* and BFT harvested one time per year was greater than *sativa* harvested twice a year. While not statistically significant *falcata*

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produced numerically more shoots plant^{-1} within a season as harvest is delayed and additional

observations may explain the DMY advantage of *falcata* over *sativa* when harvest is delayed.

This contrasts the findings of Small and Brookes (1984) who observed a greater number of

shoots per crown in *sativa* than *falcata* at an unspecified period in the seeding year. While mass

shoot^{-1} of *falcata* harvested once per year is actually lower than that of *sativa* (Fig. 1.4), the

shoots m^{-2} (Fig. 1.5) are greater and may explain the greater DMY (Fig. 1.2c). While an

understanding of why a forage species produces greater DMY is important, ultimately, forage

quality is the determinate parameter of animal performance.

Table 1.6. Regression equations, R^2 values, and *P* values for the influence of plants m^{-2} (avg. of spring and fall counts) on total annual DMY (2006-2008), and mass shoot^{-1} and shoots m^{-2} on first harvest DMY (2008) at East Lansing, MI.

Year	Plants m^{-2}	R^2	*P* value			
all years	y=0.036x + 3.68	0.38	<0.0001			
2006	y=0.029x + 6.13	0.40	<0.0001			
2007	y=0.034x + 2.54	0.46	<0.0001			
2008	y=0.022x + 4.33	0.07	0.016			
	Shoots m^{-2}	R^2	*P* value	Mass shoot^{-1}	R^2	*P* value
2008	y=0.020x + 2.11	0.14	0.01	y=1.467x + 3.71	0.10	0.046
2008	Both shoots m^{-2} and Mass shoot^{-1}				0.38	0.0012
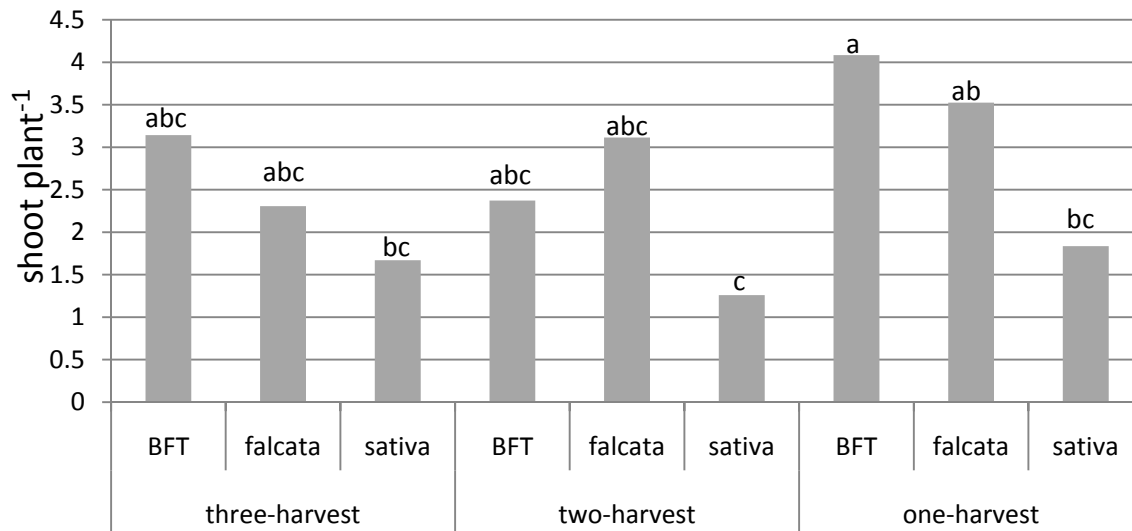
Figure 1.6. Shoots per plant of birdsfoot trefoil (BFT), *falcata*, and *sativa* in the first harvest of 2008 for three harvest frequencies in East Lansing, MI. Means with the same letter are not significantly different at the 0.05 level of probability.

Forage quality

The main effects of harvest frequency and species were significant for all measured

constituents (Table 1.3). The harvest frequency x species interaction was significant for ADF and

NDF, but not for CP. However, because comparison between species within a harvest frequency

is one of the objectives of the trial, all forage quality values are presented for species within a

harvest frequency. A highly significant ($P< 0.01$) location x year x harvest frequency interaction

required a separate analyses of locations and years for both CP and fiber components. These

interactions may be due to variation in GDD accumulation at harvest (Table 1.1) or precipitation

(Fig. 1.1) over years at both locations.

Crude protein

In each year of both locations, CP concentration declined with fewer harvests (Fig. 1.7)

with the exception of EL in 2008 and LC in 2006, where there were no significant differences

from two to one harvest per year. A decline in CP with advancing maturity is well-documented

and is primarily associated with decreased leaf:stem ratio and increase in stem lignin and cell wall materials (Kilcher and Heinrichs, 1974; Kalu and Fick, 1983). Because leaves contain two to three times the CP concentration of stems (Mowat et al., 1965) and less fiber than stems, a reduction in total CP and an increase in total fiber occurs as the leaf-to-stem ratio decreases (Hatfield et al., 2007).

The CP concentration of *sativa* at LC decreased with fewer harvests (Fig. 1.7) from an average of 157 g CP kg^{-1} for three harvests to an average of 123 g CP kg^{-1} for one harvest. The greatest decrease (56 g kg^{-1}) in CP concentration from three to one harvest per year of *sativa* occurred in LC in 2007 when the one-harvest cutting was not taken until 20-Jul (1170 GDD's). The CP concentration loss from three to two harvests varied from as little 7 g kg^{-1} at LC in 2006 to as much as 30 g kg^{-1} at EL in 2008. Reducing the number of harvests of *sativa* from two to one per year usually resulted in decreased CP from 3 to 39 g kg^{-1}, except for LC in 2006 when CP concentration increased in the one harvest treatment. Above-average rainfall and temperatures prior to the Jul harvest may have encouraged formation of vegetative shoots increasing CP concentration.

The CP concentration of *falcata* was similar to that of *sativa* within a harvest frequency in all but EL in 2006 when it was lower than *sativa* in the two harvest treatment (Fig. 1.7). While leaf retention appears to be greater in *falcata* than *sativa* when harvests are delayed (personal observation), *falcata* exhibited similar leaf-to-stem ratios to *sativa* in all but the two-harvest treatment where it was lower than *sativa* (Fig. 1.3). Our leaf-to-stem ratios were taken

chronologically later than, and contrast those, of Lenssen et al. (1991), who found that *falcata* harvested at two to three bud stage, had a greater leaf-to-stem ratio (w/w) than *sativa* harvested at the same stage. It may be that *falcata* has numerically more leaflets, but they are lower in surface area than *sativa*. The CP concentration of two-harvest, *falcata* was consistently lower (9 to 36 g kg^{-1}) than that of three-harvest, *sativa* in all but LC in 2008, which may be explained by the 26% greater leaf to stem ratio of *sativa* (Fig. 1.3). Although lower than *sativa* harvested three times or BFT at any of the cutting frequencies tested, the CP concentration of *falcata* harvested twice (avg. 134 g kg^{-1}) meets the dietary needs of most livestock on maintenance diets.

The highest CP within a harvest frequency was typically obtained from BFT. Although not statistically greater than *sativa* or *falcata* in all comparisons, the CP concentration of BFT harvested one, two, or three times per year was numerically greater than *sativa* or *falcata* in 13 of 18 possible comparisons (locations [2] x harvest frequencies [3] x years [3]). BFT produced biomass with significantly greater CP concentrations than *falcata* when harvested three times at EL and LC in 2007 and greater than *sativa* at LC in 2007 and 2008. BFT was also greater in CP concentration than *falcata* in two harvests per year at EL in 2006 and LC in 2007 and greater in one harvest per year at LC in 2006 and 2008. When harvested three times per year, the CP concentration of BFT was greater than *sativa* at LC in 2007 and 2008 and exceeded CP concentration in *sativa* in two harvests at LC in 2006. Crude protein levels of one-harvest, BFT only exceed those of *sativa* at LC in 2008. High forage quality from stockpiled BFT is well-documented (Marten and Jordan, 1979; Collins, 1982; Alison and Hoveland, 1989).
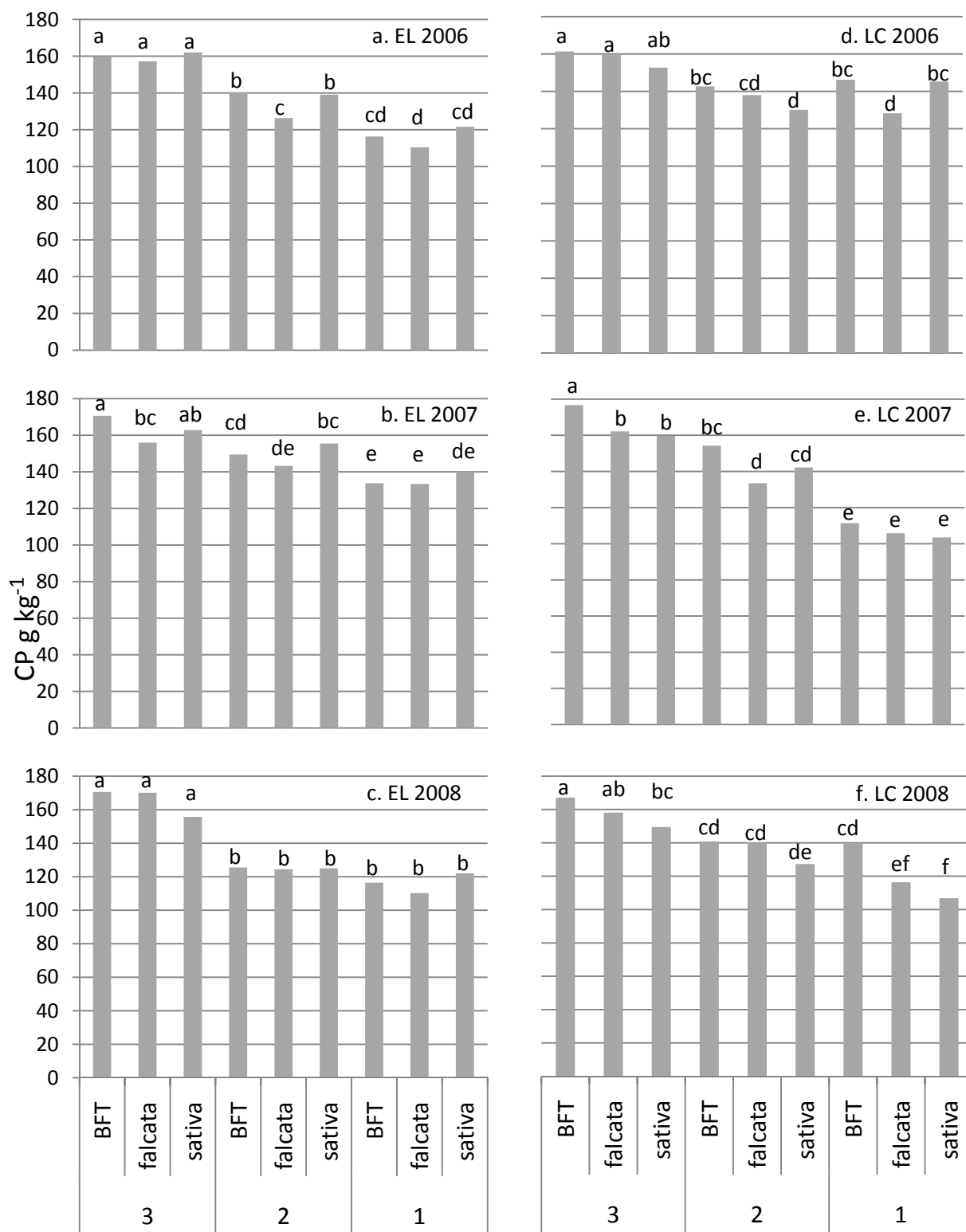
Figure 1.7. Weighted average crude protein (CP) concentration for Birdsfoot trefoil (BFT), *falcata* and *sativa* for three harvest frequencies (three, two and one per year) for two locations (East Lansing [EL], Lake City [LC], MI) and three years (2006-2008). Means with the same letter are not significantly different at the 0.05 level of probability.

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Fiber components

Harvests usually occurred earlier (by calendar date) at EL than LC and the crop may have matured more rapidly with higher temperatures (Table 1.3), but average fiber levels were still slightly higher (ADF >14 g kg^{-1}; NDF >11 g kg^{-1}) at EL than LC Forage quality is usually greater in forage crops that grow in cooler climes, because lignin deposition is increased by higher temperatures (Marten et al., 1988; Collins and Fritz, 2003). Concentrations of ADF and NDF were lower on average at EL in 2007 than any other year. This may be due to PLH damage, which stunted the first cutting of each harvest frequency in that year due to a missed insecticide application. The ADF and NDF concentrations increased for all species with fewer harvests per year at both locations as a greater, more fibrous, proportion the total dry matter yield was obtained from the first harvest. This was evident in the decrease in leaf to stem ratio with fewer harvests at EL in 2008 (Fig. 1.3). The ADF and NDF concentration of *sativa* harvested three times over three years at EL (ADF= 343 g kg^{-1}, NDF= 456 g kg^{-1}) (Figs. 1.8 & 1.9) were similar to expected values for *M. sativa* harvested at full-bloom (ADF= 350 g kg^{-1}, NDF= 450 g kg^{-1})(Hatfield et al., 2007) and as expected, the ADF and NDF concentration increased with decreasing harvest frequency. Many trials have documented increased fiber concentration when harvest of *M. sativa* is delayed (Anderson et al., 1973; Kallenbach et al., 2002; Brink et al., 2010). In 2006 at LC, *sativa* ADF and NDF concentrations were lower in the one-harvest than the two-harvest treatment (Fig.1.10 & 1.11), possibly due to new shoot growth that occurred at the crown of the one-harvest treatment (pers. obs.). Greater ADF and NDF levels were observed from *sativa* harvested once per year at LC in 2007 and 2008 than in 2006, probably due to greater GDD at harvest in 2007 and 2008 (Table 1.1).

Biomass produced by BFT had the lowest ADF and NDF (248 and 335 g kg^{-1}, respectively) concentrations of the trial in three harvests per year at LC in 2007 (Fig. 1.10 & 1.11).   By date, this was the earliest first cutting of any location or year, but only the second earliest by GDD. The ADF concentration of BFT, when harvested twice a year, was less than *falcata* or *sativa* at LC in all three years, as well as, at EL in 2006. The lower average temperatures at LC may result in lower fiber levels of BFT. Two-harvest, BFT ADF concentration at EL in 2007 was similar to *sativa* and 60 g kg^{-1} less than that of *falcata*. The PLH damage incurred during the first cutting of 2007 may explain lower ADF concentrations in *sativa* for that year. It may be that PLH caused more stunting of *sativa* than *falcata*, because *falcata* was developed from *M. falcata* genotypes selected for greater PLH tolerance (Boe et al., 1998). In 2008, BFT harvested twice at EL was similar in ADF concentration to *falcata* and 50 g kg^{-1} less than *sativa*. A single harvest per year of BFT produced lower ADF concentrations than *sativa* or *falcata* at EL in 2006 and LC in 2006 and 2007. The NDF concentration of BFT biomass harvested once per year was lower than *sativa* and *falcata* at EL in 2006 and at LC in 2007 and 2008. In most cases, fiber levels of BFT were lower than those of *sativa* or *falcata* when first harvest was delayed until mid-June or later. Other research has shown BFT *in vitro* dry matter digestibility is not greatly affected by stockpiling (Mays and Washko, 1960; Collins, 1982; McGraw and Marten, 1986). Yields of BFT were lower than *falcata* or *sativa* in these harvest regimes, but concentrations of CP, ADF, and NDF of BFT were greater. Forage fiber concentrations of *M. falcata* have been shown to be lower than *M. sativa* in harvests at the early-bud stage (Juiler et al., 1996). However, in this study both species were harvested at the same time (past one-tenth bloom for *M. sativa*) and the concentration of ADF and NDF of *sativa* and

falcata, when three harvests were taken per year, were statistically similar. Acid detergent fiber

concentration of two harvests per year of *falcata* was lower than the same harvest frequency of

sativa at EL in 2008 (42 g kg^{-1} less) and at LC in 2006 (35 g kg^{-1} less). Conversely, in 2007 at

EL ADF concentration of two-harvest, *falcata* was actually greater (46 g kg^{-1}) than two-harvest

sativa, which may be attributed to reduced stem lengths due to PLH damage. The NDF

concentrations of *falcata* and *sativa* harvested two times per year were similar in most years,

with the exceptions of LC in 2007 and 2008. In those years, *falcata* NDF was higher than *sativa*

(Fig. 1.11). While there may not be a NDF concentration advantage of *falcata* over *sativa* in

delayed harvest frequencies, lower ADF concentrations were obtained from *falcata* than *sativa*

when a single harvest was taken per year at EL in 2006 and at LC in 2007 and 2008 and similar

ADF contents in other years. The exception was at LC in 2006, when greater (60 g kg^{-1}) ADF

and (90 g kg^{-1}) NDF concentrations of *falcata* were obtained. This may be attributed to greater

rainfall and soil moisture which spurred new crown shoot growth in *sativa*. It may also be due to

an earlier (279 and 153 GDD's earlier than 2007 or 2008, respectively) harvest date than for any

other year or location, which may have not been late enough for leaf senescence in *sativa*.
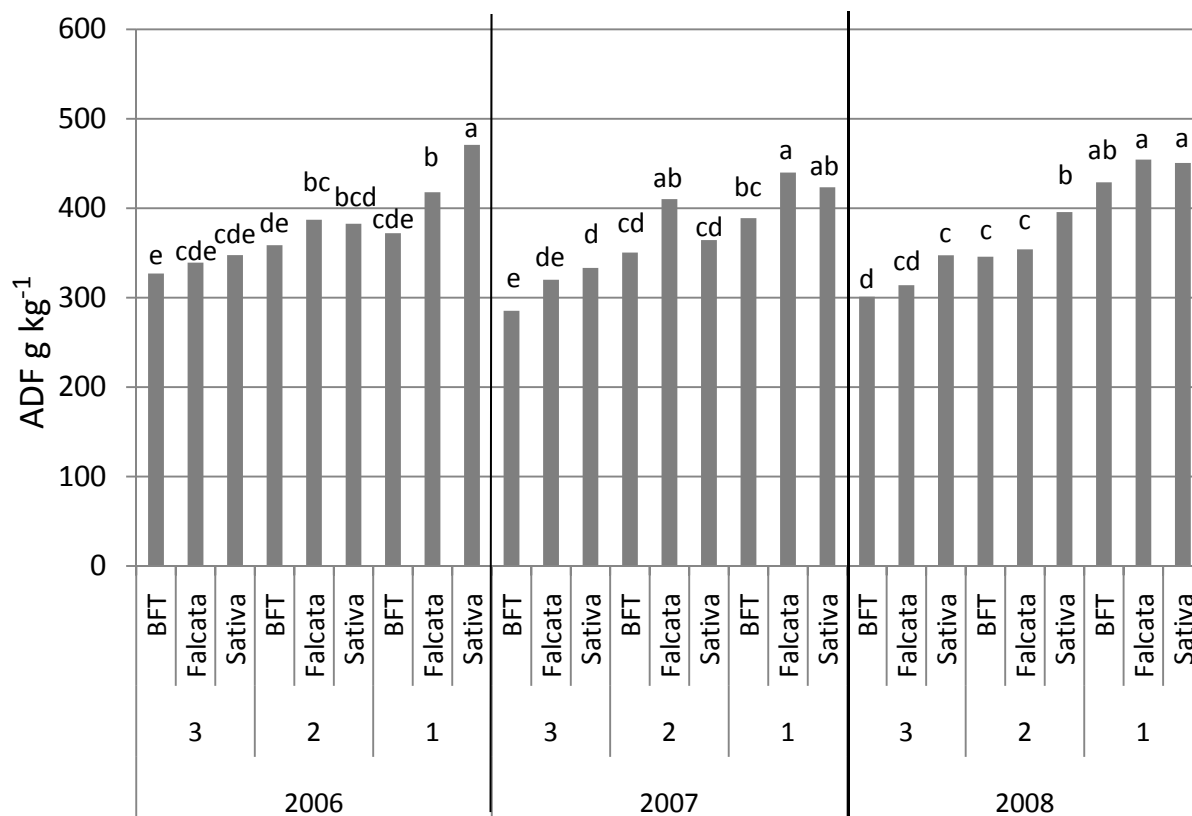
Figure 1.8. Weighted average acid detergent fiber (ADF) concentration for three species and three harvest frequencies (three, two and one per year) at East Lansing, MI over three years (2006-2008). Means with the same letter are not significantly different at the 0.05 level of probability.
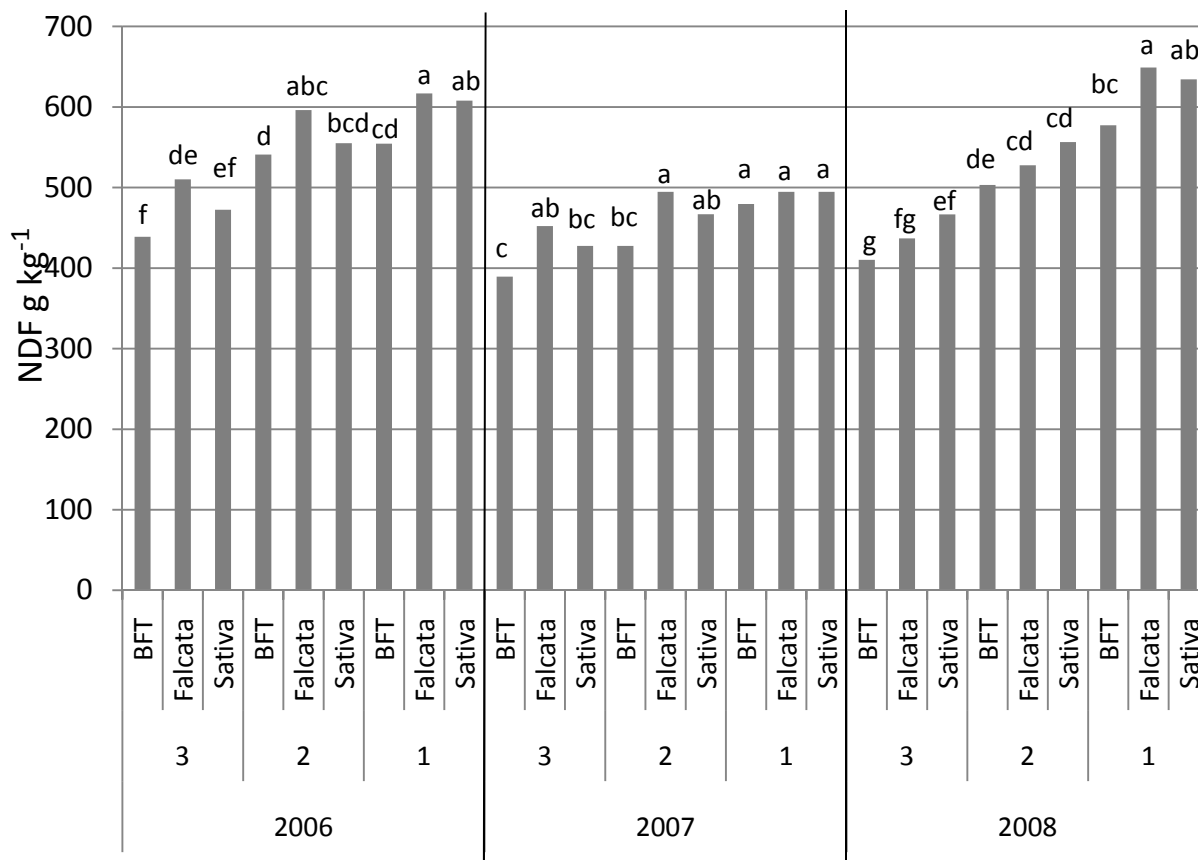
Figure 1.9. Weighted average neutral detergent fiber (NDF) concentration for three species and three harvest frequencies (three, two and one per year) at East Lansing, MI over three years (2006-2008). Means with the same letter are not significantly different at the 0.05 level of probability.
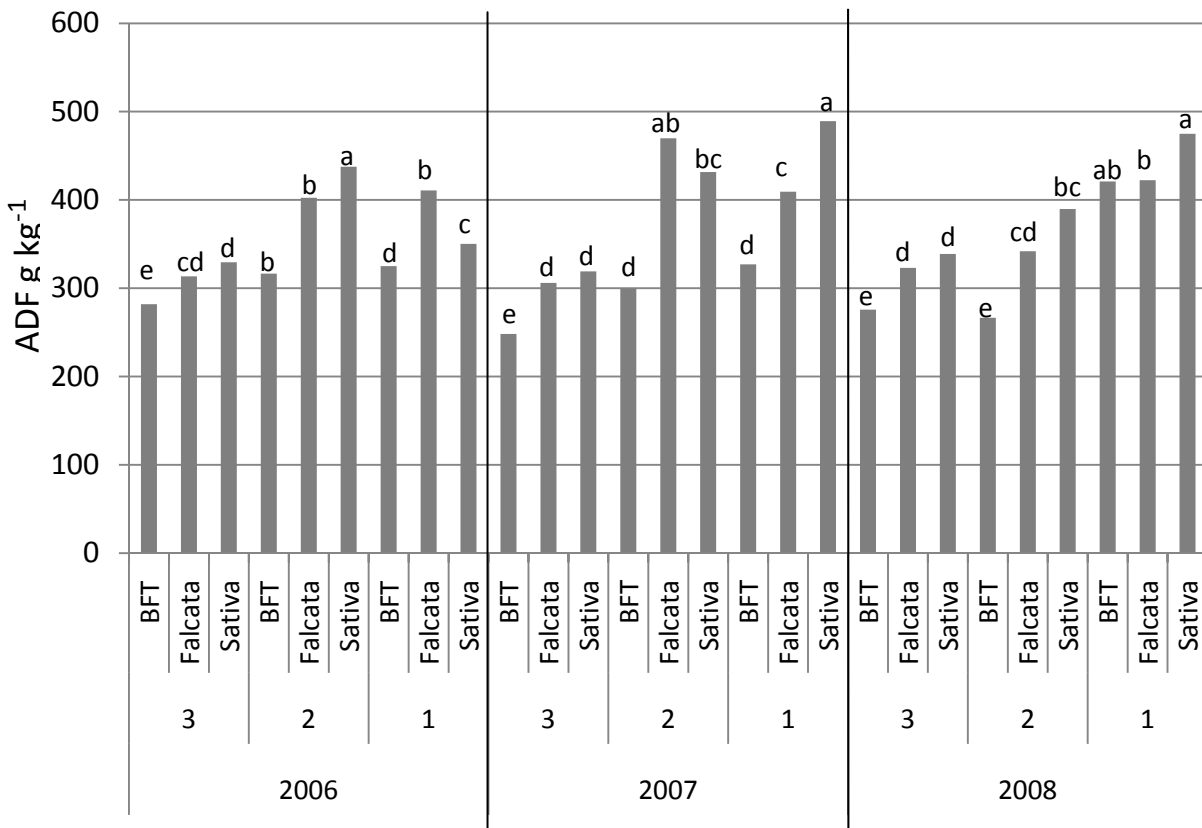
Figure 1.10. Weighted average acid detergent fiber (ADF) concentration for three species and three harvest frequencies (three, two, and one per year) at Lake City, MI over three years (2006-2008). Means with the same letter are not significantly different at the 0.05 level of probability.
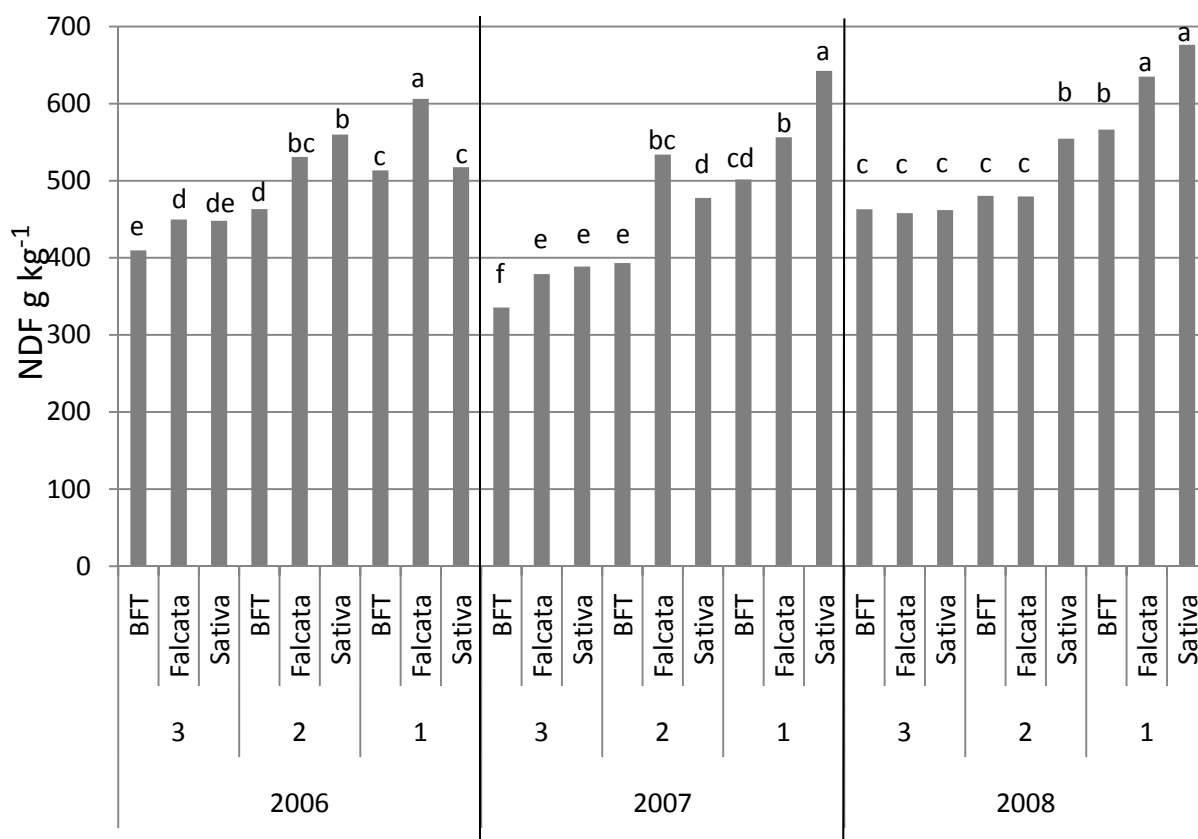
Figure 1.11. Weighted average neutral detergent fiber (NDF) concentration for three species and three harvest frequencies (three, two, and one per year) at Lake City, MI over three years (2006-2008). Means with the same letter are not significantly different at the 0.05 level of probability.

Nutrient Yield

The annual CPY of *falcata* harvested two times per year was equal to *sativa* harvest three times per year in all locations and years except at EL in 2007 (Fig. 1.12). Greater DMY of falcata harvested twice compensated for lower CP concentrations. The CPY of one harvest per year of *falcata* was lower than three harvests per year of *sativa* in all comparisons except at LC in 2007. This is as expected because CP concentration of *falcata* harvested once per year was lower and DMY was usually similar to *sativa*. A similar trend was observed in TDNY (Fig. 1.13). Two harvests per year of *falcata* produced equivalent TDNY as three harvests per year of *sativa*. One harvest per year of *falcata* resulted in lower TDNY than three harvests per year of

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sativa in all but LC in 2007 and EL in 2008. Although forage quality of BFT was typically equal or greater than *sativa* or *falcata*, low DMY of BFT reduced CPY and TDNY of BFT.

While there was not a CP or NDF concentration advantage for *falcata* harvested two times per year over *sativa* harvested three times per year, the quantity of DMY produced by *falcata* was greater and contributed to greater nutrient yield per year. Producers growing *falcata* may delay harvest into late-June, take two harvests per year, and expect to obtain the same forage nutrient yield as three harvests per year of *sativa*.
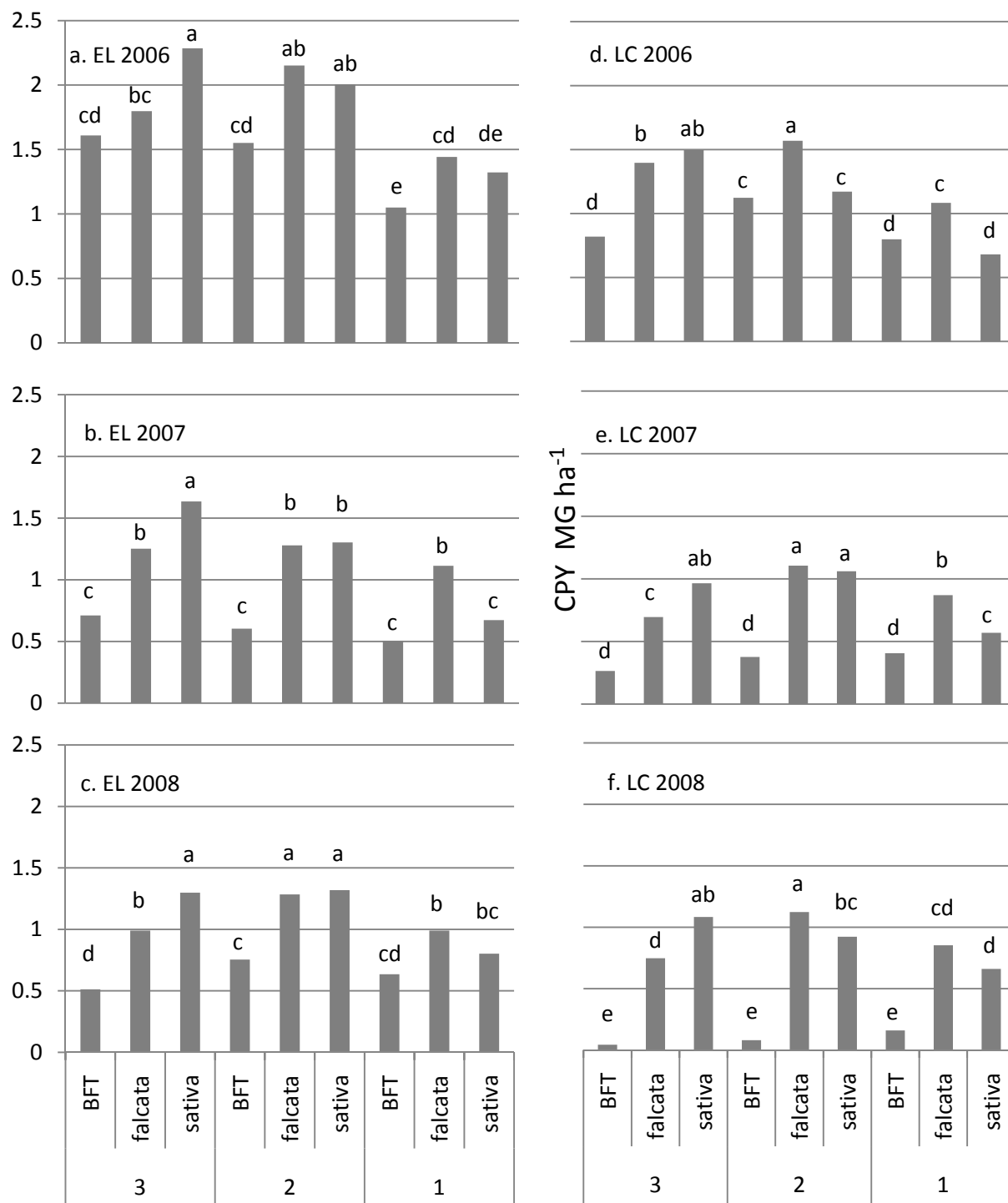
Figure 1.12. Weighted average crude protein yield (CPY) for Birdsfoot trefoil (BFT), *falcata* and s*ativa* for three harvest frequencies (three, two and one per year) for two locations (East Lansing [EL], Lake City [LC], MI) and three years (2006-2008). Means with the same letter are not significantly different at the 0.05 level of probability.

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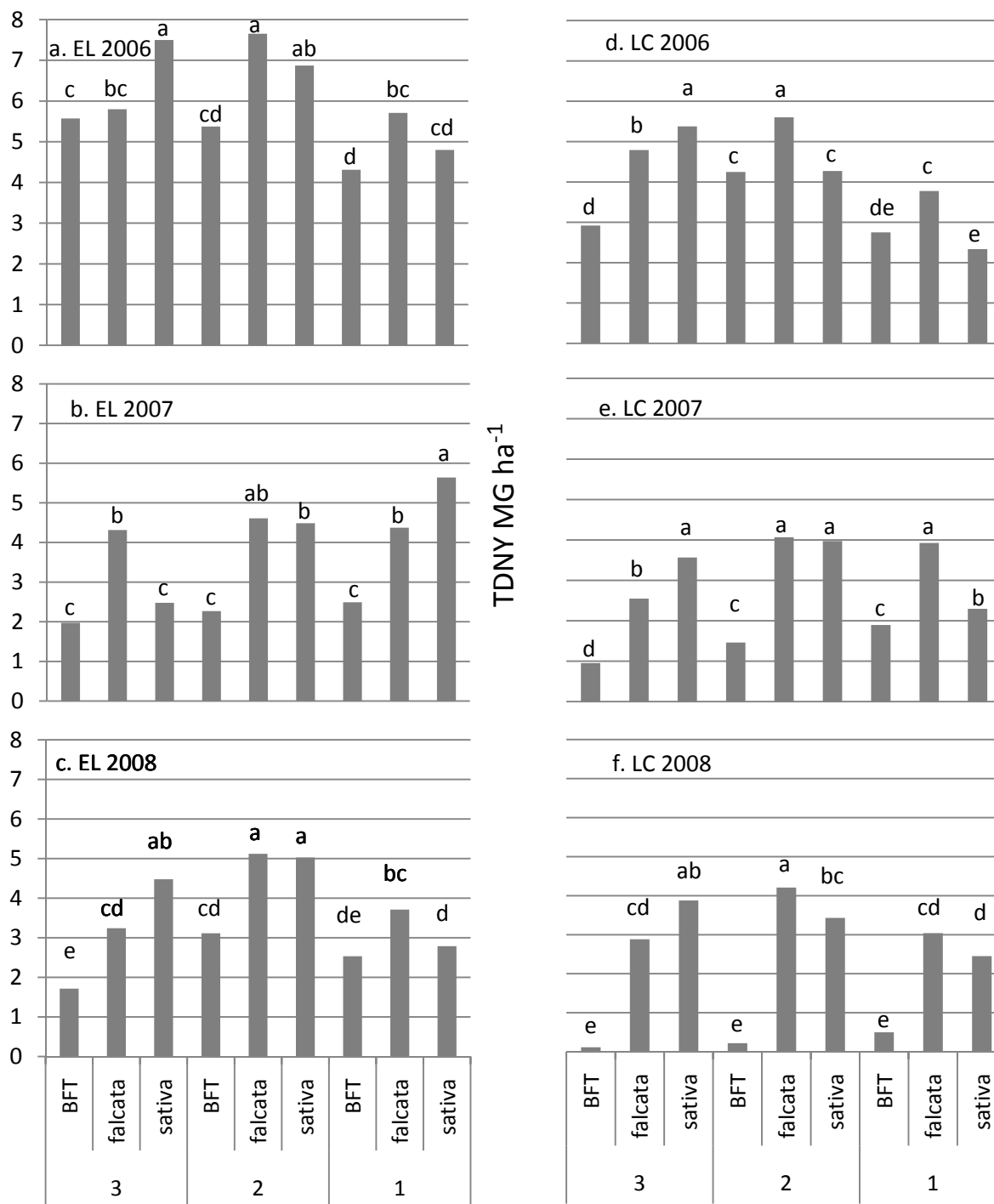
Figure 1.13. Weighted average total digestible nutrients (TDNY) yield for Birdsfoot trefoil (BFT), *falcata* and s*ativa* for three harvest frequencies (three, two and one per year) for two locations (East Lansing [EL], Lake City [LC], MI) and three years (2006-2008). Means with the same letter are not significantly different at the 0.05 level of probability.

Persistence

The main effect of species was significant but harvest frequency was not (P=0.3) for plants m^{-2}. The interaction species x harvest frequency x location was significant and, as expected, plant density decreased over years and there was an interaction between species x year. Stand density data is presented for spring and fall plant counts within a year and location for each species (Fig. 1.14a & b).

Sativa had greater plant density than BFT in all but the spring 2008 and was greater than *falcata* in all but the final year (spring and fall 2008) at EL. In both locations, with the exception 2008, greater loss of *sativa* occurred in the summer rather than the winter, supporting the findings of Stout et al. (1992) and Berg et al.(2005). Harvest frequencies evaluated here, did not influence this pattern of greater stand loss in the summer than winter. Single yearly defoliation of alfalfa in MN has been shown to reduce the persistence of alfalfa when compared to three or four defoliation events per year (Sheaffer et al., 1997), but there was no harvest frequency effect on stand density in our trial. The only significant reduction in *sativa* stand density for the winter period was from fall 2007 to spring 2008 at EL. The final plant density was 75 plants m^{-2} at EL and 109 plants m^{-2} at LC and both were above the recommended minimum (43 plants m^{-2}) for hay production in *M. sativa* alfalfa stands (Tesar and Marble, 1988).

Stand density of *falcata* at EL was initially lower (69 plants m^{-2} fewer) and remained lower than *sativa* until the spring of 2008. However, the rate of stand decline for *falcata* was less (19 plants m^{-2} yr^{-1}) than that of *sativa*. There were no differences in stand density between *falcata* and *sativa* at LC for the duration of the study. Root and crown diseases of *falcata* were expected to be worse than those of *sativa*, because selections for SD201 were made in a drier climate; however, there was no evidence of greater stand loss due to disease. As in *sativa*, *falcata* stand density declines were greater in summer than winter. The greatest reduction in stand density was between spring and fall 2007 (61 plants m^{-2}) and may be attributed to dry summer conditions (Fig. 1.1). The final stand density of *falcata* (65 plants m^{-2}) remained above the recommended minimum (43 plants m^{-2}) for *M. sativa*. (Tesar and Marble, 1988).

Stand density of BFT was lower than *sativa* and *falcata* in LC than EL in several sampling events. This study supports other findings that suggest that BFT can be difficult to establish (MacAdam et al., 2006). Initial density at LC was approximately half that of EL and approximately equivalent to the stand density of the spring 2008 values for EL, possibly due to drier conditions in the seeding year and lower water holding capacity of soils at LC. Stand densities of BFT were lower than *falcata* and *sativa* at most sampling events in both locations. However, at EL in the fall of 2007 there was no difference in stand density between BFT and *falcata* and the spring of 2008 there were no differences among species as the plant densities of both alfalfas had dropped to similar levels as BFT. Stand density of BFT decreased the least (89 plants m^{-2} at EL and 39 plants m^{-2} at LC) of any species over the three years of the study and

may be due to natural reseeding. But, there were no significant increases in BFT plants over years and observations of new plants were not taken. Similar to the alfalfas, BFT plant losses were usually greater in the summer as suggested by Allison and Hoveland (1989) who observed greater stand loss of two BFT cultivars in the summer than in the winter in GA. Fusarium *(Fusarium oxysporum* f.sp. *loti*) is typically a causal agent for plant death of BFT (English, 1999) in the northeastern USA, and conditions for this disease may have been more favorable at EL resulting in greater stand loss.
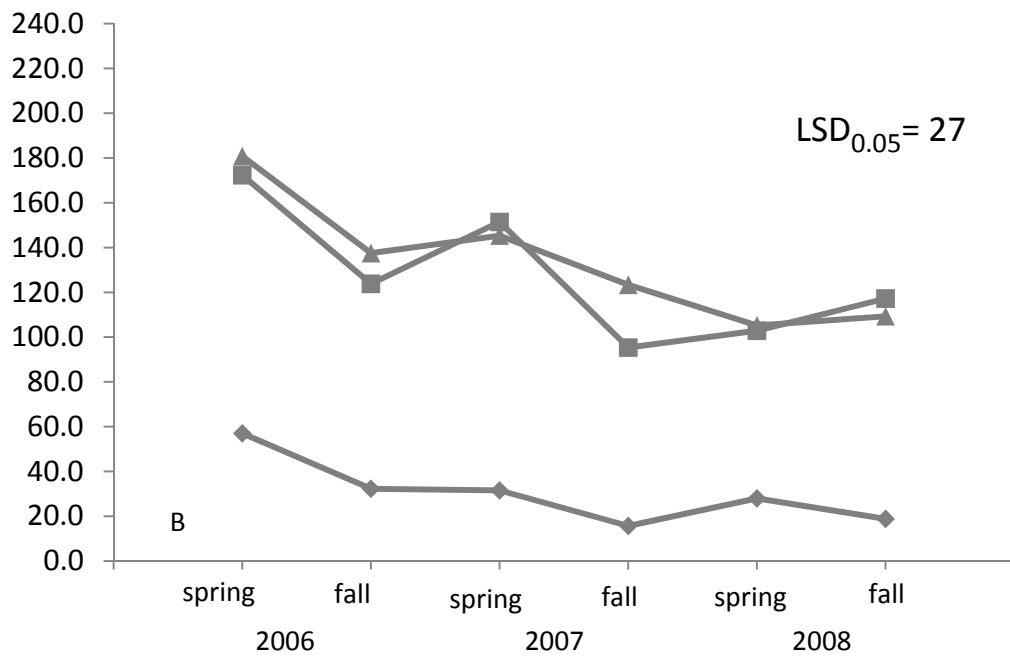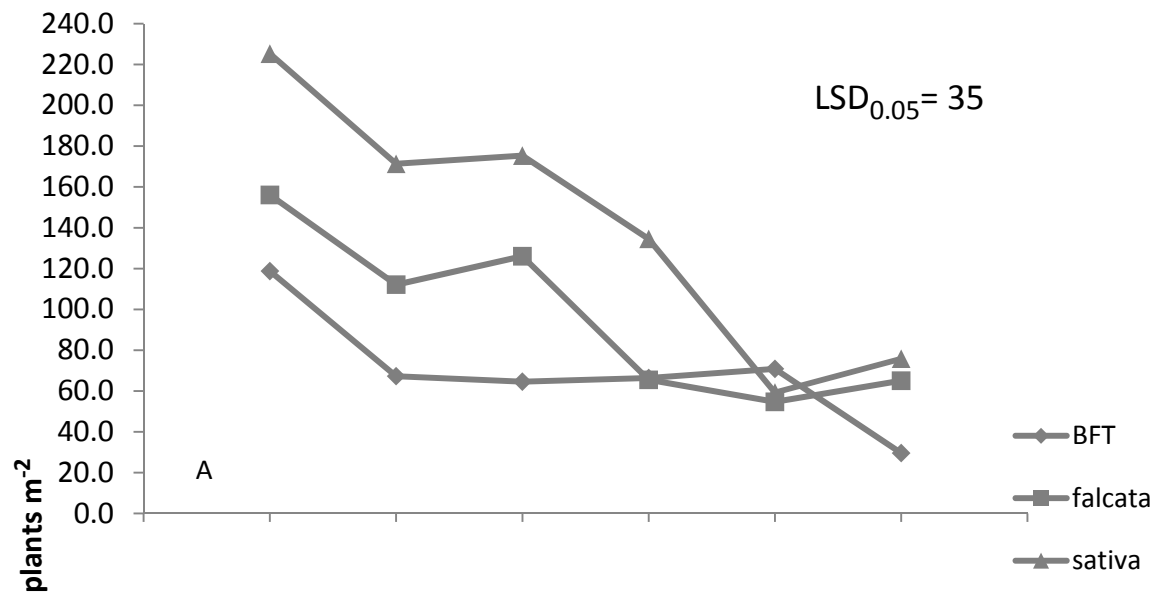
Figure 1.14. Stand density of *falcata*, *sativa*, and birdsfoot trefoil (BFT) averaged over three harvest frequencies over 3 yrs. in East Lansing (A) and Lake City (B), MI.

CONCLUSIONS

1. *Medicago sativa* spp. *falcata* 'SD201' may provide as much or more DMY in one or two harvests per year as'WL346LH' *M. sativa*, in three harvests per year. In subsequent years, greater DMY should be expected from *falcata* over *sativa* for a single annual harvest in July, but expect equivalent DMY when two harvests are taken annually, the first in mid- to late-Jun and the second in Sept. Producers should expect greater DMY from *falcata* than BFT, like BFT, in one, two, or three harvests per year in Michigan for similar harvest dates. Greater DMY of *falcata* over *sativa* in a single Jul harvest may be due to greater late-season shoot production by *falcata*.

2. *M. sativa* spp. *falcata* had similar CP and NDF to *M. sativa* on the three, two, or one harvest per year schedules in this study. When both species are harvested a single time in July, the ADF concentration of *falcata* may be lower than *sativa*. Greater CPY and TDNY are obtained from two harvests per year of *falcata* than three-harvests per year of *sativa*.

3. *M. sativa* spp. *falcata* plant density was equivalent to *M. sativa* and greater than BFT when harvested one, two, or three times per year for four years. Greater plant losses of *falcata* occur in summer than in winter. Although breeding selections of the *falcata* cultivar evaluated were made in a drier climate, diseases were expected to be a concern, but based upon persistence data, there was no evidence of greater occurrence than *sativa*.

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CHAPTER 2

DEFOLIATION EFFECTS ON TAPROOT CARBON AND NITROGEN RESERVES OF
MEDICAGO SATIVA SPP. *FALCATA* AND *MEDICAGO SATIVA*

ABSTRACT

Regrowth of *Medicago sativa spp. falcata* following defoliation is slower than *M. sativa,* but the physiological reasons for this difference are unknown. Several studies have investigated the role of total non-structural carbohydrate (TNC) and N reserves in *M. sativa* regrowth in the spring or following defoliation, but limited information is available about the role of taproot energy reserves on growth of *falcata* following defoliation. This two-year study assessed the concentrations of taproot sugar, starch, total soluble protein (TSP) and amino acid-nitrogen in *falcata* (SD201) and *sativa* (WL346LH) grown in East Lansing, Michigan. Sampling occurred when plants were defoliated in June and 3, 6, 9, 15 and 30 days, thereafter. Taproot sugar concentrations were usually similar between species, but sugar concentrations of both species were greater when greater rainfall was received. Taproot starch, TSP and amino N concentrations of *sativa* were almost always greater than *falcata*. An earlier decline in TSP and amino-N concentration following defoliation was observed in WL346LH. We conclude that greater starch, TSP, and amino N concentration are factors contributing to the difference in growth rate following defoliation of *falcata* and *sativa.*

INTRODUCTION

Medicago sativa spp. *falcata* produces greater dry matter yields than *M. sativa* in the

seeding and second year when first harvest is delayed until late-June and only one or two harvest

are taken per year (Dietz, 2011). The growth habit of *falcata* differs from *sativa* with *falcata*

increasing above ground biomass even after inflorescence, possibly due to a shoot density (shoot

m^{-2}) increase over time (Dietz, 2011). Three harvests per year, with the first occurring in early-

Jun, was found to decrease total annual above-ground biomass production of *falcata* when

compared to one or two harvests per year. The regrowth rate of *falcata* following defoliation is

slower than that of *sativa* (Dietz et al., 2008). The physiological reasons for differing responses

to defoliation are not understood.

Harvest or defoliation of alfalfa removes most of the photosynthetic tissue, and

subsequent shoot regrowth requires mobilization of C (sugars) and N (nitrate, amino acids,

proteins) from root reserves to synthesize new photosynthetic tissue (Hodgkinson, 1969; Kim et

al., 1993; Avice et al., 1996b; Volenec et al., 1996). Previous studies suggested that TNC in

taproots were the main contributor of C for shoot regrowth (Smith and Silva, 1969), but in the

1990's, researchers with the stable isotopes ^{13}C and ^{15}N determined that most of the TNC was

lost to root respiration (Ta et al., 1990; Avice et al., 1996a). Volenec (1988) reported similar root

TNC concentrations present in both the slow regrowing diploid and faster regrowing tetraploid

alfalfas. This study revealed greater shoot tissue in the crown and larger mass of individual

growing shoot tips of tetraploid alfalfa as compared to diploid alfalfa. Positive associations have

been found between sugars and N reserves and spring regrowth potential of alfalfa (Volenec et

al., 1996). More current studies suggest that concentrations of vegetative storage proteins (VSP),

specifically, four polypeptides (15, 19, 32, and 57 kDa) (Boyce and Volenec, 1992; Cunningham

and Volenec, 1996), are the major contributing factors to alfalfa regrowth rate following shoot removal (Avice et al., 1997). Avice et al., (1996b) isolated these polypeptides to the periphery of starch granules within taproot vacuoles and determined that they comprise up to 40% of the total soluble protein (TSP). Mobilization of these VSP's occurs at a rate 2x that of other taproot proteins 10 d following harvest (Hendershot and Volenec, 1993; Avice et al., 1996b). The phytohormone, abscisic acid (ABA) has been reported to induce accumulation of VSP in taproots either by water deficit, cold temperatures, or by defoliation (Avice et al., 2003; Erice et al., 2007). The presence of ABA may also suppress axillary bud development. White and Mansfield (1977) applied ABA to axillary meristems following defoliation, and demonstrated suppression of axillary bud development. Indole acetic acid (IAA) may also play a role in bud development. Suppressed axillary bud growth was observed when IAA was applied to the cut stem surface of *Phaseolus vulgaris* (Hall and Hillman, 1975). The ratio of growth regulators has also been suggested as a determinant factor in axillary bud dormancy and growth (Beveridge et al., 1994). Alas, the role of growth regulators in axillary bud dormancy or correlative inhibition is still unknown, but the presence of nutrients required for regrowth is a metric that suggests the potential for regrowth.

Taproot reserves may affect regrowth rates of *falcata* are not known and an understanding of the physiological and biochemical differences of *falcata* and *sativa* will allow for more accurate cutting management recommendations and may provide a focus for improvement-efforts by plant breeders. The objectives of this study were to compare taproot starch, sugar, protein, and amino acid concentrations of *falcata* to *sativa* following defoliation to determine levels at a mid-June harvest and the regrowth period, as possible causes for slower regrowth in *falcata*.

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MATERIALS AND METHODS

Site Description and Sample Collection

A trial was established at East Lansing (42.4 N, 84.3 W), Michigan in 2007 of *Medicago sativa* spp. *falcata* 'SD201' was obtained from Arvid Boe, of the South Dakota State University and commercially available *sativa* 'WL346LH' from WL Research of Madison, WI. Seed was inoculated with a commercial inoculant of *Sinorhizobium meliloti* (Urbana Laboratories, Urbana, IL) and planted in potting medium in 3.8 x 21-cm super cells (Stuewe and Sons, Inc., Corvalis, OR) on March 15, 2007. Plants were grown in the greenhouse at 26 ± 5°C with incident solar radiation and irrigated with tap water. Plants were transplanted with a spade shovel on 13-Jun into a Capac soil (fine loamy, mixed, mesic Hapladauf) (pH: 7.4, P: 26 ppm, K: 124 ppm) with 0.5-m spacing within the row and 1-m between rows. Each row was comprised of a single species. Transplants were irrigated on 26-Jun 2007 but natural rainfall supplied water to the plants thereafter. A single application of 58 kg ha^{-1} of P and 156 kg ha^{-1} of K was made 3-Jul, 2007. Biomass above 6 cm was harvested on 29-Aug, 2007 to reduce weeds. Biomass above 6 cm in height was harvested on 20-Jun, 2008 (656 GDD base 5°C) and 26-Jun, 2009 (811 GDD base 5°C) from all plants. In both years, defoliation occurred when WL346LH had reached mean stage count of 6. Four plants of *M. falcata* and *M. sativa* were collected by digging to a depth of approx. 25 cm at 0, 3, 6, 9, 15 and 30 d following harvest. In 2008, the four plants of each variety that were taken were next to each other, while in 2009, the plants were selected at random across the trial. Roots were separated from remaining above-ground biomass at the cotyledonary node and placed immediately in cold water. A root subsample from the crown to length of 20 cm was rinsed in cold water and divided into two segments: the uppermost 5 cm immediately below the

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crown; and the remaining 5 to 20 cm segment. Both segments were diced, frozen in liquid

nitrogen, and stored at -70°C until lyophilized. Dry root tissues were ground in a Christy-Turner

lab mill (Christy-Turner LTD, Ipswich, UK) to pass a 1-mm screen and stored at -70°C until

analysis.

TNC and N Analyses

Sugar and starch analyses followed procedures according to Smith (1981). Sugars were

extracted from ca. 30 mg of taproot tissue with 1 mL of 800 mL L^{-1} ethanol in 1.5-mL microfuge

tubes. Tubes were shaken for 10 min at 25°C, microfuged at 14,000 x g for 5 min at 4°C, and

the supernatant was retained. The ethanol extraction was repeated twice and the combined

supernatants diluted to a final volume of 10 mL with 800 mL L^{-1} ethanol. Sugar concentrations

in the ethanol extracts were determined with anthrone reagent according to Koehler (1952) using

glucose (0 to 200μg mL^{-1} in 800 mL L^{-1} ethanol) as a standard. The ethanol-extracted residue

was oven-dried at 70°C. Water (500 μL) was added to each tube, and the tubes water heated in a

boiling water bath for 10 min to gelatinize starch. The pH of the solution was adjusted, when

necessary, to 5.1 by adding 0.2 N Na acetate buffer. Starch was digested by adding 0.2 U of

amyloglucosidase (Sigma Chemical Co., St. Louis MO; product A3514 from *Aspergillus niger*)

and 40 U of α-amylase (Sigma Chemical Co., St. Louis MO; product A0273 from *Aspergillus*

oryzae) in 100 μL of 0.2 N Na acetate buffer (pH 5.1). Tubes were incubated at 55°C for 24

hours with occasional shaking. Tubes were centrifuged and glucose in the supernatant was

determined using glucose oxidase (Glucose Trinder, Sigma Chemical Co., St. Louis MO;

Product 315-100). Starch concentration was estimated at 0.9 x glucose concentration.

Protein analysis was conducted at 4°C unless otherwise stated. Soluble proteins were extracted by suspending 30 mg of freeze-dried taproot tissue in 1 mL of 100 mM sodium phosphate buffer (pH 7.5) containing 1 mM phenylmethylsulfonylfluoride and 10 mM 2-mercaptoethanol. Tissue suspensions were vortexed four times for 30 s at 5-min intervals and then centrifuged at 14,000 g_n for 10 min. The supernatants were retained and soluble protein was estimated using protein dye-binding reagent as described in Bradford (1976) using bovine serum albumin as a standard. Buffer-soluble protein concentrations were expressed on a structural dry weight basis (dry weight minus sugar and starch concentrations). Total amino-N was determined in triplicate from supernatant from the aforementioned buffer soluble protein extraction using glycine standards. Colorimetric analysis at 570 nm was performed using ninhydrin (Rosen, 1957) to determine total amino-N.

Statistical Analysis

Samples taken in 2008 were not randomly selected across the trial, as they were in 2009, and were taken from two areas. For this reason, years were analyzed separately, even if there was no species or sampling day x year interaction. The experimental design was a randomized, complete-block with four replications in a split-plot design. Species was the main plot and Days after defoliation was the sub-plot. Analysis of variance was conducted PROC MIXED procedure software version SAS 8.2 (SAS Institute, 2003) using the Kenward-Roger method for determining degrees of freedom. Years were considered fixed effects. Main effects and interactions were considered significant when $P < 0.05$. Means were calculated and are presented as least square means. The SAS macro 'PDmix80' (Saxton, 1998) was used to generate letter values for means separation based upon contrasts.

RESULTS AND DISCUSSION

The main effects of species and days were significant for each constituent (Table 2.1).

Species x day interactions were significant for taproot sugar, starch and TSP, but not for amino

N. The three-way interaction of year x species x day was significant for sugar and starch.

Because of unequal variances between years, each constituent will be presented by year.

Table 2.1. Results from ANOVA for taproot sugar, starch, soluble protein (TSP), and amino N of
M. falcata and M. sativa for six sampling events (0, 3, 6, 9, 15, and 30 d after defoliation) over
two years 2008-2009.

source	Sugar	Starch	TSP	Amino N
Species (S)	*	***	***	***
Day (D)	***	***	**	**
S x D	***	***	***	ns†
yr x S x D	***	***	ns	ns

*Significant F tests at the P< 0.05 level
**Significant F test at the P<0.01 level
***Significant F test at the P<0.001 level
†ns = not significant

Taproot sugar concentrations were similar for *sativa* and *falcata* in all sampling events in

2008 (Fig. 2.1A) and in all but Day 0 and 9 in 2009 (Fig. 2.1B). Sugar concentrations of *sativa*

on Day 0 were 25 mg g^{-1} less than Day 30 in 2008, while in 2009, Day 0 was 65 mg g^{-1} greater

than Day 30. Pembleton et al. (2010) observed greater sugar concentration for three alfalfa

cultivars (winter-dormant, winter-active, and highly winter-active) in Australia grown under

irrigated conditions as compared to those supported by natural rainfall. Lower sugar

concentration (76 g mg^{-1}) on Day 0, 2008 following drier conditions in May and Jun, and higher

sugar concentration (143 g mg^{-1}) in 2009 following the same period with greater rainfall was

observed in this study (Fig. 2.2) which concurs with the findings of Pembleton et al. Growing

conditions may have a greater effect on taproot sugar concentrations than alfalfa species.

Figure 2.1. Sugar concentration of *falcata* and *sativa* taproots sampled 0, 3, 6, 9, 15, and 30 d after defoliation in 2008 (A) and 2009 (B). *Significant difference between species (*P*<0.05).

Table 2.2. Average air temperature and total precipitation by month for each year of the study and the 30-yr. avg. at East Lansing, MI. Temperatures are averages of daily maximums and minimums.

	2008	2009	Normal*	2008	2009	Normal*
		°C			cm	
JAN	-2.6	-9.0	-5.9	5.1	0.3	4.0
FEB	-5.2	-2.5	-4.4	3.1	4.1	3.5
MAR	0.6	3.2	1.1	4.0	7.6	5.5
APR	10.3	8.8	7.6	5.5	16.5	8.3
MAY	12.7	14.2	14.1	3.5	10.9	6.9
JUN	20.6	19.1	19.2	12.2	12.6	8.1
JUL	21.9	19.2	21.4	9.4	6.1	7.6
AUG	20.9	20.2	20.3	1.3	16.8	8.7
SEP	18.4	17.1	16.1	21.4	1.9	8.9
OCT	9.9	9.2	9.7	4.1	9.2	6.3
NOV	4.3	6.3	3.3	3.2	2.4	6.7
DEC	-3.5	-2.7	-2.8	4.4	3.4	5.1

* 30- yr average (1971-2000) source: Michigan Automated Weather Network
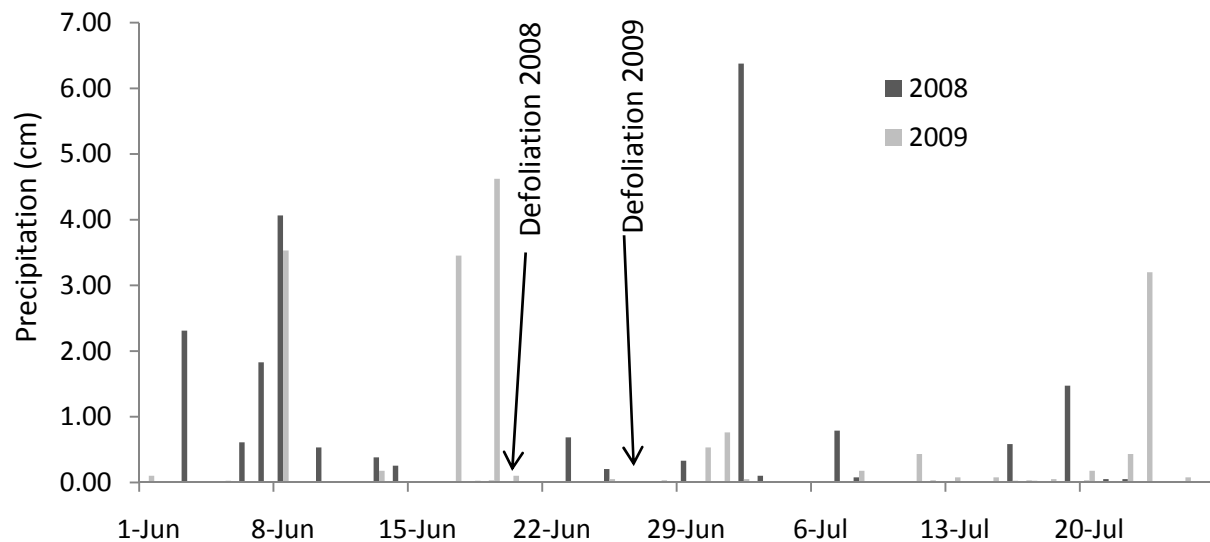
Figure 2.2. Daily precipitation at East Lansing, MI from 1-Jun to 26-Jul in 28 and 2009. Source: Michigan Automated Weather Network, http://www.agweather.geo.msu.edu/mawn/

The average taproot starch concentration in 2008 was 132 mg g^{-1} greater than 2009 (Fig.2.3 A,B). Erice et al. (2007) observed an increase in taproot starch concentration in water-deficit grown alfalfa in glasshouse conditions after 60 days of growth and 30 days after defoliation when ambient temperature was increased by 4 °C. Greater taproot starch accumulations in drier conditions were also observed by Pembleton et al. (2010). In 2008, starch concentration of *sativa* was greater than SD201 in every sampling event, and in 2009, it was greater in all but two events (Day 0 and Day 9). In 2008, both cultivars increased in starch concentration from Day 0, however, the concentration of *sativa* declined earlier (Day 3 to 6) than *falcata* (Day 6 to 9). This may suggest a more rapid regrowth response following defoliation by *sativa*. *Sativa* starch concentrations remained unchanged between Day 9 and 15 but increased by Day 30, while *falcata* starch concentrations continued to decrease through Day 30 in 2008. The plant height of *sativa* was approximately 38 cm at Day 15, while *falcata* remained less than 38cm by Day 30 in 2008. Adequate growth of *falcata* may not have occurred by Day 30 to enable excess sugar production for the activation of starch synthase and an increase in taproot starch. Less variation in starch concentration was observed in 2009. Starch concentrations of *sativa* still increased from Day 0 to Day 3, then remained at the same level to Day 6, and decreased on Day 9, which was later than in 2008. An increase in starch concentration of *sativa* was observed on Day 15. A decrease of starch concentration on Day 30 in *sativa* may have been the result of greater rainfall prior to this sampling date (Fig. 2.2).

Starch concentrations were almost always greater in *sativa*. While taproot starch has been found to be mainly support respiration of root and crowns following defoliation (Ta et al., 1990; Avice et al., 1996a; Barber et al., 1996), starch granules have been associated with four VSP's

(Avice et al., 1996b) identified as having a key role in N reserve storage in taproots (Hendershot and Volenec, 1993).
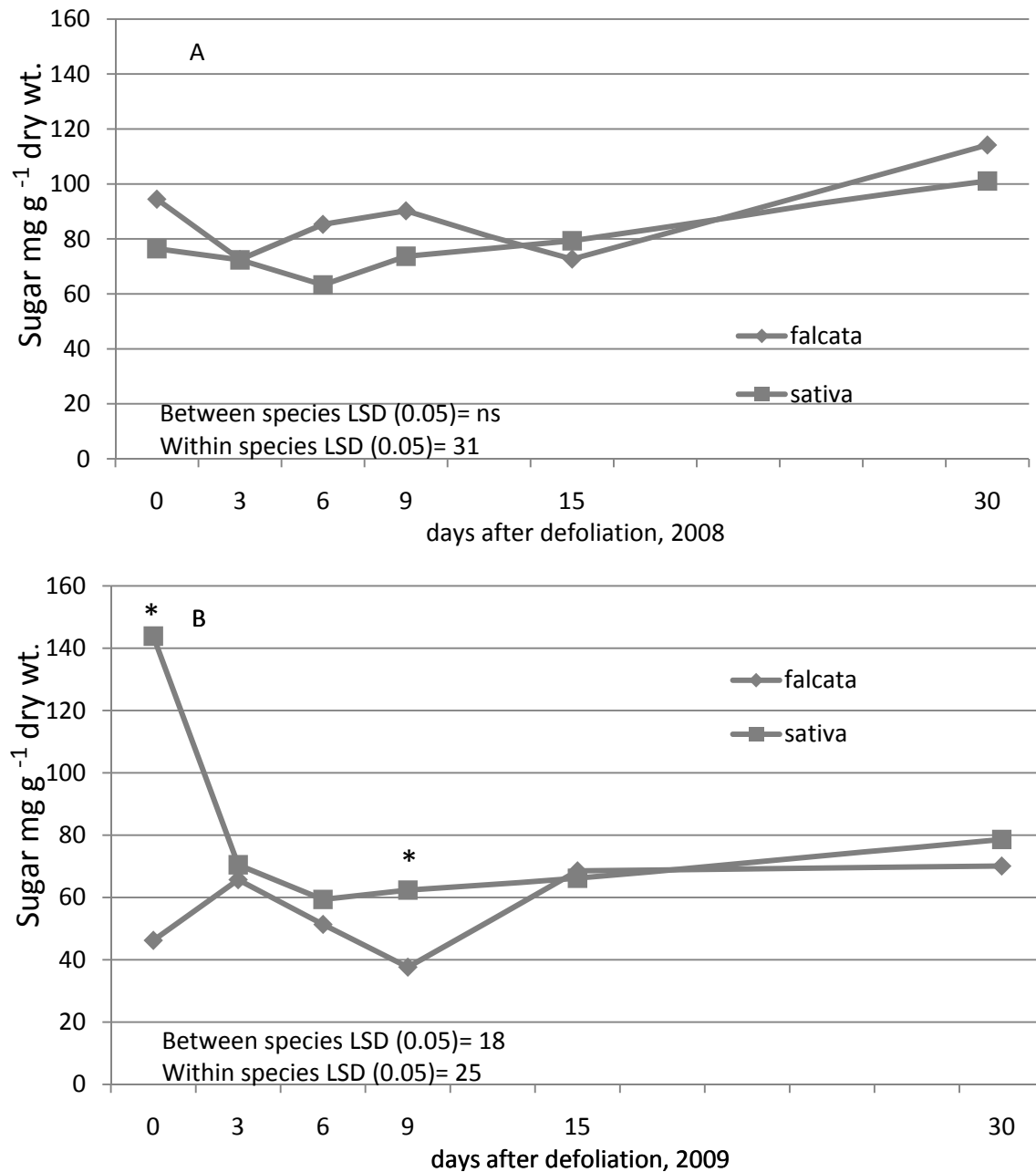

Figure 2.3. Starch concentration of *falcata* and *sativa* taproots sampled 0, 3, 6, 9, 15, and 30 d after defoliation in 2008 (A) and 2009 (B). *Significant difference between species ($P<0.05$).

Greater TSP concentrations were found in *sativa* than *falcata* at every sampling event (Fig. 2.4A, B). This finding concurs with findings of Cunningham and Volenec (1998) who found greater TSP concentrations in alfalfa cultivars selected for decreased fall dormancy (FD). The FD of *sativa* has been classified as 4.0 and the winterhardiness (WH) as 2.0, while *falcata* has not been classified for FD or WH. But the *falcata* cultivar ceased growth much earlier in the fall than *sativa*, and because the germplasm originated in Siberia, it would presumably be very dormant and winter hardy. Concentrations of TSP in *sativa* were greatest on Day 0 in both years of the study, and levels in each year decreased from 28.4 to 21.2 mg g^{-1} in 2008 and 27.5 to 19.1 mg g^{-1} in 2009 from Day 0 to Day 30, respectively. Our 2009 results show a similar decreasing trend to those observed by Berg et al.,(2009) with taproot protein concentrations greatest at Day 0, but, contrasting Berg et al findings, neither species in our study showed an increase by Day 30. In the Berg study, TSP of *M. sativa* under four fertilizer treatments continued to decrease to Day 14 and then increased thereafter, but did not attained the concentrations observed on Day 0. Concentrations of TSP of *sativa* in this study in 2009 decreased to Day 9 and then increased on Day 15 but then returned to a level not different than Day 9 or Day 30. The TSP levels of *falcata* varied less across sampling day and were always lower than *sativa*. The greatest TSP of *falcata* was not on Day 0 in either year. Numerically, the greatest protein contents of *falcata* were Day 9 and 3 in 2008 and 2009, respectively. The only differences observed between sampling days for *falcata* TSP were Day 6 and 9 which were greater than Day 30 in 2008, and Day 3 and 9 which were greater than Day 30 in 2009. There was a decreasing trend in TSP concentration for *falcata* after Day 9 in each year that may have been a delayed response to defoliation; the decrease in TSP for *sativa* was on Day 6 in 2008 and Day 3 in 2009.

The TSP concentration of *sativa* was greater for the period observed and decreased earlier than *falcata*. This would suggest that TSP plays a key role in differing rates of regrowth for *sativa* and *falcata*. Further comparisons of ABA concentrations of each species may reveal the specific cause for the delayed regrowth and decrease in TSP of *falcata*.
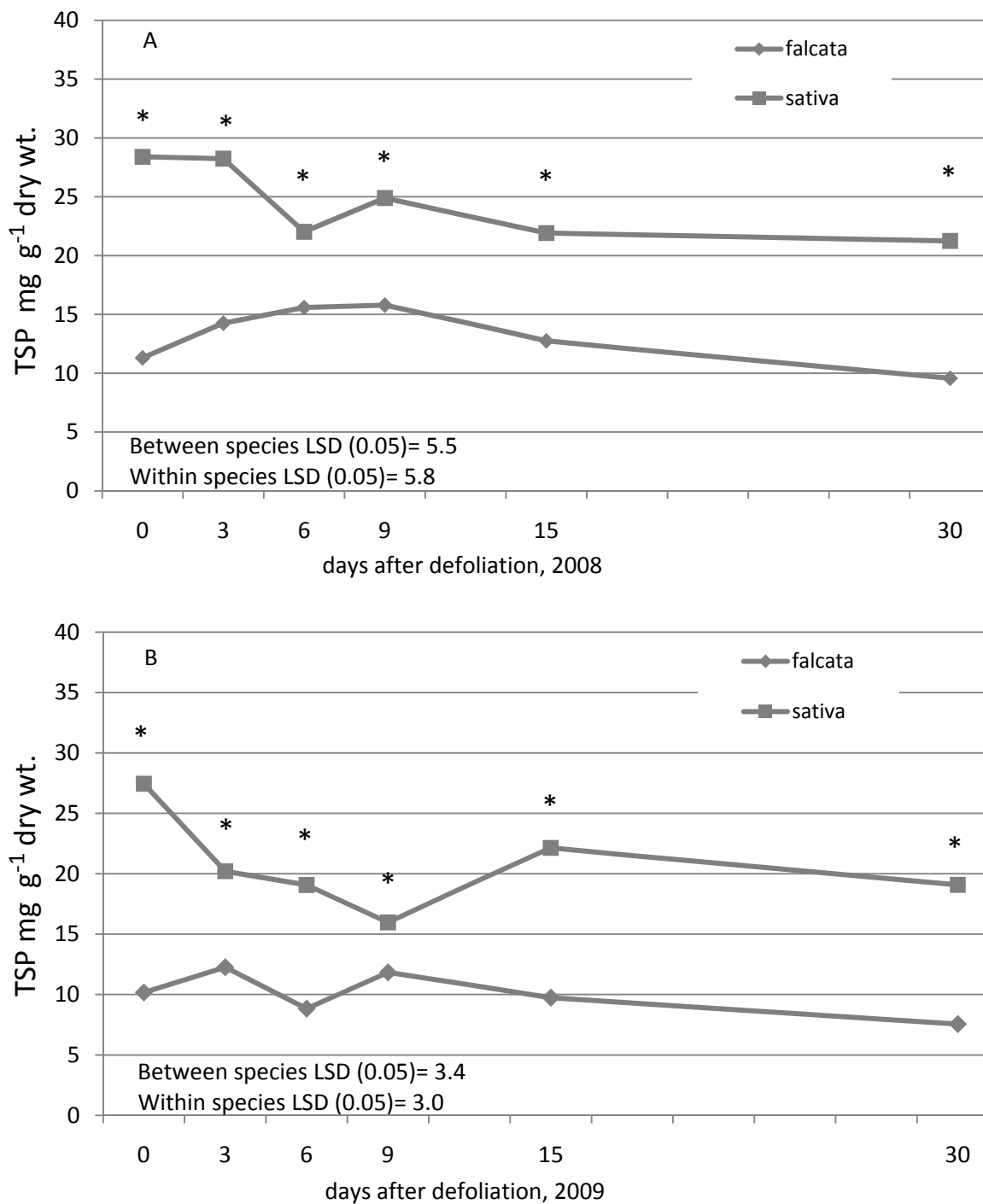
Figure 2.4. Total soluble protein (TSP) concentrations of *falcata* and *sativa* taproots sampled 0, 3, 6, 9, 15, and 30 d after defoliation in 2008 (A) and 2009 (B). *Significant difference between species (*P*<0.05).

Root amino-N concentrations of *sativa* were numerically greater but statistically similar in most sampling events in 2008 (Fig. 2.5A). In 2009, *sativa* amino-N levels were greater than *falcata* (Fig. 2.5B). This supports and adds to the findings of Cunningham and Volenec (1998) and Pembleton et al. (2010) who observed greater amino-N content of alfalfa selected for decreased FD. Within years, amino N levels of *falcata* and *sativa* followed similar trends, with the exception of an increase in amino-N for *sativa* from Day 15 to 30 in 2008 and a decrease from Day 0 to 3 in 2009. The only significant difference in 2008 was Day 30, when amino-N concentration of *sativa* was greater than *falcata*. However, in 2008, there is a trend for amino-N to increase from Day 0 to 6, and then decrease from Day 6 to 15 for both *sativa* and *falcata*. The greatest amino-N concentration for *sativa* in 2009 was at Day 0 and lowest were Days 3, 9, 15, and 30. Amino-N concentration of *falcata* reached the numerically greatest level on Day 6, as in 2008, but Days 3, 6, 9 and 15 were not significantly lower. On the final day (30), the *falcata* amino-N concentration was not different than Day 0 in 2008 and 2009. This was a measurement of total amino-N and specific amino acids may have had different trends.

Taproot amino-N concentrations, like TSP concentrations, were usually greater in *sativa* than *falcata*. Amino acids are the mobile building blocks of proteins and increases may predicate increasing TSP levels.
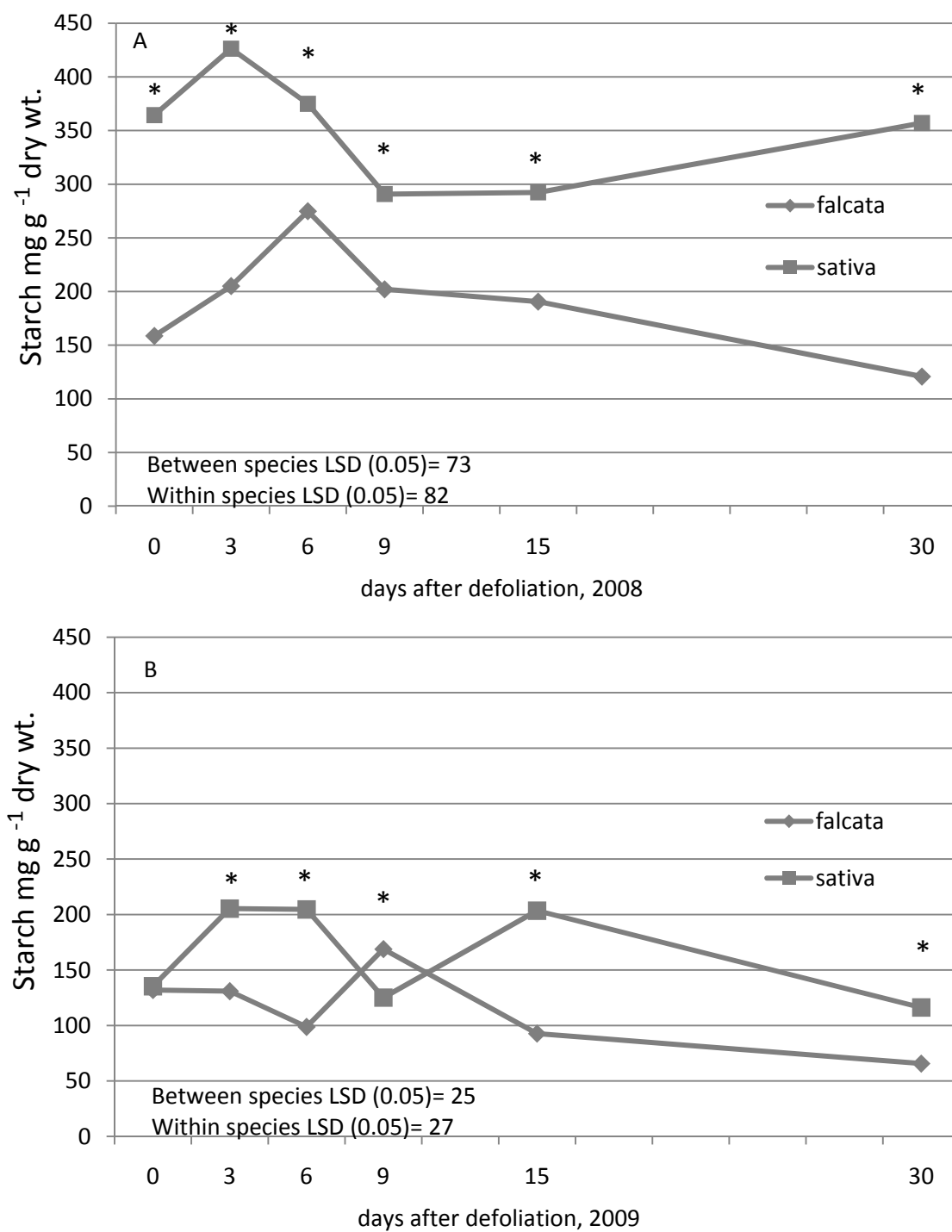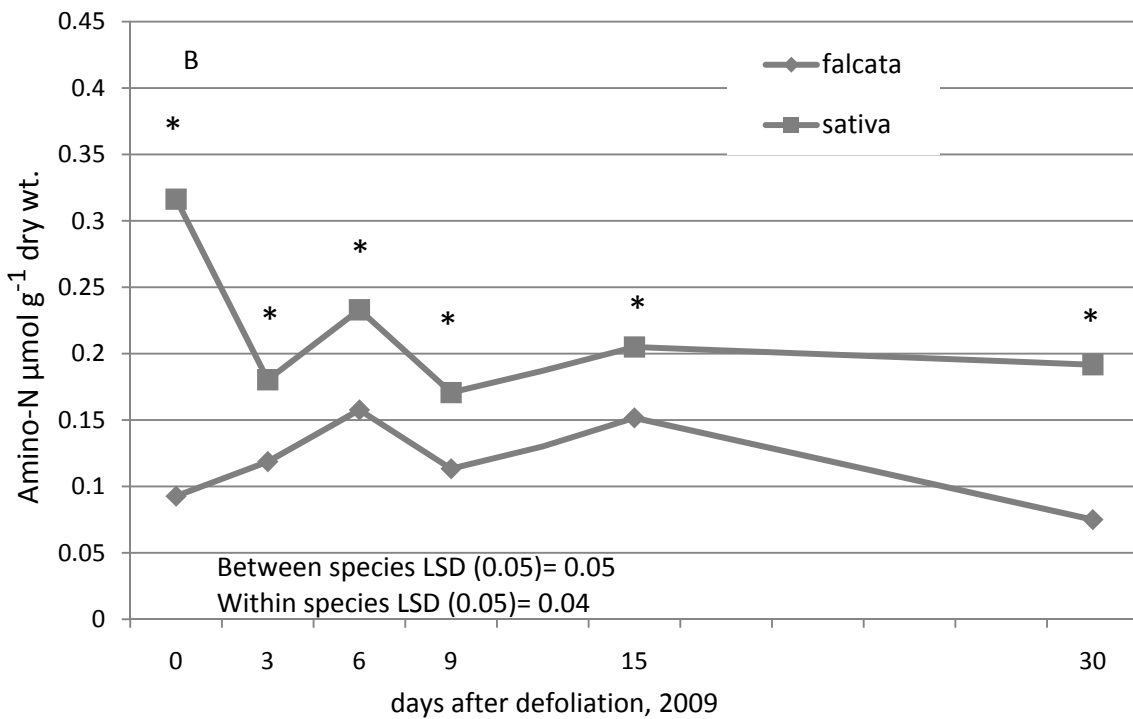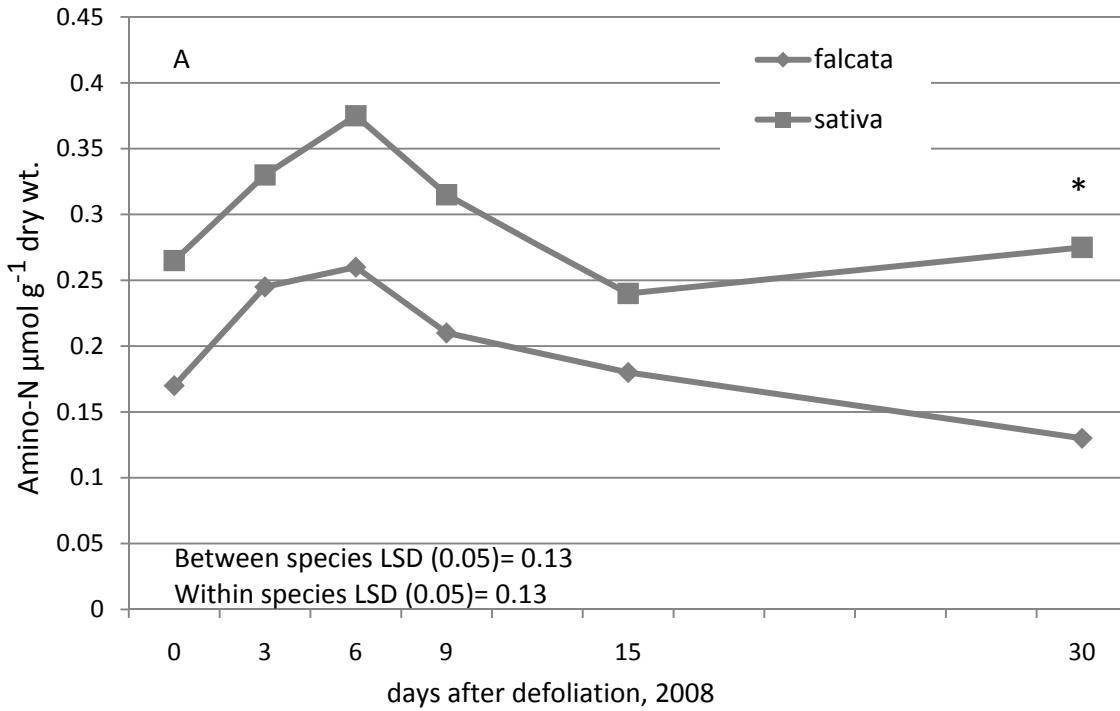
Figure 2.5. Amino N concentrations of *falcata* and *sativa* taproots sampled 0, 3, 6, 9, 15, and 30 d after defoliation in 2008 (A) and 2009 (B). *Significant difference between species (*P*<0.05).

CONCLUSIONS

Taproot sugar concentrations did not differ greatly between *falcata* and *sativa* but moisture conditions did influence levels of sugar. Greater moisture prior to sampling resulted in an increase in sugar content, while dry conditions increased starch. Taproot starch concentrations of *sativa* were generally greater than *falcata* in Jun and during the 30 days following defoliation. Taproot starch concentrations of the *falcata* cultivar evaluated, recovered more slowly than that of *sativa* following defoliation. Our results also indicate greater concentrations of amino-N and TSP in *sativa* than *falcata* in a mid to late-Jun harvest and for the 30 days following. Greater energy N reserves in *sativa* than *falcata* are likely the cause for the more rapid regrowth rate in *sativa* following defoliation.

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CHAPTER 3

AGRONOMIC PERFORMANCE OF SELECT *MEDICAGO SATIVA*, *M.SATIVA* SPP. *FALCATA* ALFALFAS AND BIRDSFOOT TREFOIL UNDER REDUCED HARVEST FREQUENCY

ABSTRACT

M. falcata alfalfa (*Medicago falcata* L. or *M. sativa* spp. *falcata* [L]. Arcang.) is a perennial legume which may have potential as a summer stockpiled forage crop in Michigan. The *M. falcata* experimental cultivar, SD201, has produced as much, or more dry matter yield (DMY) in one or two harvests per year as a conventional *M. sativa* alfalfa cultivar (WL346LH). We compared DMY, crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF) and pest resistance of SD201 to cultivars with *M. falcata* parentage, Norcen Birdsfoot trefoil (*Lotus corniculatus*)(BFT), and, WL346LH, a conventional potato leafhopper (*Empoasca fabae*) resistant *M. sativa*. Evaluation of DMY and pest resistance was conducted at Chatham (CH) and Lake City (LC), Michigan from 2005-2008, while DMY, CP, ADF, NDF, and pest resistance were compared at East Lansing (EL), Michigan from 2005-2008. The DMY of SD201 in the seeding year (2005) at LC and first production year (2006) at EL was greater than almost all others. The DMY of SD201 in subsequent years was usually similar to other alfalfas and greater than Norcen BFT. Forage quality parameters measured (CP, ADF, & NDF) were not affected by insecticide application. The CP, ADF and NDF concentration of SD201 was usually similar to other alfalfa cultivars, but CP was lower and the ADF and NDF were usually greater than that of Norcen BFT.

INTRODUCTION

Alfalfa (*Medicago sativa* L.) is a preferred species for hay production in the US due to consistently high yields of good quality forage. However, a great deal of time and effort are required to produce multiple harvests of good quality hay. Current per harvest cost estimates based upon custom harvest rates for cutting, raking and baling in Michigan is approximately $74 ha^{-1} (Stein, 2009). Furthermore, temperatures and day-length in late-May in Michigan are less conducive to curing hay than in mid to late-Jun. Therefore, many growers who produce hay for livestock on maintenance diets opt to delay harvest to minimize drying time. However, conventional alfalfa cultivars (*M. sativa*) are poorly suited for these delayed harvest systems lose forage quality when harvest is delayed.

A synthetic tetraploid cultivar of *M. sativa* spp. *falcata*, 'SD201' from South Dakota (Boe et al., 1998), has been shown to produce greater yields than *M. sativa* in one harvest per year in SD (Boe et al., 1994) and in Saskatchewan in wide (90cm) row spacing (Irvine and Jefferson, 1984). In Michigan, SD201 produced greater yields in fewer harvests than a conventional cv. 'WL346LH', alfalfa (*M. sativa* spp. *sativa* L.) and 'Norcen' birdsfoot trefoil (*Lotus corniculatus* L.) in the first and second production year; with forage quality levels sufficient for livestock on maintenance diets (Dietz, 2011). Greater yields appear to be the result of prolonged vegetative growth and indeterminate growth habit where development of new shoots of *M. falcata* continues after inflorescence. By comparison, *M. sativa* shoot production ceases (Dietz, 2011). While a prolonged vegetative growth may increase forage yield, it is a detrimental characteristic for commercial seed production, since seed pods ripen at different times.   It may be possible to utilize available cultivars of *M. sativa* spp. *falcata*, *M. falcata* or *M.*

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varia. for a delayed-harvest or stockpiled forage system. SD201 may be similar in yield and forage quality characteristics to older conventional cultivars (*M. sativa* spp. *varia*) with greater *falcata* parentage such as: Yellowhead, Travois, and Spredor 3 than conventional cvs. 'Yellowhead' is a tetraploid *M. sativa* spp. *falcata* cv currently marketed which may produce equivalent DMY to SD201 in the seeding and first production year. Yellowhead increased total forage yield in Canada when seeded with smooth bromegrass (*Bromus inermis* L.) (Bittman et al., 1991) and when it was space-planted into rangeland (Hendrickson and Berdahl, 2003). The objectives of this study were to compare dry matter yield, forage quality, and pest resistance of commercially available cultivars of *M. sativa* spp. *falcata*, *M. varia*, *M. sativa* and one BFT cultivar to 'SD201', experimental *M. falcata* cultivar.

MATERIALS AND METHODS

The eight alfalfa cultivars evaluated in the study were 'SD201', 'Yellowhead', 'Travois', 'Spredor 3', 'Ladak', 'Vernal', 'WL346LH' and 'Norcen' BFT. The synthetic cultivar SD201 (*M. falcata*) was developed from 41 genotypes selected for vigor, Potato leafhopper yellowing (PLY) tolerance, leaf retention, and semi-erect to erect growth habit by Arvid Boe, South Dakota State Univ. Yellowhead (*M. sativa* spp. *falcata*) alfalfa developed by Agriculture and Agri-Food Canada, Swift Current, SK was included as a commercially available *M. falcata* and is characterized as having 97% yellow flowers (McLeod, 2009). Travois developed by crosses of Cossak x Semipalatinsk (*M. falcata*) x Rambler (45% *M. falcata*) which was released by South Dakota Agriculture Experiment Station in 1963 was included as an older *M. falcata*-type. Spredor 3 (Northrup King) has approximate germplasm sources of *M. falcata-35%,* Ladak-31%, *M.varia-31%,* Flemish-2%, and Chilean-l%. Ladak is an Indian *M. sativa* (Barnes et al., 1977) or

M. sativa x *falcata* varia (Quiros and Bauchan, 1988). Vernal, a conventional cultivar that is believed to have 25% *M. falcata*, has been used as a standard for conventional cultivar testing. WL346LH is a sixth generation (2004 release), PLH resistant cultivar (*M. sativa*) developed by WL Research (Madison, WI). Norcen, a European-type (upright growth habit) BFT, was included because BFT maintains good forage quality when harvests are delayed (Mays and Washko, 1960; Collins, 1982).

Trials were conducted at East Lansing (EL) (42.4 N, 84.3 W) and Lake City (LC) (44.2 N, 84.1 W) in the Lower Peninsula, and Chatham (CH) (46.3 N, 86.9 W) in the Upper Peninsula and were established 17-May, 27-May, 3-Jun 2005, respectively. The EL trial was arranged in a split-plot design with insecticide (Warrior [lambda-cyhalothrin] 0.017 kg a.i.ha^{-1}) (IT) and untreated (UT) as the main plot and cultivar/species as the sub-plot. Insecticide was applied at EL to the IT block on 12-Jun, 2006; 31-Jul, 2006; 25-May, 2007; 19-Jul, 2007; 15-May, 2008; and 11-Jul, 2008. The LC and CH trials were arranged as a randomized, complete-block design and were treated with insecticide, after the seeding year, when PLH were present. Insecticide was only required once at LC and was applied 19-Jul, 2007 and not needed in CH. Yield was collected with a rotary flail harvester (Carter Manufacturing Co. Inc., Brookston, IN) set to harvest 0.9 by 7.6 m plots at a cutting height of 9 cm from the soil surface. Dry matter content of harvested alfalfa was determined by collecting a subsample of harvested biomass which was weighed wet, dried at 60° C for 72 h, and weighed again. The DMY was determined as: wet weight of harvested biomass *(DM content [%] = Dry [g]/ Wet [g] x 100). A portion of this material was retained for nutritive evaluation. A single harvest was taken in the seeding year (2005) at LC (13-Sep.) and EL (24-Aug.), the CH trial was clipped for weed control and weights

were not taken. Harvest dates and growing degree day accumulations (base 5°C) for production

years (2006-2008) are reported in Table 3.1.

Table 3.1. Harvest dates (growing degree days base 5°C since 1-Mar or since previous harvest for second harvest) for the years (2006-2008) at Chatham, East Lansing, and Lake City, MI.

	2006		2007		2008	
Chatham	27-Jun (582)	24-Aug (872)	10-Jul (809)	4-Oct (1058)	14-Jul (631)	12-Sep (774)
East Lansing	20-Jun (738)	21-Aug (1026)	20-Jun(823)	29-Aug (1113)	23-Jun (753)	2-Sep (1094)
Lake City	22-Jun (648)	22-Aug (849)	28-Jun(859)	5-Sep (1056)	24-Jun (627)	14-Oct (941)

Forage Quality

Dried sub-samples obtained from the harvester at EL in 2006-2008 were ground to pass

through 1-mm screen in a Christy-Turner Lab Mill (Ipswich, Suffolk, UK) and a minimum

subsample of 30 g was retained for nutritive analyses. Each sample was scanned with a FOSS

6500 near-infrared spectrophotometer (NIRS)(FOSS NIRSystems, Inc., Eden Prairie, MN) with

wavelengths between 800 and 2500 nm and reflected wavelengths were recorded using the

WinISI v. 1.5 software. Crude protein (CP), acid detergent fiber (ADF), and neutral detergent

fiber (NDF) were predicted from equations developed by regression of chemically derived and

spectral data using a modified partial least squares regression method (Shenk and Westerhaus,

1991). Two randomly selected subsets were compiled to calibrate and validate the NIRS

prediction of dry matter (DM), CP, NDF, ADF and ash content. Dry matter content was

determined by drying 0.5 g of sample in ceramic crucibles at 100°C for 24 h. Samples were then

ignited in a muffle furnace at 500°C for 6 h to determine ash content. Total nitrogen was

determined by the Hach modified Kjeldahl procedure (Hach et al., 1985; Watkins et al., 1987),

and CP was estimated by multiplying total N by 6.25. The Van Soest and Robertson method

(1985) for fiber determination was used for NDF and ADF determination with the addition of 1

ml of alpha-amylase to the neutral detergent solution at the onset of boiling. The calibration and

validation statistics are presented in Table 3.2. The weighted average for each nutritive

constituent was calculated as (using CP as example): $WACP=(CP_i \times DMY_i)/\Sigma DMY_i$ where

WACP represents the weighted average of CP (g kg^{-1} DM), CP_i represents the CP concentration

of harvest i, and DMY represents dry matter yield of the harvest i.

Table 3.2. Near infrared reflectance spectroscopy calibration and validation statistics for crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF) for legume stands harvested over three years (2006-8) in Lake City and East Lansing, MI.

Constituent	n	Mean	SEC†	SECV‡	r^2§	1-VR¶
			------------g kg^{-1}-------------			
CP	73	141	4.2	4.9	0.95	0.91
ADF	54	314	11.0	10.9	0.97	0.94
NDF	47	487	6.2	12.7	0.99	0.97

†SEC, standard error of calibration.
‡SECV, standard error of cross validation
§r^2, coefficient of determination for calibration
¶ 1-VR, 1 minus the variance ratio in cross validation during modified partial least squares regression

Visual Ratings- Insect damage

Potato leafhopper (*Empoasca fabae*) (PLH) yellowing (PLY) was visually scored prior harvest using a 1 to 5 scale, 1 being 0-10% leaves exhibiting PLY symptoms and 5 being 81-100% from all plots when any one plot received a score of 2 or greater. Alfalfa weevil damage (AWD) was determined prior to the first cutting by counts of the number of damaged stems out of 20 when damage was present.

Statistical Analysis

The trials at LC and the CH were randomized complete block designs (RCBD) with four replications. The EL trial was a RCBD in a split-plot arrangement with insecticide treated or untreated as the main plot and cultivar as the sub-plot. Since the trial design differed, LC and CH trials were analyzed and presented separate from EL. All data was tested, based on the Shapiro-Wilk statistic, for normality and unequal variances using PROC UNIVARIATE. Analysis of variance was performed on all data with the PROC MIXED procedure software version SAS 8.2 (SAS Institute, 2003) using the Kenward-Roger method for determining degrees of freedom. Main effects and all interactions were considered significant when $P < 0.05$. The SAS macro 'PDmix80' (Saxon, 1998) was used to generate letter values for means separation based upon contrasts.

RESULTS AND DISCUSSION

The Yellowhead cultivar seeded in this trial did not express 97% yellow flowers that is characterized in the cultivar description by Agriculture and Agri-Food Canada (Swift Current, SK) (McLeod, 2009), so we suspect that *M. falcata* comprises a lower percentage of the

parentage for the seed that was used. Cooler temperatures prevailed in CH compared to EL or

LC (Table 3.3). Greater average monthly temperatures were observed as latitude decreased (CH

>LC>EL) for the years of the study (2005-2008). Below-average precipitation in Jul and Aug

was received in CH in 2007.

Table 3.3. Monthly precipitation (A) and average temperatures (B) 2005-2008 and the 30-yr. avg. (Norm) at Chatham (CH), East Lansing (EL), and Lake City (LC).

A

	CH					EL					LC			
	Norm	2005	2006	2007	2008	Norm	2005	2006	2007	2008	Norm	2005	2006	2007	2008
	mm														
Jan	55.1	55.1	90.2	81.5	113.8	40.1	67.3	88.4	38.9	51.8	40.1	85.9	83.1	35.6	74.2
Feb	33.8	47.7	68.3	54.1	82.0	35.3	45.0	27.4	3.6	31.2	30.7	43.9	32.8	28.7	52.6
Mar	52.1	48.0	90.9	102.8	53.6	54.6	15.7	60.5	56.4	39.9	51.6	38.9	69.6	87.9	31.2
Apr	62.5	60.2	50.5	163.3	160.0	71.4	30.2	59.2	47.0	4.0	73.2	26.4	93.0	88.1	88.6
May	80.0	49.0	145.0	55.1	92.5	69.3	47.8	142.5	105.7	34.5	67.8	65.5	140.5	56.4	45.5
June	91.7	28.2	21.8	52.3	97.8	89.9	113.5	74.4	141.2	121.9	78.5	52.1	127.0	95.0	181.6
July	90.4	81.8	53.8	44.2	44.7	76.7	163.1	93.5	13.2	94.5	82.8	93.7	55.4	30.5	99.8
Aug	90.2	48.8	84.1	19.6	27.2	79.2	27.7	142.7	132.1	12.7	76.5	113.8	116.3	63.2	54.9
Sept	102.0	105.2	64.8	126.5	96.0	63.5	97.3	74.9	53.3	213.9	82.6	103.1	83.3	47.8	74.9
Oct	82.3	83.6	96.3	133.4	58.9	55.9	6.1	97.0	114.8	40.9	67.3	18.3	118.9	62.2	68.3
Nov	78.7	83.3	82.8	47.8	86.4	67.3	67.6	96.5	36.1	29.2	63.0	111.5	58.2	49.0	56.9
Dec	57.2	86.9	146.6	114.8	151.6	50.5	26.7	51.1	40.6	44.5	46.0	40.9	57.7	69.6	120.1

B

	CH					EL					LC			
	Norm	2005	2006	2007	2008	Norm	2005	2006	2007	2008	Norm	2005	2006	2007	2008
	°C														
Jan	-8.8	-9	-3.3	-6.3	-7.5	-5.9	-5.9	1.7	-2.1	-2.6	-8.2	-6.9	-1.8	-5.2	-5.8
Feb	-7.3	-5.6	-7.1	-10.8	-9.9	-4.4	-2.7	-2.3	-8.2	-5.2	-7.2	-2.8	-6.1	-10.1	-9.2
Mar	-2.6	-4.1	-0.9	-0.7	-5.4	1.1	-1	2.6	4.9	0.6	-2.1	-4.4	-1.1	0.5	-4.4
Apr	4.1	6.2	6.9	3	3.9	7.6	8.2	10.5	7.6	10.3	5.2	7.4	7.6	4.2	7.4
May	11.2	9.8	11.1	12.3	8.2	14.1	12.6	15	16.1	12.7	12	10.1	12.9	13.2	10.3
Jun	16	18.4	15.9	17.2	15.5	19.2	22.6	19.7	20.9	20.6	16.9	20.7	17.2	18.7	17.9
Jul	18.7	19.7	20.9	19.1	18.4	21.4	22.7	23.2	21.1	21.9	19.4	20.4	20.8	19.4	19.6
Aug	18.1	19.1	18.3	18.3	17.9	20.3	22.7	21.8	22.2	20.9	18.2	19.1	19.1	19.7	18.6
Sep	13.8	16	12.8	14.9	14.1	16.1	19.3	15.5	18.4	18.4	13.6	16.3	13.3	15.1	14.8
Oct	7.8	9.4	5.7	10.6	7.3	9.7	11.7	8.9	13.5	9.9	7.6	9.4	6	11.4	7.1
Nov	0.6	-0.4	2.4	-0.3	0.3	3.3	5.1	6.3	4.2	4.3	1.2	2.2	3.5	0.7	1.7
Dec	-5.8	-6.9	-3	-6.8	-8.9	-2.8	-4.4	2.6	-1.9	-3.5	-5.1	-5.6	-0.4	-5	-6.5

Source: Michigan Automated Weather Network

Forage Yield

The main effect of cultivar and the interactions between location x cultivar and year x cultivar were significant for DMY for trial at LC and CH (Table 3.4), therefore, results will be presented by location and year. Significant main effects of cultivar and insecticide were observed at EL, as were the interactions of cultivar x year and insecticide x year (Table 3.5). Results for DMY at EL will be presented by year and, although there was not a cultivar x insecticide interaction, by cultivar within insecticide treatment.

Table 3.4. Results from ANOVA for dry matter yield (DMY), alfalfa weevil damage (AWD), and potato leafhopper yellowing (PLHY) of *M. falcata*, *M. sativa cultivars*, and birdsfoot trefoil over two locations (Lake City, Chatham, MI) and three years (2006-2008).

Source	DMY	AWD‡	PLHY‡
Cultivar (C)	***	*	***
Location (L)	**	-	-
Year (Y)	***	-	-
C x Y	***	-	-
C x L	**	-	-
C x L x Y	*	-	-

*Significant F tests at the P< 0.05 level
**Significant F test at the P<0.01 level
***Significant F test at the P<0.001 level
†ns = not significant
‡Observations at Lake City in 2006 only

Table 3.5. Results from ANOVA for dry matter yield (DMY), crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF) of *M. falcata*, *M. sativa cultivars*, and birdsfoot trefoil at East Lansing, MI over three years (2006-2008) and potato leafhopper yellowing (PLY) over two years (2005-2006).

Source	DMY	CP	ADF	NDF	CPY	TDNY	PLY‡
Cultivar (C)	***	ns	***	***	***	***	***
Insecticide (I)	*	ns	ns	ns	ns	ns	**
C x I	ns†	ns	ns	ns	ns	ns	**
Year (Y)	***	***	***	***	***	***	ns
C x Y	***	**	ns	***	***	***	ns
I x Y	*	ns	ns	ns	ns	ns	ns
C x I x Y	ns	ns	ns	ns	ns	ns	ns

*Significant F tests at the $P< 0.05$ level
**Significant F test at the $P<0.01$ level
***Significant F test at the $P<0.001$ level
†ns = not significant
‡Observations in 2005 and 2006 only

In the seeding year (2005), DMY measurements were not taken in CH, due to weeds present that would have altered crop yield. A single harvest was taken in the seeding year (2005) at LC. The greatest yielding cultivar in that harvest was SD201, while Spredor 3 produced the least DMY. While seedling vigor of SD201 appeared lower than other cultivars at each location in the early summer (pers. obs.), the DMY of SD201 at LC taken 13-Sep. 2005, was greater than all other cv.s except Vernal. The greatest average DMY production at LC and CH was in 2006 and the greatest decrease in DMY in both LC and CH occurred after the first production year. But the average DMY production over species was actually lower in 2007 than in 2008 at CH. Dry conditions in Jul and Aug of 2007 at CH may have been the cause. In all production years at LC and CH, the alfalfa cultivars produced more DMY than Norcen BFT. The DMY of BFT is typically less than alfalfa in three or more harvests per year (Beuselinck and Grant, 1995) SD201 produced as much or more DMY in each year as other alfalfa cultivars at LC, but was only in the

highest yielding group in 2006 at CH. The DMY production of SD201 at CH was 80% of the greatest yielding cultivar, WL346LH, in 2007, and 81% of Spredor 3 in 2008. Lower DMY of SD201 at CH may be due to fewer GDD's that had accumulated at first harvest, as compared to LC, in all years except 2008 (Table 3.1). Similar results were observed in a trial comparing SD201, WL346LH, and Norcen under three harvest frequencies. Three harvests per year of SD201 resulted in less total annual DMY than two harvests per year (Dietz, 2011). This may be due to delayed regrowth following defoliation, thereby, reducing second harvest DMY.

The average DMY across cultivars at EL was nearly twice that of LC in the seeding yr. (2005) (Fig. 3.1 and 3.2). This is probably a result of greater precipitation and higher temperatures (Table 3.3) at EL in Jun and Jul. Insecticide application contributed to greater DMY across cultivars at EL in 2005, 2006, and 2007, but not in 2008. Potato leafhopper populations were only at damaging levels and resulted in PLY ratings in 2005 and 2006 (Table 3.6), but carryover effects from damage may have impacted DMY in some cultivars in 2007. In the seeding yr (2005), Vernal DMY increased the most (1.88 Mg ha^{-1}) due to insecticide treatment and SD201 DMY increased the least (0.54 Mgha^{-1}), which may be due to breeding selection for PLH resistance/tolerance in SD201 by Boe et al. (1998). Other cultivars with equivalent DMY to SD201 in 2005 were IT-Vernal, IT-Yellowhead, and IT-Norcen. In 2005, the lowest DMY's were produced by UT: Ladak, Spredor 3, Travois, Vernal, WL346LH and Yellowhead. As with trials at LC and CH, the first production year (2006) at EL produced the greatest DMY. Total annual DMY decreased for each cultivar, each year, but Norcen DMY decreased the least. Norcen was among the lowest yield in 2006 and 2007 and produced the least DMY in 2008. In 2006, the *M. falcata* cultivar, SD201 treated with insecticide was the highest

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yielding at EL, but was not significantly greater than UT-SD201. The IT-Vernal, IT-Ladak, and

IT and UT Spredor 3 produced equivalent DMY to UT-SD201 in 2006. These findings support

those of Dietz et al.(2011) that SD201 will produce greater DMY in the first production year

than WL346LH in two-harvests per year, but that no DMY advantage was seen in subsequent

years. Yellowhead, a conventional *M. falcata* cultivar, was among the lowest yielding with the

UT not different than UT-Norcen, the lowest yielding. However, in 2007, IT and UT Yellowhead

DMY production was equivalent to IT and UT SD201. Lower DMY was obtained from SD201

in 2008, with UT-SD201 only being greater than Norcen. Insecticide treated-Ladak, IT and UT

Spredor 3, and UT-Travois were highest yielding in 2008. After two years without PLH pressure,

there was no advantage to the insecticide application in all cultivars except Ladak.

Table 3.6. Potato leafhopper yellowing (PLY) ratings of seven alfalfa cultivars and one birdsfoot trefoil in 2005 and 2006 (two-yr. average) with and without insecticide in East Lansing and in 2005 at Lake City and alfalfa weevil damage counts for 2006 at Lake City, MI.

	East Lansing		Lake City	
	PLY*			
	2-yr. avg.		PLY*	AWD**
	IT†	UT‡	2005	2006
Ladak	1.0	3.3	2.5	14.50
Norcen	1.0	1.1	1.0	4.25
SD201	1.0	1.3	1.0	10.25
Spredor3	1.1	3.1	2.6	12.00
Travois	1.1	3.4	2.9	19.50
Vernal	1.0	3.4	2.9	13.25
WL346LH	1.1	1.5	1.0	19.25
Yellowhead	1.4	3.1	2.5	15.25
LSD (0.05)			0.4	8.20
within spray	NS	0.8		
between spray		0.5		

* Potato leafhopper yellowing severity rating (1=no hopperburn [hb], 2=slight hb, 3= moderate hb, 4= moderately high hb, 5= severe hb)
**Alfalfa weevil damage: number of stems showing leaf feeding per 20 stems
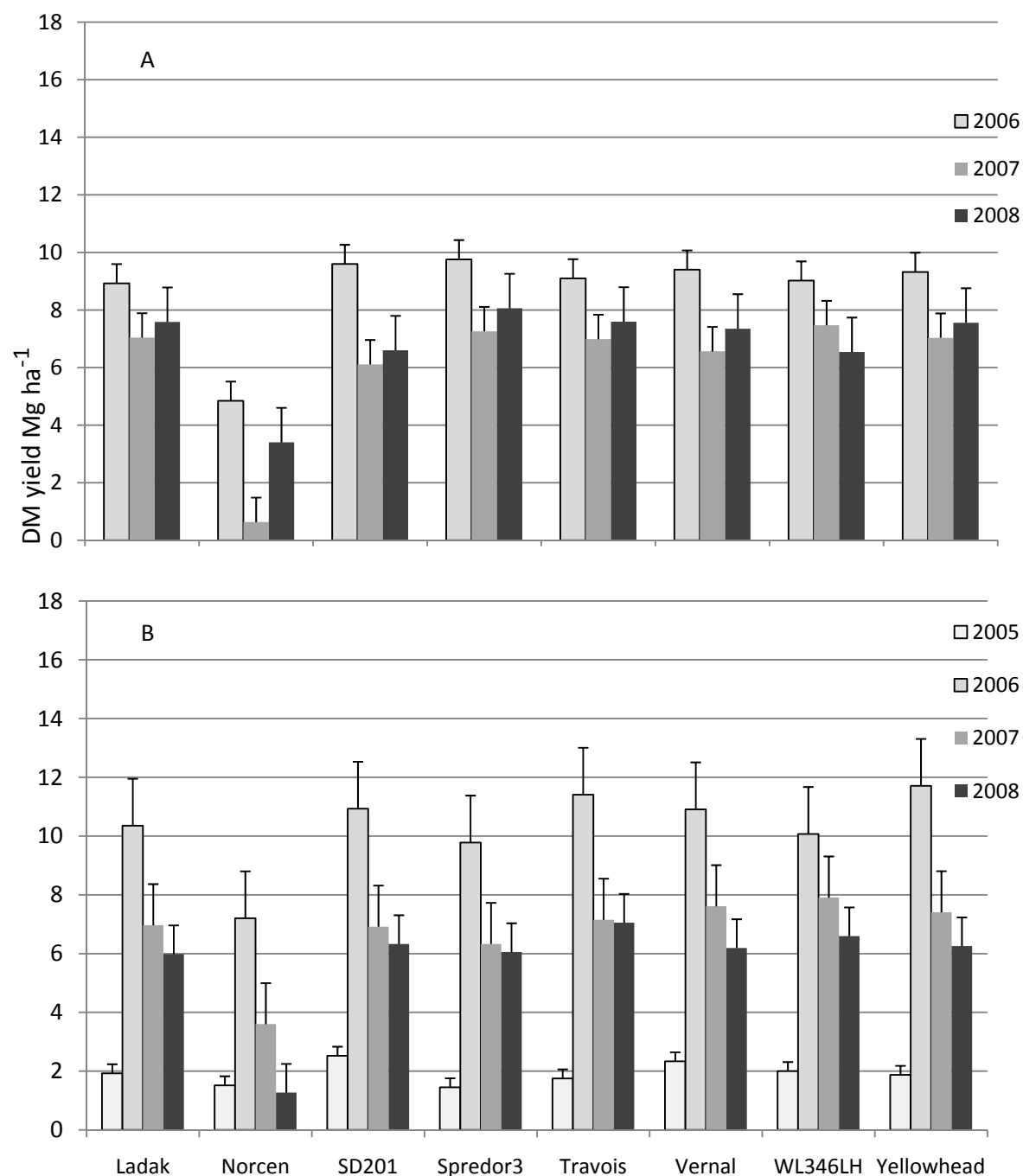† Insecticide treated
‡ Untreated

Figure 3.1. Forage dry matter yield (kg ha^{-1}) of seven alfalfa cultivars and one birdsfoot trefoil (Norcen) at Chatham (A) from 2006 to 2008 and Lake City (B), MI from 2005-2008. Error bars represent least significant difference ($P < 0.05$) for comparisons within a year and location.

Figure 3.2. Forage dry matter yield (kg ha^{-1}) of seven alfalfa cultivars and one birdsfoot trefoil (Norcen) under insecticide treated (IT) and untreated (UT) conditions at East Lansing, MI from 2005-2008. Error bars represent the least significant difference ($P < 0.05$) for comparisons within a year.

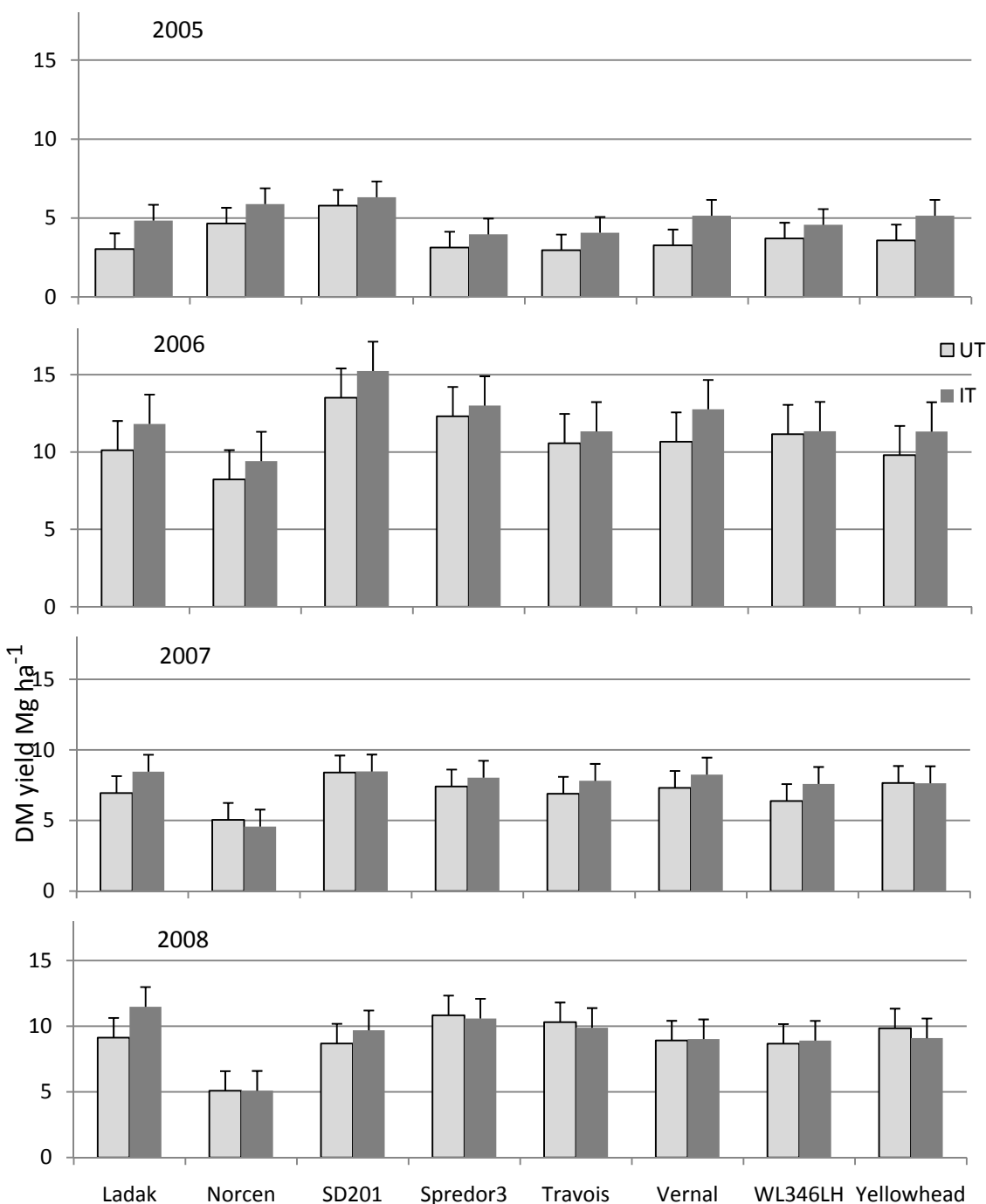While there were interactions between cultivar and year, total DMY over years is

important when comparing perennial forage performance. All alfalfas evaluated produced greater

total DMY than Norcen at all locations (Table 3.7). There was no total DMY advantage for

SD201 over other alfalfas at LC or CH. While in EL, IT-SD201 produced greater total DMY

than all cultivars except Ladak, Spredor 3 and Vernal. For the UT comparison, SD201 and

Spredor 3 produced greater total DMY than all others. We had expected Yellowhead and SD201

to produce similar DMY as they did at LC, since this was believed to be the cultivar with

greatest percentage of *M. falcata* parentage, but EL results suggest that SD201 will produce

greater total DMY than Yellowhead under IT or UT conditions. Conversely, Yellowhead

produced greater total DMY than SD201 at CH. The difference between total DMY results in

LC/CH and EL may be a result of almost always greater GDD's at EL at harvest. The growth

habit and seasonal production of Yellowhead may be more similar to *M. sativa* or *varia* cultivars

and less similar to SD201.

Table 3.7. Total dry matter yield (Mg ha^{-1}) over four years (2005-2008) at East Lansing (EL) and Lake City (LC) and three years (2006-2008) at Chatham (CH), MI of seven alfalfas and one birdsfoot trefoil. The EL location included insecticide treated (IT) and untreated (UT) evaluation.

	LC	CH	EL	
			IT	UT
	--------------------Mg ha^{-1}--------------------			
Ladak	25.22	23.59	36.04	28.81
Norcen	13.57	9.16	24.60	23.27
SD201	26.72	22.33	39.65	36.98
Spredor 3	23.61	25.09	35.44	34.12
Travois	27.35	23.68	32.03	30.46
Vernal	27.04	23.36	34.94	30.22
WL 346LH	26.57	23.05	32.86	30.35
Yellowhead	27.31	23.90	33.06	30.69
Mean	24.68	21.77	33.58	30.62
LSD (0.05)	3.40	2.20	4.65	3.27
LSD between			2.68	

Forage Quality

A year x cultivar interaction required CP concentration comparisons of cultivars by year (Table 3.5). There was no effect of insecticide spray on CP and no interaction between cultivar and spray within each year. There was a significant main effect of cultivar but there was no effect of insecticide on ADF and NDF, nor was there a cultivar x insecticide interaction. A cultivar x year interaction for NDF required results presented by year, and since ADF and NDF are closely related, ADF results are also presented by year.

The CP concentration of Norcen was less than all alfalfa cultivars in 2006 (Fig. 3.3), possibly due to the presence of grass weeds (primarily Yellow foxtail [*Setaria glauca* L.]). Weed presence also affected ADF and NDF concentrations of Norcen which were similar to several alfalfa cultivars in 2006, while all other years were Norcen was lowest (Fig. 3.4). Norcen forage quality is typically greater than alfalfa when harvest is delayed. Among alfalfa cultivars there were no differences in CP concentration in 2006. The ADF and NDF concentrations of SD201 in 2006 and 2008 were among the lowest. The ADF concentration of SD201 was only 30 g kg^{-1} greater than Norcen in 2008 and NDF concentration was only 52 g kg^{-1} greater.   In 2007, WL346LH was 10 g kg^{-1} greater in CP concentration than SD201 but similar to all others including Norcen. As in Dietz et al. (2011), WL346LH crowns may have initiated growth of new shoots once growth of seed pods had ceased, increasing leaf area and CP concentration. In 2008, Norcen CP concentration was the greatest (135 g kg^{-1}) but not significantly greater than Vernal and WL346LH. Travois CP concentration was the lowest (121 g kg^{-1}) but not

significantly different than Ladak, SD201, Spredor 3, and Yellowhead. In 2008, Norcen BFT produced greater CP concentration than *M. falcata* cultivars in this delayed harvest schedule.

In 2007, the ADF and NDF concentrations of SD201 and Yellowhead were the greatest and equal to that of Vernal (Fig. 3.4 and 3.5). Both harvests in 2007 were taken after more GDD had accumulated than 2006 or 2008. The first harvest in 2007 was at 823 GDD's which was 85 and 70 GDD later than 2006 and 2008, respectively. The second harvest that year was also later at 1113 GDD since the first harvest which was 87 and 19 GDD later than 2006 and 2008, respectively. This may have been long enough for *M. sativa* alfalfas to initiate new shoot growth.
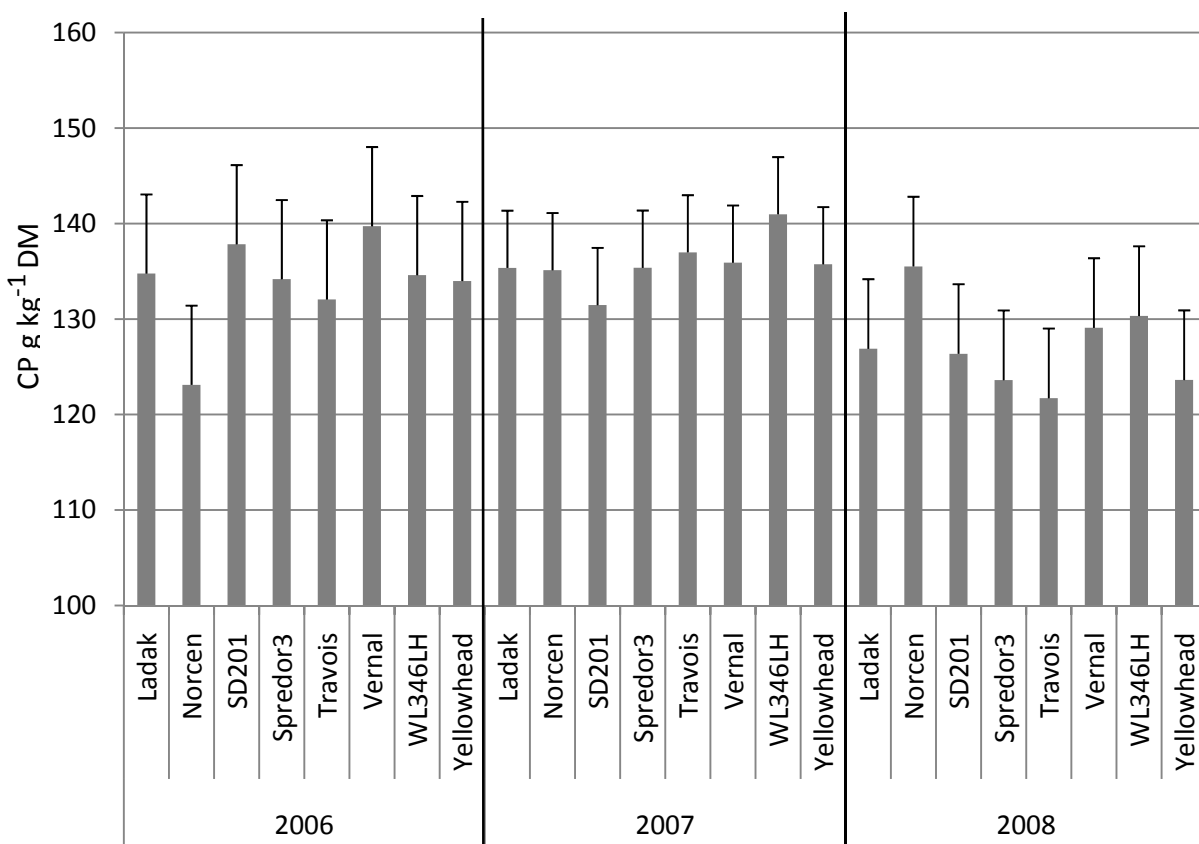

Figure 3.3. Weighted average crude protein content (g kg^{-1}) for seven alfalfa cultivars and one birdsfoot trefoil (Norcen) average of insecticide treated and untreated for three years (2006-2008) at East Lansing, MI. Error bars represent the least significant difference ($P < 0.05$) for comparisons within a year.

Figure 3.4. Weighted average acid detergent fiber (ADF) concentration (g kg^{-1}) for seven alfalfa cultivars and one birdsfoot trefoil (Norcen) average of insecticide treated and untreated for three years (2006-2008) at East Lansing, MI. Error bars represent the least significant difference ($P < 0.05$) for comparisons within a year.
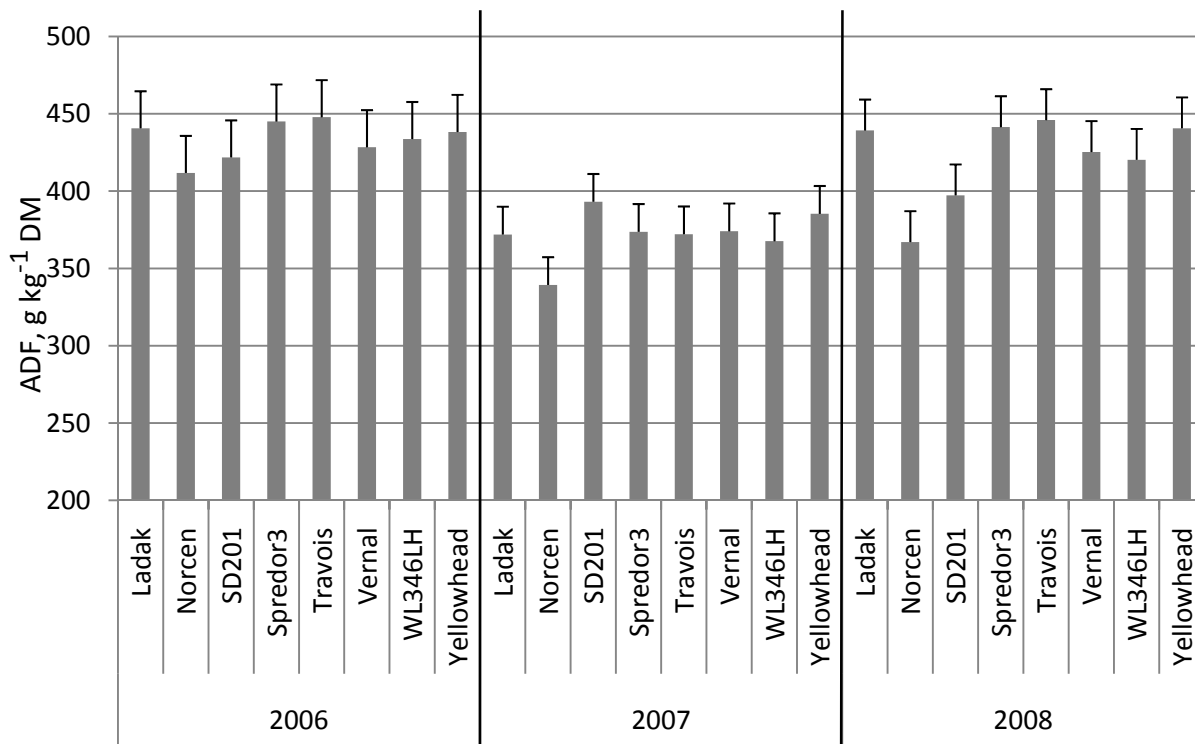
Figure 3.5. Weighted average neutral detergent fiber (NDF) concentration (g kg^{-1}) for seven alfalfa cultivars and one birdsfoot trefoil (Norcen) average of insecticide treated and untreated for three years (2006-2008) at East Lansing, MI. Error bars represent the least significant difference ($P < 0.05$) for comparisons within a year.
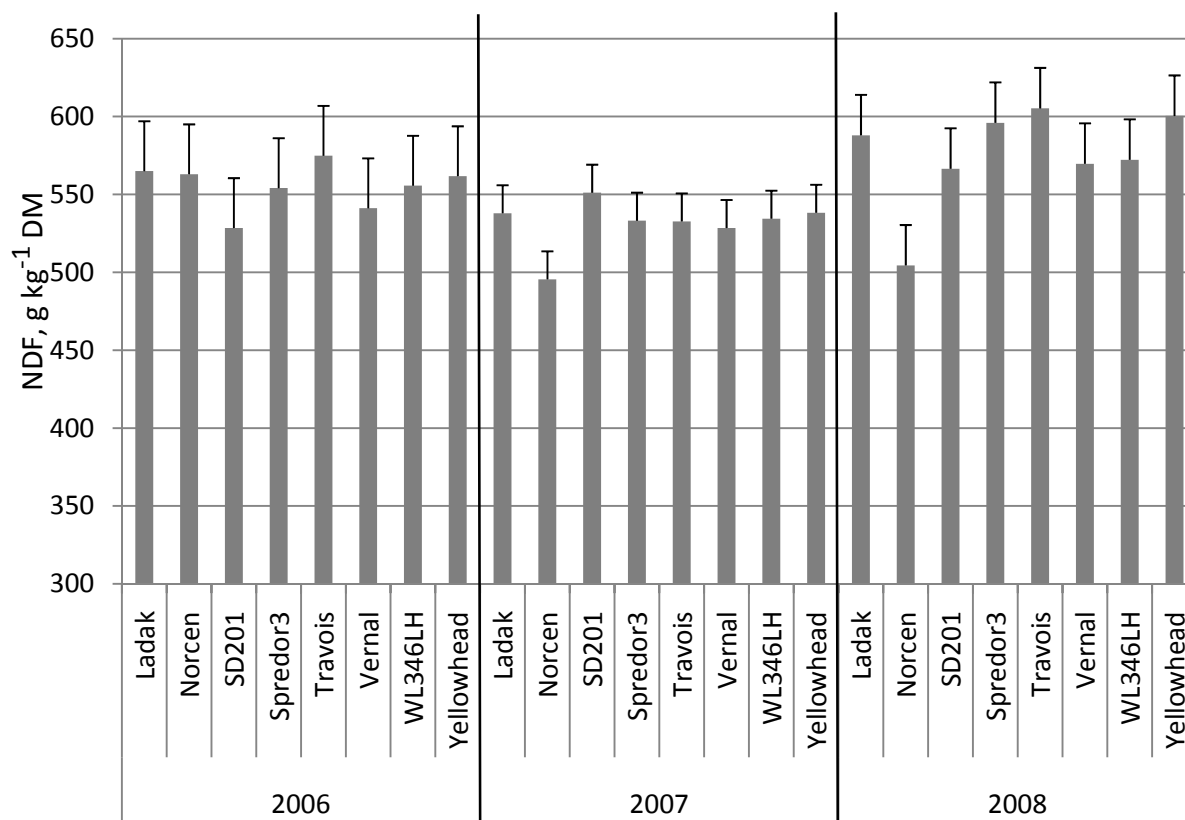
The data suggests that SD201 alfalfa does not produce forage of greater quality (CP, ADF, and NDF) than *M. sativa* or *varia* cultivars tested when harvested two times per year. This concurs with the findings of Bortem et al (1994) who found germplasm with high levels of *M. sativa* spp. *falcata* were lower in quality (lower in CP and *in vitro* dry matter digestibility) than *M. sativa.* harvested once in July in South Dakota.

Nutrient Yield

While CP, ADF, and NDF concentrations of SD201 were similar to other alfalfas in most years, greater CPY (Fig. 3.6) and TDNY (Fig. 3.7) were obtained from SD201 in 2006 due to

greater DMY. In 2006, the CPY of SD201 exceeded that of WL346LH and Yellowhead by 0.4 and 0.5 Mg ha^{-1}, and the TDNY was greater by 2.6 and 3.2 Mg ha^{-1}, respectively. In other years, there were few CPY or TDNY differences within alfalfas but BFT was typically lower. Regression analysis of DMY on TDNY (TDNY= 0.84 (DMY) + 0.001, R^2=0.99, P<0.0001) and CPY (CPY= 0.13 (DMY) + 0.007, R^2=0.93, P<0.0001) revealed strong relationships and suggests that DMY greatly affects these measurements of nutrient yield. Regression of CPY on CP resulted in an insignificant relationship (P=0.06), while TDNY on NDF (the parameter used to estimate TDN) revealed a weak, but significant relationship (TDNY=0.018(NDF) - 2.19, R^2=0.13, P<0.0001). Strong relationships were also observed by Lissbrant et al (2009) between yield and digestible nutrient yield (IVDMD x yield).
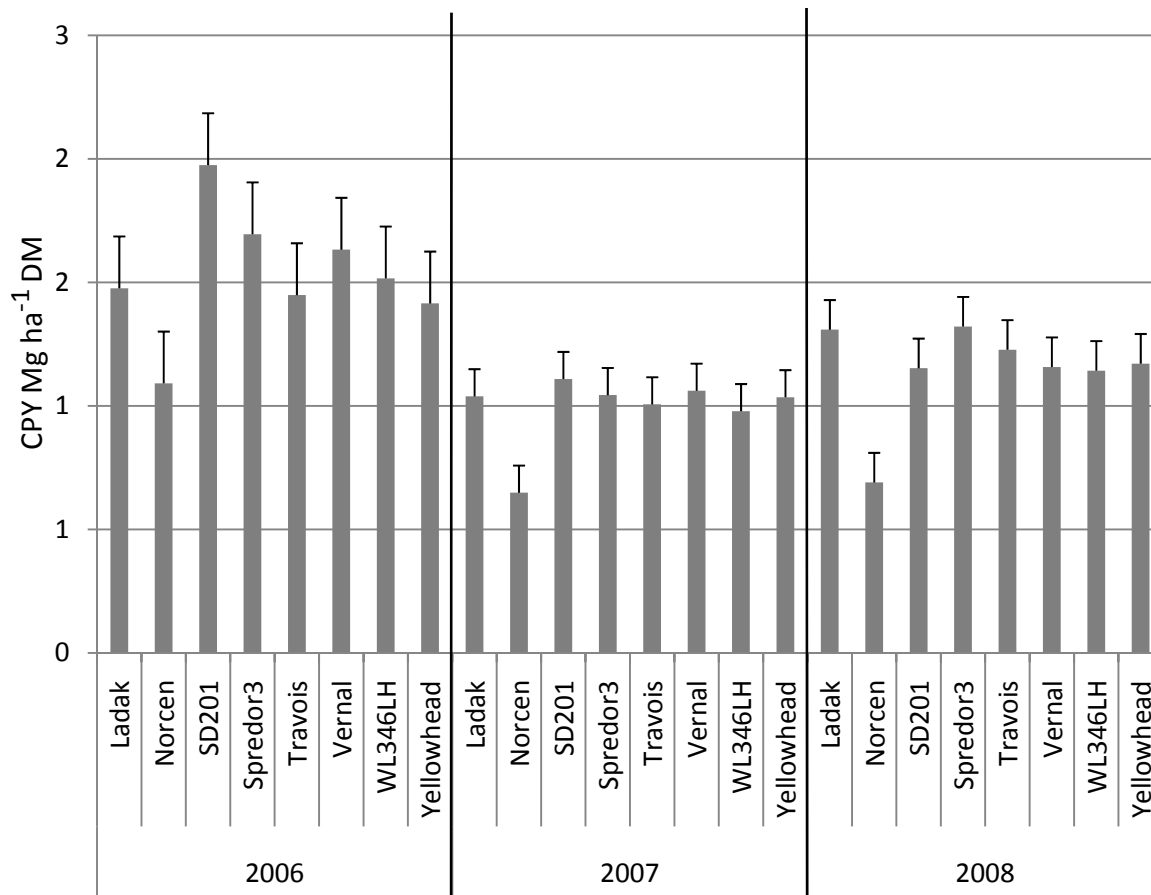
Figure 3.6 Weighted crude protein yield (CPY) (Mg ha^{-1}) for seven alfalfa cultivars and one birdsfoot trefoil (Norcen) average of insecticide treated and untreated for three years (2006-2008) at East Lansing, MI. Error bars represent the least significant difference ($P < 0.05$) for comparisons within a year.

Figure 3.7 Weighted total digestible nutrient yield (TDNY) (Mg ha^{-1}) for seven alfalfa cultivars and one birdsfoot trefoil (Norcen) average of insecticide treated and untreated for three years (2006-2008) at East Lansing, MI. Error bars represent the least significant difference ($P < 0.05$) for comparisons within a year.
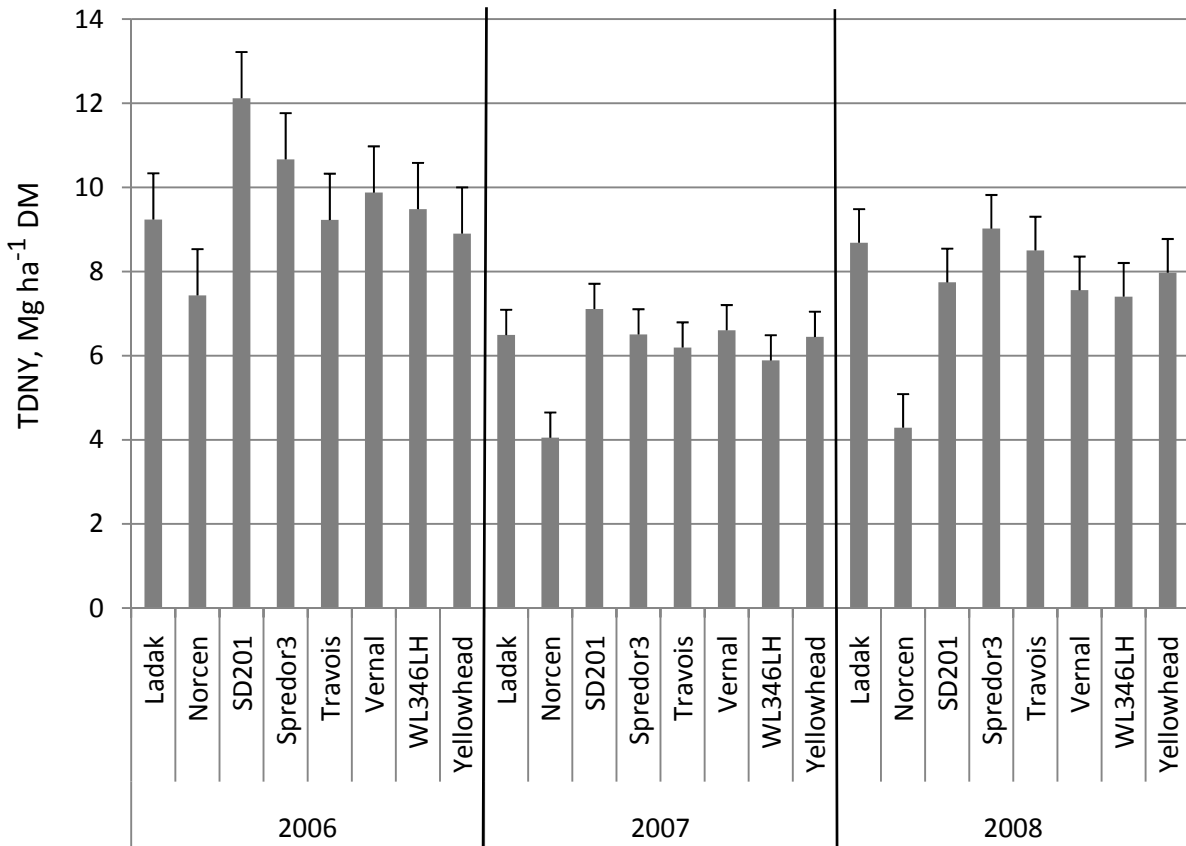
Visual Ratings- Insect damage

Potato leafhopper yellowing was measured at EL in 2005 and at EL and LC in 2006. The main effects at EL of cultivar, insecticide, and interaction between them were significant but year and the interactions between cultivar x year and insecticide x year were not (Table 3.4). The effect of cultivar was significant for PLY at LC (Table 3.3). Insecticide effectively reduced PLY for all cultivars except Norcen, SD201 and WL346LH which already had low PLY ratings than other cultivars in the UT treatment (Table 3.6). WL346LH and SD201 were bred and selected for

greater PLH resistance and visually there was little sign of PLY in these cultivars (Figure 3.8).

Yellowhead did not score higher than known PLH-susceptible alfalfa cultivars.

Alfalfa weevil damage was measured at LC in 2006 and the effect of cultivar was significant (Table 3.3 and Fig. 3.9). As with PLY measurements, Norcen had very little evidence of damage from alfalfa weevil. Resistance to PLH did not confer resistance to alfalfa weevil for WL346LH with 19.25/20 stems showing feeding damage. The SD201 cultivar, however, did have significantly less AWD than other alfalfa cultivars with 10.25/20 stems damaged. The pubescence or glandular hairs visible on WL346LH were not visible on SD201, suggesting that another factor may confer tolerance, resistance, or non-preference of PLH and resistance or non-preference of alfalfa weevil. Although AWD were highest for WL346LH and Travois, DMY of these cultivars was not significantly lower than that of the greatest DMY of Yellowhead in that year.
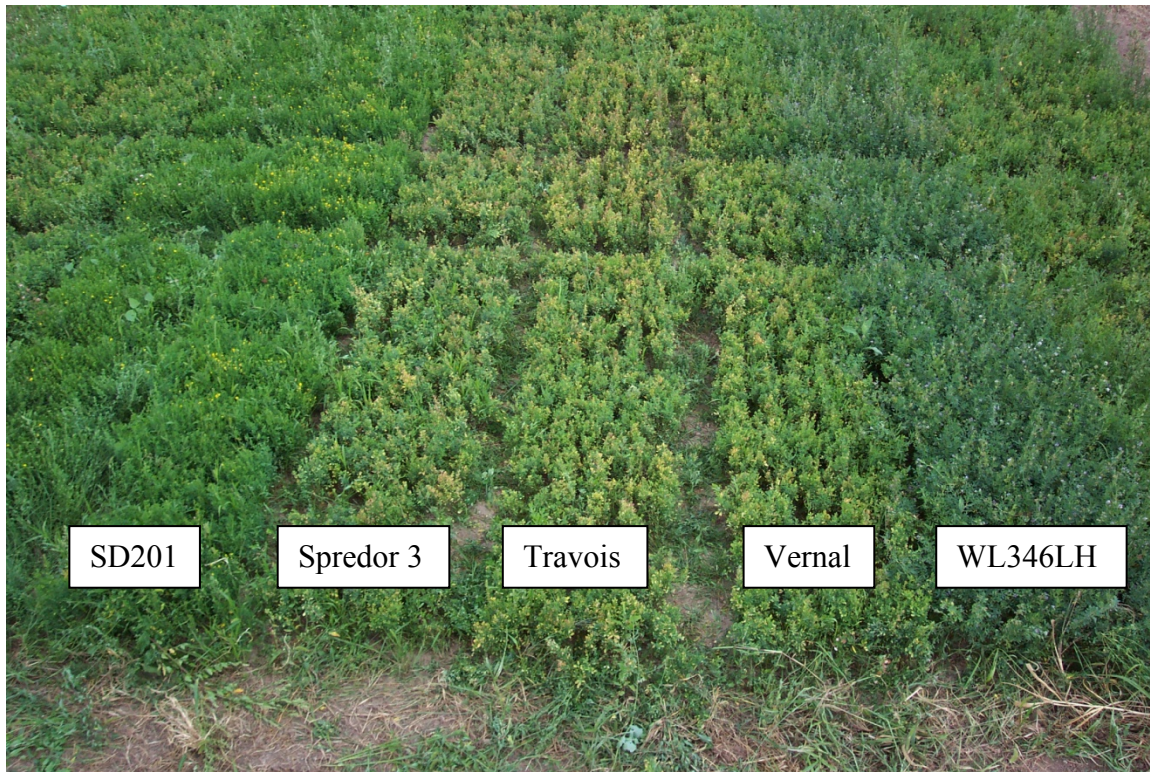
Figure 3.8. The effect of Potato leafhopper on cultivars not treated with insecticide (UT) at East Lansing, MI 8-Aug, 2005. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this document.

Fig. 3.9 Comparison of *sativa* (left) and *falcata* (right) above-ground biomass at Lake City at first cutting in 2006.

Although SD201 was more fall dormant than *M. sativa* cultivars tested, observations of leaf senescence in the late-fall suggest that SD201 leaf material remains viable to lower temperatures than *M. sativa* cultivars. Greater sugar concentrations of above-ground biomass of SD201 may depress the freezing temperatures, reducing cell rupture. Photos taken 29-Nov, 2005 at EL show no frost/freeze damage of SD201, while leaves of WL346LH are discolored and flaccid (Fig 3.9). At EL, November 2005 had an average temperature of 5.1°C and a minimum temperature of -11.8°C on 11-Nov, 2005. Fall/winter stockpiling of SD201 may produce greater quality forage than *M. sativa*.

Figure 3.10. A comparison of SD201 (left) and WL346LH (right) in the field and cut biomass on 29-Nov, 2005 at East Lansing, MI.

CONCLUSIONS

The DMY performance of *M. sativa* spp. *falcata* 'SD201' varied when compared to other cultivars tested. SD201 produced greater DMY than other alfalfas in select years and locations and almost always produced greater DMY than Norcen BFT. Greater DMY was typically obtained from SD201 over other cultivars when first harvest was taken later. There is evidence that SD201 may be resistant to alfalfa weevil or possess non-preference characteristics which may reduce the need for insecticide applications. There was no advantage in CP, ADF, or NDF for SD201 over other cultivars tested. The CP concentration and ADF and NDF levels of SD201 were usually equal to *M. sativa* and *M. varia* cultivars tested, but the CP concentration was lower, and ADF, and NDF were higher than Norcen BFT when harvest was taken earlier. The CPY and TDNY of SD201 usually exceeded that of other alfalfas and BFT in the first full production year. Cryogenic compounds such as sugar may reduce the freezing point of cellular liquids, thereby prolonging above-ground biomass quality later in the growing season than *sativa or varia.* The SD201 characteristics of alfalfa weevil resistance/non-preference and sustained growth in low temperatures are important attributes that should be further investigated.

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