EFFECTS OF ABIOTIC GROWTH FACTORS ON GLUCOSINOLATE LEVELS, SENSORY QUALITY AND YIELD COMPONENTS IN CABBAGE (*BRASSICA OLERACEA* GROUP CAPITATA)

DISSERTATION

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By

Theodore J.K. Radovich, M.S.

* * * *

The Ohio State University

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Dissertation Committee:

Dr. Matthew D. Kleinhenz, Advisor

Dr. Mark A. Bennett

Dr. Jeannine F. Delwiche

Dr. A. Raymond Miller

Dr. John G. Streeter

Approved By

_____

Advisor

Department of

Horticulture and Crop Science

ABSTRACT

Glucosinolate concentrations, flavor and physical characteristics comprise cabbage quality, and are thought, based primarily on results from single factor studies, to be affected by genetics, growth stage, temperature and irrigation. However, the individual and interactive influence of these factors on cabbage quality is incompletely characterized. Therefore, the goal of this program was to integrate focused, multi-factor field and controlled environment studies in order to identify and describe: 1) components of cabbage flavor, 2) the influence of planting date and cultivar on cabbage glucosinolate concentrations, including as they relate to flavor, 3) the effect of irrigation timing with respect to head development on cabbage flavor, glucosinolate concentrations and physical traits, 4) changes in physical traits which occur during head development and 5) tissue specific changes in glucosinolate concentrations following differential soil moisture treatments, using radish as a model system. In the first study, 26 cabbage cultivars grown in 2001 were evaluated by 12-14 panelists. To panelists, flavor was more important than texture or appearance, and cultivars differed significantly in panelists' overall acceptability scores. In the second study, total glucosinolate concentrations were measured in six commercial cabbage cultivars planted in May and June of 2001 and 2002 in Fremont, OH. Glucosinolate

concentrations varied with cultivar and planting date, with glucosinolate concentrations higher in May- than June-planted cabbage. And, pungency scores and mean glucosinolate concentrations of cultivars were significantly correlated in May- but not June-planted cabbage. The cultivar x planting date interaction was not significant, suggesting that they act independently to influence cabbage glucosinolate concentrations. In Study 3, flavor, glucosinolate concentrations, sugar levels and physical traits in 'Bravo' cabbage planted at the OARDC in 2002 and 2003 were influenced by the timing of irrigation relative to the stage of head development. Glucosinolate concentrations and sucrose levels were highest in cabbage not irrigated during head development, while fructose and glucose concentrations and head size and weight were greatest in cabbage receiving irrigation during head development. The independent and interactive effects of year and irrigation treatment were largely explained by the proportion of crop evapotranspiration replaced during head development. In additional analyses of heads from Study 3, the accuracy and precision of cabbage head volume estimates based on geometric formulae were found to be high across a wider range of conditions than previously reported. In Study 4, head physical characteristics were studied at five points in development in three cultivars planted at the OARDC in 2001 and 2002. Developmentally related increases in head size and weight across both years were explained by heat unit accumulation, while changes in density were not. Finally, in Study 5, a model system including radish grown in controlled environments was employed in 2003 and 2004 to begin to isolate the effects of soil moisture regime (15, 25 and 50% volumetric soil moisture), relative humidity

and plant tissue on concentrations of glucosinolates and the activity of their hydrolytic enzyme myrosinase. On average, enzyme activity and glucosinolate concentrations increased with decreasing soil moisture and glucosinolate levels in roots and hypocotyls were negatively related. Collectively, results from these studies improve the understanding of growth factor effects on important chemical, sensory and physical indicators of cabbage quality and facilitate the development of management strategies designed to optimize it for industry and consumer benefit.

Dedicated to my wife

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VITA

August 5, 1971…...……Born, Waimanalo, Hawaii

May, 1994……..…….B.Sc. Horticulture, University of Hawaii

August, 2000……...M.Sc. Horticulture, University of Hawaii

September 2000 – Present……..Graduate Research Associate,
The Ohio State University, Columbus, OH.

Research Publications

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FIELD OF STUDY

Major Field**:** Horticulture and Crop Science.

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CHAPTER 1

LITERATURE REVIEW

1.1 INTRODUCTION

Cabbage (*Brassica oleracea* Group Capitata) is an important vegetable. Originally collected for its medicinal value, the wild progenitor of cabbage has been selected and bred to produce several groups of vegetables valued as food for their specialized tissue structures, including the inflorescence of broccoli (*Brassica oleracea* Group Italica) and the stem of kohlrabi (*Brassica oleracea* Group Gongylodes) (Rubatzky and Yamaguchi, 1997). The large, vegetative apical bud of fresh-market cabbage is consumed worldwide in salads (e.g., cole-slaw) or as a table vegetable (Dickson and Wallace, 1986). In 2003, the fresh market cabbage industry was worth $290 million and $6.1 million in the U.S. and Ohio, respectively (National Agricultural Statistics Service, 2004). Although substantial, these numbers represent a decline in value since 2001 by almost $50 million in the U.S and $700,000 in Ohio (National Agricultural Statistics Service, 2004). The development of management systems that maximize vegetable crop quality has been identified as an important step in improving the value of fresh market cabbage in

Ohio and elsewhere (Kleinhenz and Wszelaki, 2003).

Quality is the measure of the desirability of an object resulting from the evaluation of individual characteristics, also termed "qualities" (Webster's New Twentieth Century Dictionary, 1971). Accordingly, vegetable crop quality is defined here as the hedonic value assigned to a commodity based on evaluation of specific traits, termed indicators, and may be expressed as either a degree of excellence, or an absence of defects (Shewfelt, 1999). Quality is subjective, and it depends on the relative importance of indicators to evaluators. For example, yield and its components are indicators of greater importance to growers than to consumers in assessing quality, while appearance and flavor are of primary importance to consumers. Quality may be quantified as a function of the parametric measures of indicators, if the relative importance of each indicator to a target population (e.g., consumers) is known (Shewfelt et al., 1997). Therefore, integrating quantitative indicators into assessments of quality will enhance the ability to meet target levels of quality at all points between production and consumption by providing standardized and objective information used in quality assessment.

Yield and its components determine the market availability of a commodity, which can influence its price, and, subsequently, perceptions of its quality. For example, the improvement of cabbage quality has focused on indicators such as head weight, volume and core size because of their importance to buyers (Dickson and Wallace, 1986; Kleinhenz and Wszelaki, 2003; Stofella and Fleming, 1990). However, additional indicators have become important as other approaches to improving fresh vegetable

quality are employed in efforts to secure and extend shares of increasingly competitive markets (Kuchenbuch et al., 1999). Sensory quality and potential health value are now more routinely studied as indicators of product quality. Both of these attributes are determined largely by the chemical composition of plant tissues. In cabbage, for example, head glucosinolate concentrations affect flavor and are thought to reduce the risk of some cancers (MacLeod and Nussbaum, 1977; Verhoeven et al., 1997). Therefore, their potential chemo-protective properties and strong influence on flavor make glucosinolates a particularly appealing target in efforts to maximize cabbage quality.

1.2 GLUCOSINOLATES

1.2.1 Description

Glucosinolates are a group of more than 120 secondary plant metabolites found throughout several plant families, including the agriculturally important *Brassicaceae* (Fahey et al., 2001). Derived from amino acids, they are enzymatically hydrolyzed to release glucose, sulfur, and an aglucon whose structure depends on the conditions (pH, parent amino acid, cofactors) of hydrolysis (Bones and Rossiter, 1996). The aglucon is typically more biologically active than its parent glucosinolate. The breakdown products of glucosinolates contribute to plant defense, human and livestock health, and the sensory quality of vegetables (Rosa et al., 1998). As the production of vegetable crops increases, indicators of quality, such as flavor and potential health value, become

more important in differentiating growers and industries. The involvement of glucosinolates in determining crop quality and the potential for environmental factors (including cultural practices) to affect glucosinolate levels in plants make it important to better understand the factors contributing to levels of these compounds in crops.

1.2.2 Role in human health

Much work was conducted in the 1970's and 1980's to investigate the levels of glucosinolates and their breakdown products in vegetables due to the potential goitrogenic activity of 5-vinyl-oxazolidine, thiocyanate ion and certain isothiocyanates (Bradshaw et al., 1983; Chong and Bible 1974; Chong and Bible 1975; Rosa et al., 1997; Tookey et al., 1980; VanEtten et al., 1976; VanEtten et al., 1980). Although conclusive evidence that *Brassica* vegetables, glucosinolates or their breakdown products contribute significantly to human health problems is lacking (Fenwick et al., 1983; Verhoeven et al., 1997), recent studies demonstrating the health promoting properties of *Brassica* vegetables (attributed to glucosinolate hydrolysis products) have renewed interest in the levels of these compounds in horticultural crops. Consumption of Brassicacious vegetables has been linked to a reduced risk of the onset of certain cancers (Wargovich, 2000). The anticarcinogenic activity of *Brassica* vegetables, isothiocyanates and indoles is thought to be due at least in part to the induction of enzyme systems that inhibit the production or limit the toxicity of carcinogenic compounds, as well as apoptosis of cancerous cells (Srivastava et al., 2003; Verhoeven,

1997). Specifically, isothiocyanates may induce elevated activity of phase II enzymes such as glutathione S-transferase and quinone reductase (Talalay et al., 1988).

1.2.3 Biosynthesis

In cabbage, glucosinolates are derived either directly from tryptophan, methionine or phenylalanine or from side-chain elongated methionine derivatives (Fahey et al., 2001; Rosa et al., 1997). The synthesis of glucosinolates from these amino acids has been reviewed by Halkier and Du (1997) and the major intermediates are presented in Figure 1.1. The parent amino acid is first enzymatically converted to an oxime to which sulfur is added (most likely from cysteine) to form a thiohydroximate. Glucose is incorporated into the thiohydroximate via a thiohydroximate glucosyltransferase to form the desulfoglucosinolate which in turn is converted to its corresponding glucosinolate via 3'-phosphoadenosine 5'-phosphosulphate (PAPS). The side chains of these glucosinolates may then be further modified to produce a wide range of related glucosinolates. The gene family controlling expression of the enzymes responsible for conversion of amino acids to oximes has been identified (CYP79) and CYP79 transgenes may be employed to produce novel glucosinolates in cabbage and other crops (Mikkelsen et al., 2003).

1.2.4 Hydrolysis

Glucosinolates are relatively unreactive compounds and are considered storage forms of their biologically active aglucones (Rask et al., 2000). Myrosinase (thioglucoside hydrolase E.C. 3.2.1.147) is the enzyme responsible for the hydrolyzation of glucosinolates to produce glucose and sulfate as well as isothiocyanates, nitriles, thiocyanate, epithionitriles, or oxazolidine-2-thiones, depending on the parent glucosinolate, pH, and other enzymes or cofactors which may be present (Bones and Rossiter, 1996; Rosa et al., 1997) (Figure 1.2). Myrosinase is compartmentalized in the vacuoles or 'grains' of specialized (myrosin) cells, and is released to combine with glucosinolates upon tissue disruption or increased membrane permeability (Pocock, 1987). Several isozymes of the enzyme exist and they may differ among species or tissues of the same species in substrate affinity (Bones and Rossiter, 1996). Ascorbate concentrations between 1-5 μM can greatly increase myrosinase activity (Wilkinson et al., 1984). Conditions reported to be optimum for hydrolysis are pH 5-7.5 and temperatures 45-70 ˚C (Bones and Slupphaug, 1989; Yen and Wei, 1993).

1.2.5 Factors affecting plant levels

1.2.5.1 Genotype

Genotypic differences in glucosinolate concentrations and profiles within and among crop species are well documented (Daxenbichler et al., 1979; Heaney and Fenwick, 1980; Hill et al., 1987; Kushad et al., 1999; Sones et al., 1984; Rosa et al., 1996; Rosa et al., 2000; Van Etten et al., 1976). Much of the work characterizing the genetic regulation of glucosinolates has been done on the reproductive tissues of *Brassica* oil-crops as part of efforts to reduce the levels of potential toxicants in animal feed. With this information, breeders have been successful in developing low glucosinolate lines (Gland, 1984; Murphy and Mithen, 1995). Mithen et al. (1995) measured glucosinolate levels in the leaves of several wild *Brassica oleracea* populations and found aliphatic glucosinolate levels to be highly heritable but levels of indolyl glucosinolates largely determined by environment.

1.2.5.2 Ontogeny

Levels of glucosinolates and related compounds vary during plant development and among plant tissues (Clossais-Besnard and Larher 1991; Rosa et al., 1996; Smith and Griffiths, 1988). In general, concentrations are greatest in seeds and roots and higher in young relative to older tissue (Clossais-Besnard and Larher, 1991; Rosa et al.,

1996, Rosa et al., 1998). Glucosinolate levels (both total and individual) are reported to change at seed germination, early growth and flower initiation (Chong and Bible, 1974; Clossais-Besnard and Larher 1991; Cole, 1980; Smith and Griffiths, 1988).

1.2.5.3 Planting date

Reports that planting date impacts the levels of glucosinolates and related compounds also provide strong evidence that abiotic growth factors influence glucosinolate levels (Ciska et al., 2000; Gaweda et al., 1991; MacLeod and Nussbaum 1977; Qi and Longzhi, 1996). Indeed, a number of authors have suggested variations in planting date as a means to manipulate glucosinolate levels in crops (Bible et al., 1980; Coogan et al., 1999; Rosa et al., 1996; Rosa and Rodrigues, 2001). While the effect of planting date appears real, its mechanism is unknown. Some attribute the effect to changes in temperature and plant-available water, particularly during head development (Bible et al., 1980; Rosa et al., 1997; Rosa and Rodrigues, 2001).

1.2.5.4 Temperature

A limited number of reports from controlled environment studies suggest that temperature strongly influences glucosinolate levels in various plants. Rosa (1997) found diurnal fluctuations in glucosinolate concentrations in cabbage seedlings to be greater when plants were grown at 30 ˚C versus 20 ˚C (both temperatures were

constant). In another study, isothiocyanate levels were greater in watercress grown at 25 ˚C than 15 ˚C (Freeman and Mossadeghi, 1972). Studying different compounds and plant tissues, Bible and Chong (1975) found the root thiocyanate ion concentration of a white radish cultivar to be positively correlated with accumulated cold units calculated as 18 ˚C - (mean daily temperature). Working with greenhouse grown turnip roots, Shattuck et al. (1991) observed that cold treatment (exposure to temperatures <4 ˚C for 11 days) altered the levels of individual glucosinolates, but did not affect total glucosinolate concentrations. Broccoli sprout glucosinolate levels were recently observed to be greater under both sub- and supra-optimal temperatures, relative to levels in sprouts germinated under a near optimal temperature (21 ˚C) (Pereira et al., 2002). Also, increases in glucosinolate concentrations corresponded with greater quinone reductase induction potential in temperature stressed sprouts (Pereira et al., 2002).

1.2.5.5 Plant water relations

Bible et al. (1980) reported that plant water availability affects the response of cabbage thiocyanate ion (SCN-) levels to planting date. They reported that summer planted cabbage had higher SCN- concentrations than spring planted cabbage in the absence of irrigation, while no planting date effect was observed in irrigated plots. Other work also indicates that plant-available water influences the chemical makeup of vegetable crops. For example, cabbage and watercress grown under water stress

conditions exhibited a four-fold increase in allyl isothiocyanate concentrations over cabbage and watercress grown with regular irrigation (Freeman and Mossadeghi, 1973). Plant water potential (Y_{plant}) may be the most direct indicator of plant water stress and its potential to alter glucosinolate concentration in plants. Rapeseed glucosinolate concentrations were found to increase linearly at Y_{plant} below –1.4 MPa (Jensen et al., 1996). In a separate study, similar leaf water potentials were associated with increased rapeseed glucosinolates in water-stressed plants (Mailer et al., 1987). The specific mode of action for this drought effect has not been determined, although it has been proposed that amino acids, which accumulate under drought conditions, may be differentially converted to glucosinolates, rather than to protein as during normal development (Rosa et al., 1997). In support of this hypothesis, expression of the gene family (CYP79) responsible for the first step in converting amino acids to glucosinolates has recently been shown to be inducible by plant compounds (e.g., jasmonic acid) involved in stress signaling (Mikkelsen et al., 2003).

1.3 SENSORY QUALITY

1.3.1 Role of glucosinolates

Glucosinolates and their hydrolysis products are associated with important flavor attributes of brassicacious vegetables. For example, allylisothiocyanate is the primary component of pungency in fresh cabbage, while 1-cyano-2,3-epithiopropane (derived from allylglucosinolate in the presence of epithiospecifier protein) contributes

sulfurous and musty odors to products (Chin et al., 1996; MacLeod, 1976; Yano et al., 1987). In Brussels sprouts, allylglucosinolate and 2-hydroxy-3-butenylglucosinolate concentrations were demonstrated to be positively correlated with bitterness and consumer acceptance of the product (van Doorn et al., 1998). Concentrations of 4-methylthio-3-butenyl isothiocyanate are associated with pungency in radish hypocotyls (Kuchenbauch et al., 1999; Qi and Longzhi, 1996). An odor threshold of 0.375 ppm in water has been reported for allylisothiocyanate (Buttery et al., 1976). The *in vivo* threshold for the detection of pungency in fresh cabbage was associated with allylisothiocyanate concentrations of 6.4 $mmol \cdot kg^{-1}$ dw, while strong sensations of pungency were associated with allylisothiocyanate concentrations >25.6 $mmol \cdot kg^{-1}$ dw (Yano et al., 1987).

1.3.2 Other components of flavor

With important exceptions, overall, few studies have documented factors influencing fresh cabbage sensory quality. Yano et al. (1990) related desirability scores from 82 consumers to physical and chemical measures of cabbage quality; the authors found no correlation between sugars or allylisothiocyanate concentrations and desirability, but did find 'good' flavor, succulence and green color to be correlated with acceptance. Principle component analysis indicated that texture (crispness and juiciness) and flavor were more important than color or appearance in explaining the variation in sensory quality among multiple cabbage cultivars grown at several

locations over two years (Martens, 1985). It is important to note that sweetness, sulfurous flavor, and bitterness were considered less important than fruity flavor because of year to year differences in the former descriptors, which were attributed to drought conditions in one year of the study (Martens 1985). Cabbage sugar concentrations can vary with planting date and reducing sugar concentrations in cabbage heads were positively related to irrigation frequency (Janes, 1950; Rosa et al., 2001). Total sugars were lower in cabbage heads grown at 25 ºC than those grown at 20 ºC (Hara and Sonoda, 1982). Yet, the impact of these environmental factors on cabbage flavor is under studied.

1.4 YIELD AND YIELD COMPONENTS

1.4.1 Influence of temperature

Although classified as a cool season crop, cabbage is frequently grown during the hottest time of the year (Rubatzky and Yamauchi, 1997). Planting date effects on cabbage head size are generally attributed to differences in air temperature during head development, with smaller heads being produced under conditions of high temperatures (Greenland et al., 2000; Kleinhenz and Wszelaki, 2003). Head growth is particularly sensitive to stress temperatures at its later stages (Hara and Sonoda, 1982). Therefore, the response of cabbage growth to temperature is very important to researchers and crop quality managers. Optimum temperatures for cabbage growth are regarded to be 15-20 ºC, with confirmation from controlled environment studies (Criddle et al., 1997; Hara and Sonada, 1982; Wien and Wurr , 1997). Heat units are frequently used in attempts to

describe the growth and yield of cabbage and other leafy *Brassicas* in the field. The general applicability of many attempts is limited because they employ a threshold temperature (e.g. 25 ºC) that is above the temperature at which cabbage growth is retarded (21ºC), and do not account for growth retardation at temperatures approaching the threshold or some continued growth above it (Criddle et al., 1997; Singh et al., 1993; Stranberg and White, 1979). Perhaps as a result, descriptions of leafy *Brassica* growth and development using thermal concepts have been highly variable (Isenberg et al., 1975; Strandberg and White, 1979). An exception is Dufault et al. (1989), who found decreased variability in harvest estimations of collard when accounting for continued but slowing growth at temperatures above 24 ºC.

1.4.2 Influence of plant and soil water status

Irrigation has been identified as an effective tool for pre-harvest quality management in vegetables, including cabbage (Singh and Alderfer, 1966; Swaider et al., 2002). Soil tensions of < 25 kPa and soil moisture > 80% of field capacity are associated with maximum yield and individual head weight, with head development identified as the period during which irrigation has the most influence on yield (Mamman and Haque, 1999; Singh and Alderfer, 1966; Smittle et al., 1994). Similarly, replacing 100% of estimated crop consumptive use is reported to optimize yield and cabbage head weight (Sammis and Wu, 1989; Sanchez et al., 1994; Tiwari et al., 2003).

1.4.3 Influence of ontogeny

Hara and Sonoda (1979) reported a sigmoidal increase in the dry weight of cabbage head leaves 60-120 days after planting. Isenberg et al. (1975) recorded increases in weight and density over a 20-30 day period beginning approximately 100 days after planting and found that changes were cultivar dependent. Although not well documented and studied principally at horticultural maturity, major head traits and their relationships are thought to change throughout development (de Moel and Evaraarts, 1990; Wszelaki and Kleinhenz, 2003). This gap in the literature could negatively affect the ability to predict cabbage yield based on relationships between head size and weight (Kleinhenz, 2003).

1.6 RATIONALE AND SIGNIFICANCE

Cabbage glucosinolate levels are important indicators of fresh cabbage quality, but should not be considered in isolation from other indicators used to evaluate quality. Abiotic factors influence yield, flavor and glucosinolate concentrations of cabbage independently and in combination with biotic factors such as genotype and developmental stage (Kleinhenz and Wszelaki, 2003; MacCleod and Nussbaum, 1977). The influence of factors such as planting date, air temperature and irrigation, as well as their interaction with cultivar and crop developmental stage are poorly documented, thus restricting our understanding of cabbage crop physiology and decreasing our ability to develop and implement efficient strategies to increase both crop yield and

quality.

Work to quantify the contribution of individual quality attributes of fresh cabbage to consumer acceptance has not yet been reported in the U.S. In Japan, Yano et al., (1990) employed a 3-point hedonic scale to quantify the acceptability of shredded samples from five fresh cabbage cultivars to consumers with regard to appearance, color, juiciness, firmness, taste and overall desirability. These researchers detected differences among cultivars in acceptability and related the physical and chemical properties of samples to acceptability scores. The predominant influence on consumer acceptability of fresh cabbage is thought to be flavor (Schutz et al., 1984; Yano et al., 1990), although no statistical relationship between flavor and overall acceptability has been described.

Planting date and cultivar are reported to affect glucosinolate concentrations in cabbage (Bible et al., 1980; Rosa et al, 1996), yet their independent and interactive effect on total glucosinolate concentrations are under-studied. Overall, investigations of the planting date effect on cabbage glucosinolate concentrations are few, include none in the U.S., and employ a limited number of current commercial cultivars (Bible et al., 1980; MacLeod and Nussbaum 1977; Rosa et al, 1996).

Although soil moisture availability strongly influences plant physiology, the effect of irrigation on cabbage flavor has not been studied in detail. The single study reported in the literature used a variety not currently of commercial importance and failed to account for the crop developmental stage at which irrigation was applied or corresponding effects on important physical head traits (Freeman and Mossadeghi,

1973).

Bible et al. (1980) reported that irrigation can eliminate seasonal variation in head concentrations of thiocyanate (a glucobrassicin hydrolysis product), as Freeman and Mossadeghi (1973) had earlier reported that soil moisture stress increased the concentrations of allyl-isothiocyanate (a product of sinigrin hydrolysis) four-fold relative to well watered plants. However, the relative response of individual glucosinolates in cabbage heads to supplemental irrigation and the importance of head development as a targeted period for management of glucosinolate levels are unknown. Likewise, despite the substantial contribution of sugars to the physical mass of heads and their metabolic importance, little information exists on the factors influencing their concentrations in cabbage, with a single study of irrigation on cabbage sugar levels found in the literature. It is also important to note that measures of glucosinolate levels are more common than measures of myrosinase activity and few studies include measures of both system components. As a result, the literature is deficient since the levels and biological activity of glucosinolates are tied to myrosinase activity (Rosa et al., 1997). Likewise, irrigation is frequently scheduled based on soil moisture measurements made with instruments such as TDR sensors and tensiometers (Paschold et al., 1997). Nevertheless, studies correlating glucosinolate levels to soil moisture measured with these instruments are lacking (Bible et al., 1980; Bourchereau et al., 1996; Freeman and Mossadeghi, 1973). Finally, glucosinolates and myrosinase may move in the plant via the symplastic network and transpiration stream, respectively (Brudenell et al., 1999; Chen and Andreasson, 2001; Hoglund et al., 1991). Despite the

mobility of the glucosinolate-myrosinase complex, changes in the system among tissues of plants exposed to differential root zone moisture treatment have not been characterized. Clearly, there is a strong need to couple rigorous control over experimental variables with measures of major components of the glucosinolate-myrosinase system in different plant tissues.

While head development is a continuum, major head traits and their relationships have been more thoroughly studied at stages associated with market readiness (Isenberg et al., 1975; Swaider and Ware, 2002; Wien and Wurr, 1997). The gap in our knowledge about events and relationships among key traits early in head formation restricts our fundamental understanding of cabbage crop development and impairs our ability to manage crop yield and quality.

As part of a larger effort to develop management systems that maximize vegetable crop quality, the goals of this program were to integrate focused, multi-factor field and controlled environment studies in order to identify and describe: 1) the components of cabbage flavor, 2) the influence of planting date and cultivar on cabbage glucosinolate concentrations, including as they relate to flavor, 3) he effect of irrigation timing with respect to head development on cabbage flavor, glucosinolate concentrations and physical traits, 4) changes in physical quality which occur during head development and 5) tissue specific changes in glucosinolate concentrations following differential soil moisture treatments, using a radish model system.

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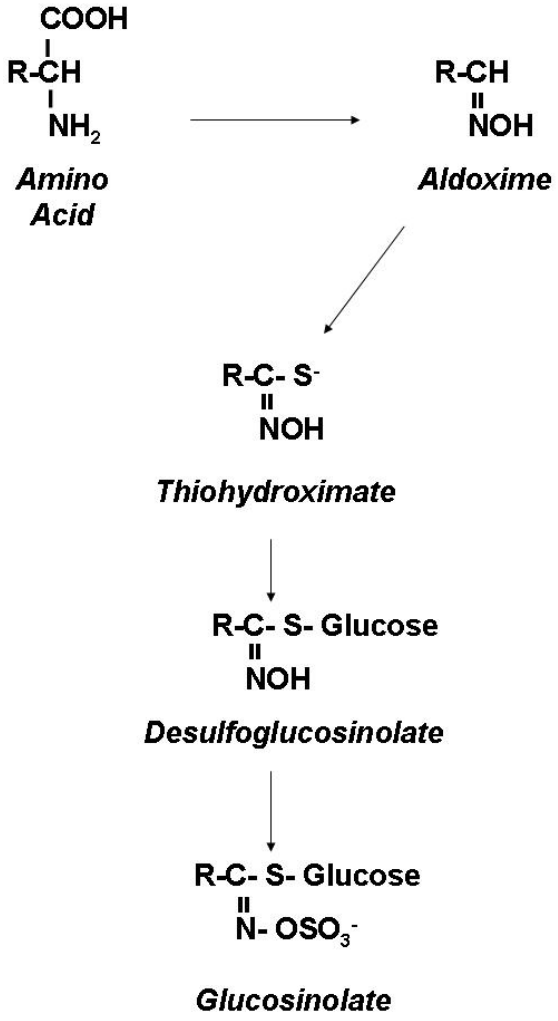

Figure 1.1. The biosynthetic pathway of glucosinolates. R- represents the side chain of the parent amino acid. After Rosa et al. (1997).

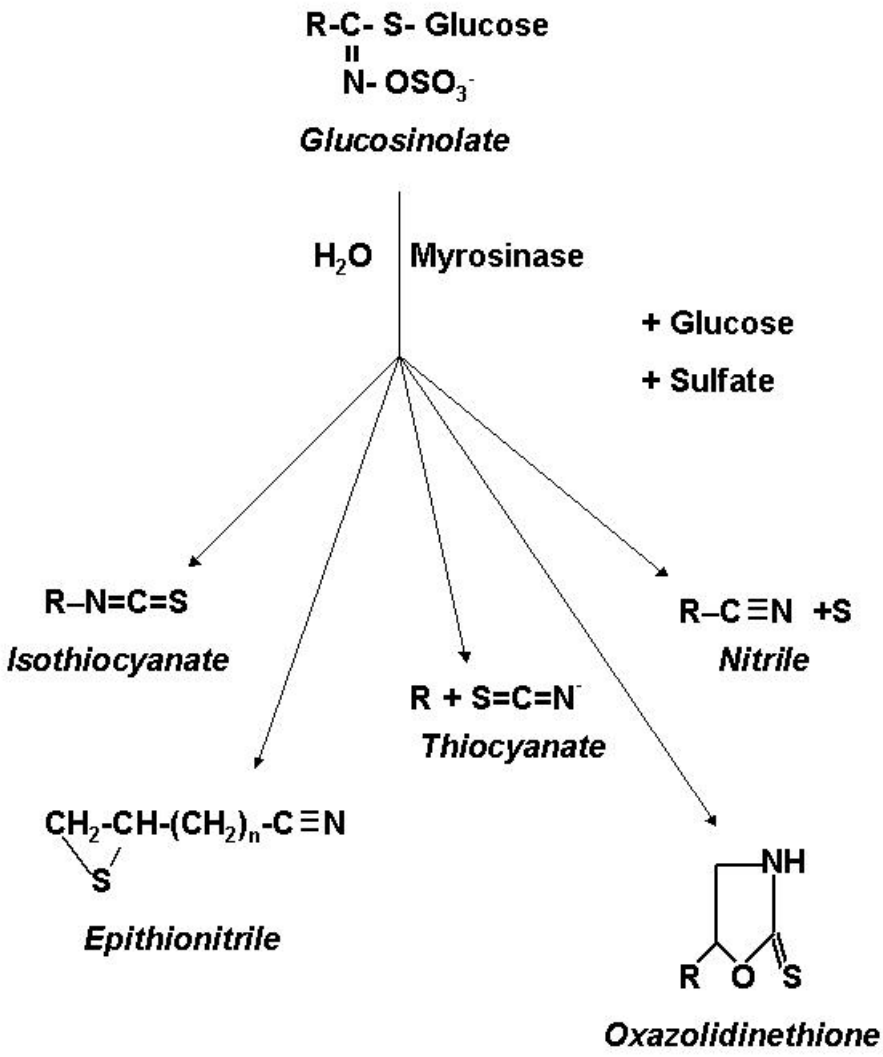

Figure 1.2. Hydolysis of glucosinolates by myrosinase (thioglucoside glucohydrolase, E.C. 3.2.1.147). R- represents the side chain of the parent amino acid. After Rosa et al. (1997)

CHAPTER 2

FRESH CABBAGE SENSORY QUALITY: COMPONENTS AND THE IMPACT OF PRODUCTION FACTORS

T.J.K. Radovich, M.D. Kleinhenz, A. Sanchez-Vela, J.C. Scheerens and B. Schult

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2.1 ABSTRACT

Strong anecdotal evidence suggests that sensory quality is a primary driver of consumer acceptance of fresh cabbage (*Brassica oleracea* var. *capitata*). In 1999, we initiated a series of studies to better understand the mechanisms driving cabbage sensory quality, in part to develop production systems which maximize it. Results from 1999 and 2000 suggested that cultivar and planting date impact perceptions of overall fresh cabbage sensory quality. Unstructured evaluation of forty cultivars of spring- and summer-planted cabbage by a small number of experienced tasters showed a wide range in various traits among the samples. In 2001, twenty-one untrained but experienced panelists were asked to evaluate samples of 26 cultivars planted in May and June at the

OARDC Vegetable Crops Research Branch in Fremont, Ohio. Panelists scored the overall desirability of samples and their acceptability based on flavor, aroma, texture,and color. Linear scales were also used to quantitatively describe flavor and texture components (hot, sweet, bitter, crisp) relative to a known reference (cv. Bravo) which was also included as a sample. Panelists detected distinct quality differences among the cultivars. Also, multiple regression analysis revealed that variation in flavor acceptability explained 75% of the variation in overall sample desirability, while texture, aroma and color collectively explained less than 10% of the variation in overall sample desirability. The importance of individual flavor components varied with planting date. To our knowledge, this is the most comprehensive explanation to-date of the contribution of specific quality components and major production factors to fresh cabbage sensory quality.

2.2 INTRODUCTION

When the production of a commodity has reached levels adequate to meet consumer demand, maintaining high quality standards becomes necessary to secure the available market in the presence of competition and increase future demand to expand the market. Improving the sensory quality of vegetables is a process involving the characterization of relationships between consumer preference and specific quality attributes of a given crop (Kuchenbuch et al., 1999).

Work to quantify the contribution of individual quality attributes of fresh cabbage to consumer acceptance has not yet been reported in the U.S. In Japan, Yano

et al., (1990) employed a 3-point hedonic scale to quantify the acceptability of shredded samples from five fresh cabbage cultivars to consumers with regard to appearance, color, juiciness, firmness, taste and overall desirability. These researchers were able to detect differences between cultivars in acceptability and to relate physical and chemical properties of the samples to acceptability scores. The predominant influence on consumer acceptability of fresh cabbage is thought to be flavor (Schutz et al., 1984; Yano et al., 1990), though no statistical relationship between flavor and overall acceptability has been described. Cabbage flavor is dependent on the concentrations of the sulfur containing glucosinolates and related hydrolysis products which impart bitterness and pungency, and likely influence the perception of sweetness (Yano et al., 1987). Genotypic and environmental factors may influence the levels of these compounds in crops (Bible et al., 1980; MacLeod and Nussbaum, 1977; Rosa et al., 1996; VanEtten et al., 1976), and potentially impact the sensory quality of fresh cabbage.

The goals in this project were to 1) establish a preliminary evaluation protocol for fresh cabbage, 2) provide reliable estimates of post-harvest quality on numerous cabbage genotypes grown under varying environmental conditions and 3) investigate the contributions of flavor, color, texture and other quality indicators to overall consumer desirability of shredded, fresh cabbage.

2.3 MATERIALS AND METHODS

2.3.1 1999 and 2000 evaluations

Forty cabbage cultivars, grown at two planting dates as previously reported (Kleinhenz and Schult, 1999; Kleinhenz et al. 2000), were harvested at maturity and evaluated for flavor at the OSU Food Industries Center. Two to three experienced evaluators employed descriptors based on industry terms, and assigned appropriate values (i.e. positive, negative or neutral) to those descriptors. All the descriptive values generated for a cultivar were used to qualitatively describe it as positive (+), negative (-) or neutral (0) for each planting date.

2.3.2 2001 transplant production and plot establishment

Twenty-seven cultivars and experimental lines of fresh market/slaw-type cabbage were planted on May 10 and June 20, 2001 at the OARDC Vegetable Crops Research Branch in Fremont, Ohio. Plots of each entry were replicated four times per planting date and arranged in a randomized complete block design. Transplants were set into two-row plots established with a cone-type transplanter. The plots were maintained and harvested in accordance with standard practices (Kleinhenz et al., 2001).

2.3.3 Sensory quality evaluation at the OARDC

2.3.3.1 Sample preparation and presentation

At harvest, a total of eight heads (two per replication x four replications) of each cultivar were placed in nylon mesh bags and transferred to refrigerated (7 °C) storage at the OARDC and held for 22-41 days. Samples used in evaluation were prepared 15 hr prior to evaluation by removing a 7 cm thick longitudinal slice from the center of each head, discarding core and damaged tissue, and shredding using a FoodPro2 food processor (Hamilton Beach/Proctor-Silex, Washington, North Carolina, U.S.A.). Sealed plastic containers (943 cm^3 by volume) were filled with homogenized samples of each entry containing tissue from eight heads and stored at 7 °C until evaluation. Approximately 35 g of sample of each cultivar and the reference ('Bravo') were presented to untrained panelists in cups (125 cm^3) randomly numbered with a three-digit code. Presentation order was randomized. The cultivar used for the reference is a standard fresh market/slaw cabbage, and was also included in the evaluations. Panelists were asked to evaluate all samples over the course of several days. The twenty-six cultivars of May-planted cabbage were evaluated 6-7 samples per day for 4 days, August 28-31 2001. Fifteen cultivars planted in June were evaluated, 5 samples each day for 3 days, Oct 10-12 2001. Evaluations took place between 10:00 am and 2:00 p.m.

2.3.3.2 Evaluation

The procedure for scoring individual sample traits was done in two parts, with both parts scored on a single sheet. Each sample was evaluated on a separate score sheet. First, the sample was evaluated for taste, aroma, color, texture and overall desirability using a nine point hedonic scale where 1= dislike extremely and 9= like extremely. Next, the intensity of each sample relative to a reference was evaluated for the characteristics hot, sweet, bitter and crisp using a continuous line scale centered with the reference and anchored with "much less" on the left and "much more" on the right. Samples stronger in intensity than the reference for the characteristic evaluated were scored an appropriate distance to the right of the center mark, while samples of weaker intensity were scored to the left of center. The distance (mm) of the mark from the left anchor was measured with a ruler and recorded as the intensity score for that characteristic. Higher scores indicate greater intensity. Aroma acceptability was evaluated by inhaling through the nose several times just above the sample. To evaluate taste and texture acceptability and flavor characteristics, samples were chewed several times and ingested. Re-tasting was permitted. Color evaluations were done from an approximate distance of 0.50 m^{-1} from the sample. Evaluations were conducted with panelists seated at undivided tables in a room lit with 16 florescent tubes (40 W, General Electric) mounted approximately 3 m^{-1} above the tables. Panelists were asked to cleanse their palate with water and bread or crackers between samples.

2.3.3.3 Data analysis

Analysis of Variance (ANOVA) of data for each character evaluated was conducted with cultivars as the treatment and evaluators as blocks. Because panel composition differed for the evaluation of May and June planted cabbage, separateANOVAs were conducted for each data set. Multiple step-wise regression analysis was performed using the Proc Reg procedure of SAS (Statistical Analysis System, v. 7 for Windows™, Cary, North Carolina, U.S.A.).

2.4 RESULTS AND DISCUSSION

2.4.1 1999 and 2000

In 2000, Samples of high (+) quality were determined to be those with a relatively mild flavor typical of cabbage and lacking any off (earthy, grassy, musty, sulfur) flavors, bitterness, pungency or astringency. More cultivars were described as neutral than either positive or negative, and the ratio of high quality (+) cultivars to cultivars of unacceptable quality (-) was higher in June-planted cabbage than that planted in May. Similar trends for differences between cultivars and planting date were observed in the 1999 evaluations (data not shown). Detailed results of both studies were reported elsewhere (Kleinhenz et al., 2000; Radovich et al., 2001).

2.4.2 2001

Acceptance scores for overall desirability and the intensity scores for hot were significantly ($\alpha = 0.05$) affected by genotype in both evaluations (Figs. 2.1 and 2.2). Scores for color and bitter were also significantly influenced by genotype in both evaluations. Analysis of data generated by panelists common to both evaluations indicates planting date effects on color, sweet and crisp scores (data not shown). Table 2.1 describes the contribution of individual trait acceptability and intensity to overall sample desirability as determined by multiple regression analysis. These relationships were stable among and across evaluations (Table 2.1). Taste explained approximately 78% of the total variance in overall desirability scores. Variance of the individual flavor components hot and sweet scores explained small but significant ($\alpha = 0.15$) portions of overall desirability variance, although relative contributions differed between planting date. The relationship between hot and sweet scores and overall desirability were positive at both evaluations. Bitter scores were negatively related to overall desirability in the evaluation of May-planted cabbage.

Quality is an important consideration in purchasing decisions made by consumers of fresh produce (Scheerens, 2001). Our results demonstrate that genotype impacts fresh cabbage quality, including flavor and overall acceptability. Anecdotal evidence and previous research suggest that planting date may also affect cabbage quality. Although any planting date effect in this study is potentially confounded by

differences in panel composition, data from panelists participating in both evaluations indicate that planting date may play a role in cabbage sensory quality. In this study, cabbage planted on different dates experienced maximum temperatures and precipitation at different times during development (Fig. 2.3). Research-based information on how a cultivar responds to changes in management practices such as cultivar and planting date selection may assist growers in identifying cultivars largely unaffected by variations in climate.

Taste scores explained a very large portion of the variation in overall desirability scores relative to texture (~5%), aroma (~3%) and color (~1%). These results suggest that the contribution of flavor to fresh cabbage sensory quality far exceeds that made by the other quality indicators measured. In fact, the very low R^2 values associated with variables in the model other than taste (Table 1) may indicate that their contribution is negligible. However, aromatic, visual and mouth-feel stimuli may affect panelists' perception of flavor (Meilgaard et al., 1987) and taste scores were likely affected by other scored and unscored attributes (Delwiche, 1996). In addition, attributes such as color might prove most important in situations where tasting may not be feasible (i.e. supermarkets). The intensity of flavor and texture components measured by hot, bitter and sweet scores were significantly related to scores for overall desirability, indicating their importance in fresh cabbage quality. The relative weakness (model $R^2 < 0.20$, Table 1) of the relationship may be due to several factors: 1) the absence of panel training; 2) the use of different scales to measure overall desirability and individual flavor components and; 3) the contribution of other quality components to overall

scores which were not measured in this study. The positive relationship between hot and sweet scores and overall desirability contradicts the current convention of associating a negative value to any pungency in fresh cabbage and confirms the importance of perceived sweetness in consumer acceptance of cabbage.

An acceptable scientific protocol for the large-scale evaluation of cabbage sensory quality is not available. In this and previous related reports (Kleinhenz et al., 1999; Radovich et al., 2000), we have established a preliminary protocol for obtaining and reporting estimates of cabbage sensory quality. Further development of the protocol should include the use of trained panelists in Qualitative Descriptive Analysis (QDA) and the integration of independent measurements of chemical quality indicators (e.g. sugar, chlorophyll, and glucosinolate concentrations), which may affect the sensory quality of fresh cabbage.

2.5 CONCLUSIONS

Under 2001 experimental conditions, genotype impacted the sensory quality of fresh cabbage. Of the quality indicators evaluated for acceptability, flavor was by far the greatest contributor to overall sample desirability in both panels. The individual contributions of flavor components sweet, hot and bitter to overall sample desirability were important but variable and will continue to be investigated. The inclusion of sensory quality with other considerations, such as potential yield and physical quality, complicates cultivar selection in fresh market cabbage production and demonstrates the need for a comprehensive, integrated approach to germplasm evaluation. The protocol described herein is the most comprehensive integrated approach yet

reported to evaluate the sensory quality of fresh cabbage.

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Independent variable	May R^2	June R^2	Across Dates R^2	Direction of Relationship
Taste	0.81	0.77	0.78	positive
Texture	0.04	0.05	0.05	positive
Aroma	0.01	0.02	0.02	positive
Color	0.01	0.01	0.01	positive
Model	0.87	0.85	0.86	
Sweet	0.10	0.05	0.09	positive
Hot	0.03	0.05	0.03	positive
Bitter	0.03	ns	0.01	negative
model	0.16	0.10	0.13	

Table 2.1. R^2 values from multiple stepwise regression analysis with overall sample desirability as the dependent variable. Variables in the model are significant at the 0.15 level.

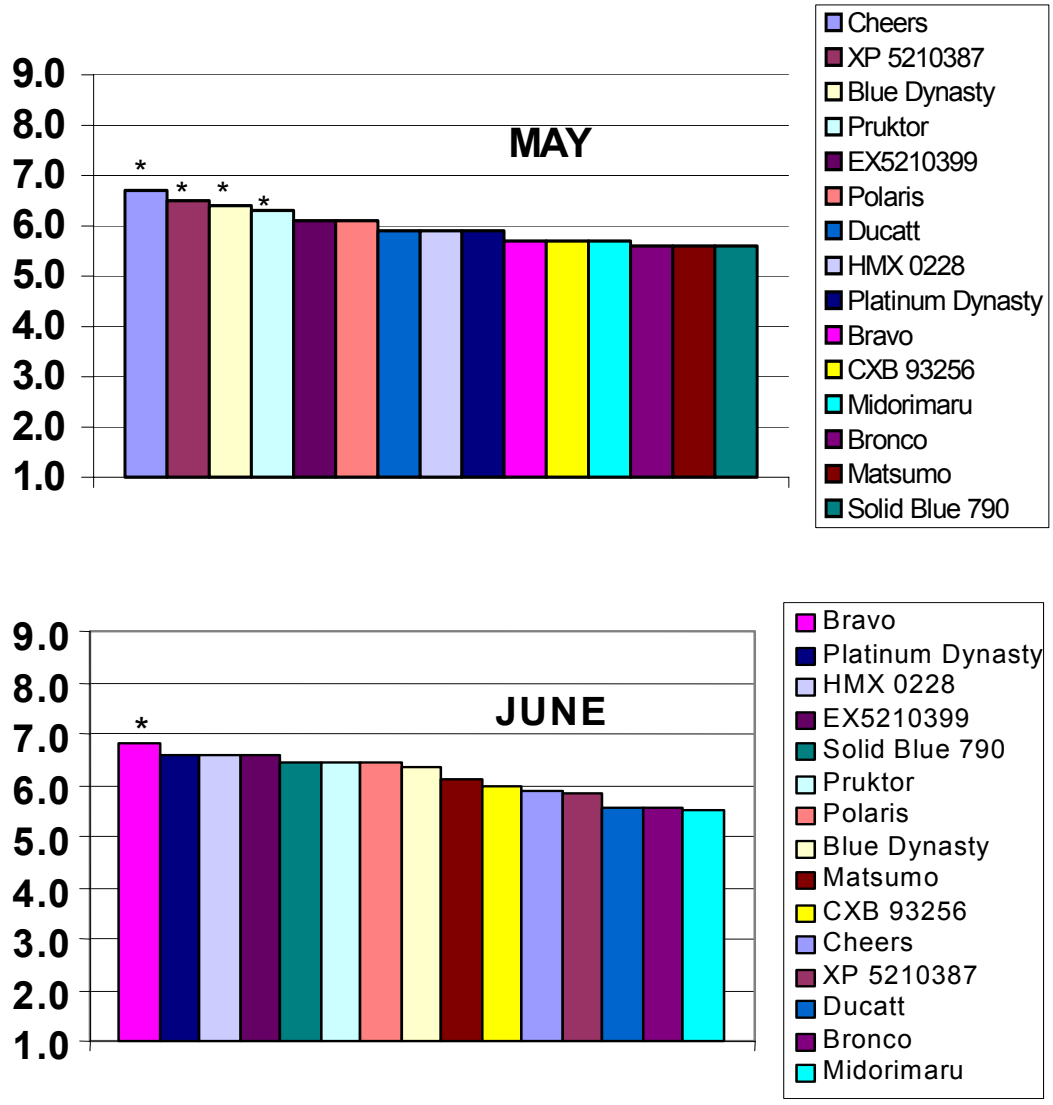

Figure 2.1. Overall acceptability scores of fifteen cabbage cultivars planted in May and June 2001 at the OARDC Vegetable Crops Research Branch in Fremont, Ohio, and evaluated in August and October 2001, respectively. Overall acceptability scores were rated on a scale of 1-9, where 1 = dislike extremely and 9 = like extremely. Bars with an asterisk (*) are significantly higher than the lowest score but not significantly different from each other.

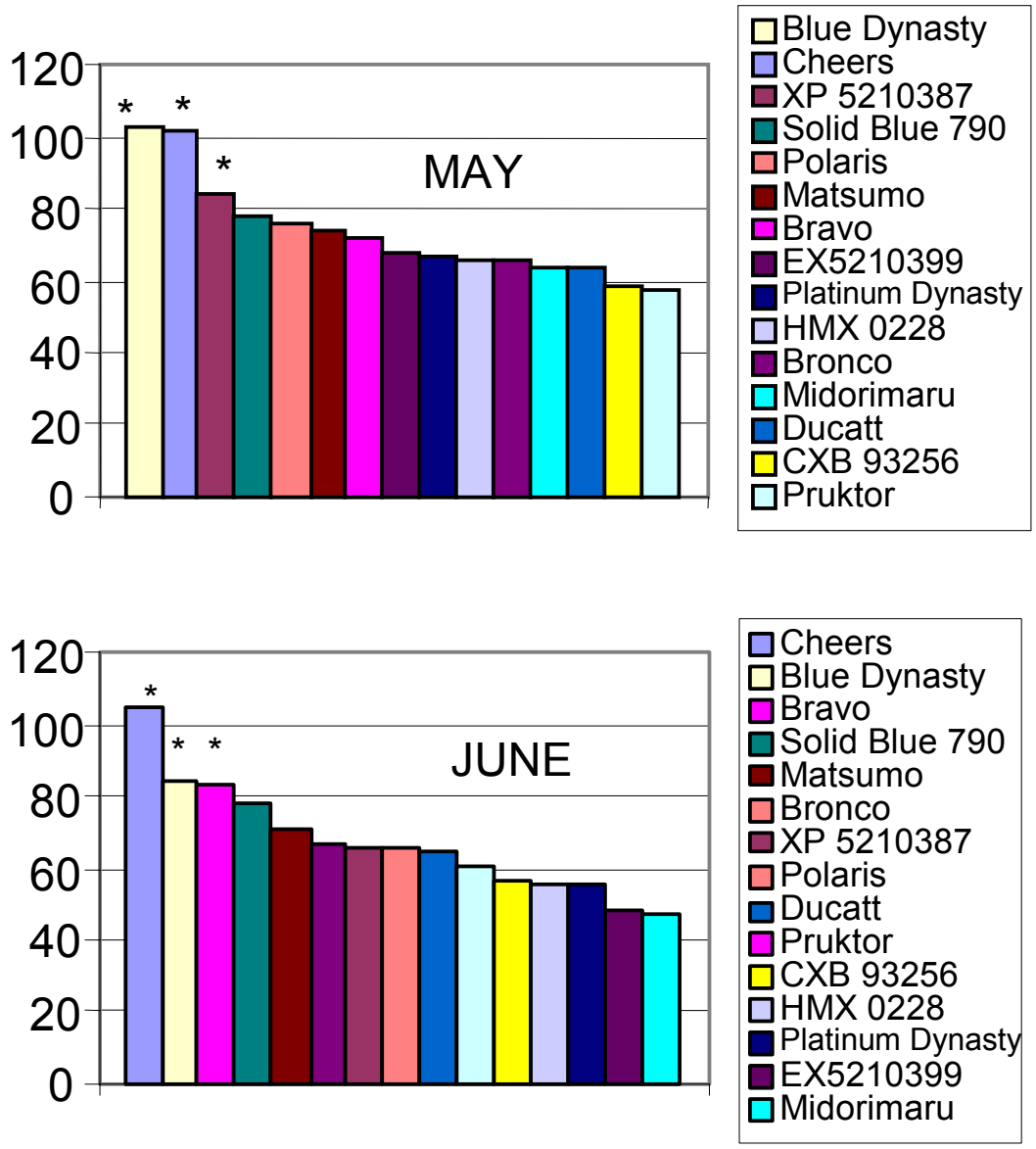

Figure 2.2. Hot (A) intensity scores of fifteen cabbage cultivars. The intensity of hot sensations were measured relative to a reference ('Bravo'). Scores increase with increasing intensity. Bars with an asterisk (*) are significantly higher than the lowest score but not significantly different from each other.

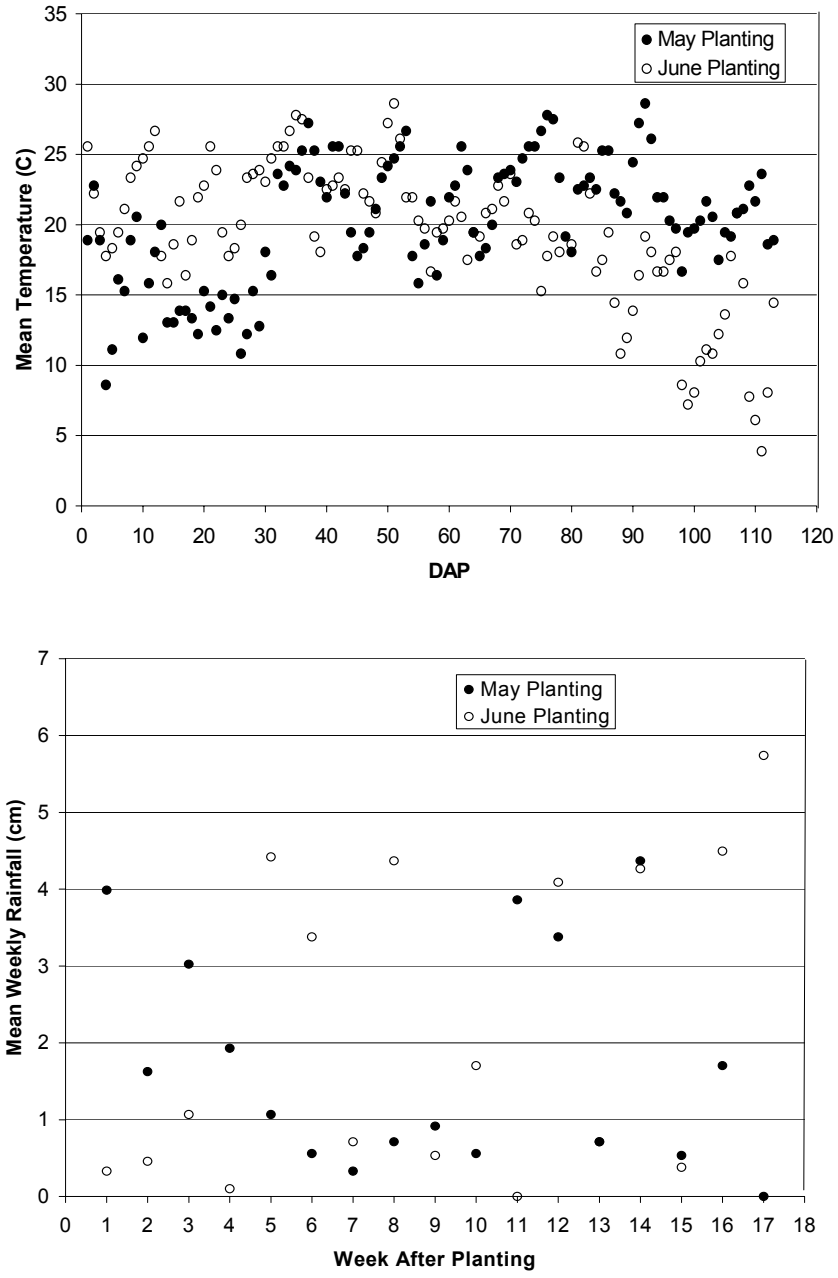

Figure 2.3. Temperature and rainfall recorded during development of cabbage planted in May and June of 2001 at the OARDC Vegetable Crops Research Branch in Fremont, Ohio. Temperatures are daily means (°C). Rainfall values (cm) are 7-day means for each week of development after planting.

CHAPTER 3

PLANTING DATE AFFECTS TOTAL GLUCOSINOLATE CONCENTRATIONS IN SIX COMMERCIAL CULTIVARS OF CABBAGE (*BRASSICA OLEREACEA* L., CAPITATA GROUP).

T.J.K. Radovich, M.D. Kleinhenz, J.G. Streeter, A.R. Miller and J.C. Scheerens

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3.1 ABSTRACT

Glucosinolates are secondary plant metabolites derived from amino acids that influence human health, pest populations and crop flavor. Our primary objective was to determine the independent and interactive effects of planting date (PD) and cultivar (C) on total glucosinolate concentrations in cabbage, in part to help develop management systems that optimize them. A second objective was to explore the reported link between total glucosinolate concentrations and pungency in fresh cabbage. Six commercial fresh market cabbage cultivars were planted in May and June of 2001 and 2002 at the Ohio Agricultural Research and Development Center (OARDC) Vegetable

Crops Research Branch in Fremont, Ohio. Total glucosinolate concentrations in horticulturally mature heads were determined using a glucose evolution procedure. In 2001, 12 to 14 experienced panelists also scored sample pungency. Total glucosinolate concentrations were significantly affected by PD and C, but the PD x C interaction was not significant. Mean glucosinolate concentrations were greater in May- than June-planted cabbage in both years. Cultivar ranking with regard to glucosinolate concentrations was similar between planting dates in both years. 'Cheers' had the highest mean glucosinolate concentrations (23.1 and 29.5 mmol · kg dry weight^{-1} in 2001 and 2002, respectively) and 'Solid Blue 790' the lowest (17.1 and 19.7 mmol · kg dry weight^{-1} in 2001 and 2002, respectively). In 2001, panelists generally scored cultivars highest in glucosinolates as more pungent than cultivars lowest in glucosinolates. These data suggest that planting date and cultivar effects on total glucosinolate concentrations in cabbage are largely independent. Climatic data suggest that higher air temperatures during head development of May- compared to June-planted cabbage induced plant stress and resulted in higher glucosinolate concentrations in May-planted cabbage.

3.2 INTRODUCTION

Glucosinolates are plant-mobile, sulfur- and glucose-containing compounds found in several families of the order Capperales, including the *Brassicacea* (Chen and Andreasson, 2001). These anionic secondary products are derived from amino acids and possess little or no biological activity. However, the enzyme myrosinase (EC 3.2.1.147)

catalyses the hydrolytic cleavage of the glucose moiety and, accompanied by the spontaneous release of sulfate from the unstable intermediate, produces a biologically active aglucon whose structure depends on the conditions of hydrolysis (Bones and Rossiter, 1996). In plants, glucosinolates are compartmentalized separately from myrosinase. Hydrolysis occurs when the two are combined as a result of tissue damage (e.g., chewing) or active membrane transport of glucosinolates by the plant (Chen and Halkier, 2000; Bones and Rossiter, 1996). The aglucones resulting from glucosinolate hydrolysis are important as they may exhibit goitrogenic, anti-carcinogenic, antibiotic or organoleptic activity (Fahey et al., 2001; MacLeod, 1976; Rask et al., 2000; Rosa et al., 1997; Verhoeven et al., 1997). Concentrations of various glucosinolates and their products associated with maximum or optimum bioactivity have been reported in both *in vivo* and *in vitro* studies. Howerver, optimal glucosinolate levels in vegetables have been established only in a relative sense (i.e., low vs. high) and depend on biological context. Specifically, maximizing glucosinolate concentrations may be considered desirable for cancer protection (Fahey et al., 1997; Verhoeven et al., 1997), while minimizing levels is expected to increase flavor acceptance by consumers of fresh vegetables (Chin et al., 1996; van Doorn et al., 1998). The influence of glucosinolates on crop and product quality is particularly important to growers, processors and researchers as glucosinolates are responsible for a range of flavor attributes, including pungency or hotness, bitterness, and sulfurous aroma (MacLeod, 1976; van Doorn et al., 1998). Glucosinolates, in particular, may contribute to the unacceptable pungency of coleslaw and other fresh shredded cabbage products (Ball et al., 1999; Yano et al., 1987). Therefore, horticultural manipulation of glucosinolate concentrations in cabbage

may be worthwhile (MacLeod and Nussbaum, 1977; Rosa et al., 1997). For example, cabbage growers alter planting dates within a season according to weather, labor and equipment availability, or market issues. These decisions, in turn, may affect crop quality and marketability due to climate changes throughout the season (Kleinhenz and Wszelaki, 2003).

Planting date and cultivar selection are thought to affect concentrations of glucosinolates and their hydrolysis products in cabbage. Bible et al. (1980), Kushad et al. (1999) and Van Etten et al. (1976; 1980) described potentially large differences in glucosinolates or their metabolites among cabbage cultivars. In a survey of 22 genotypes, Van Etten et al. (1976) reported a difference of more than 400% between cultivars with the highest and lowest total glucosinolate concentrations. Environmental effects on glucosinolate levels may also be large. Rosa et al. (1996) reported higher glucosinolate concentrations in a single cabbage cultivar of the Capitata group when planted in March compared to August in Portugal, and attributed the lower concentrations in August-planted cabbage to lower air temperatures later in plant development. Planting date had a similar effect on head concentrations of thiocyanate (a goitrogenic glucosinolate metabolite) in two cabbage cultivars in Quebec (Bible et al., 1980). Contrary to Rosa et al. (1996), Bible et al. (1980) attributed higher thiocyanate concentrations in June- compared to May-planted cabbage to low soil moisture during development of June-planted cabbage. In Great Britain, MacLeod and Nussbaum (1977) reported lower isothiocyanate concentrations in two cabbage cultivars planted and harvested late in the season, compared to earlier plantings. However, no explanation for the effect was offered. Overall, investigations of the planting date effect on cabbage

glucosinolate concentrations are few, include none in the U.S., and employ a limited number of current commercial cultivars. Planting date and cultivar are reported to influence important cabbage head traits (Kleinhenz and Wszelaki, 2003; Wszelaki and Kleinhenz, 2003), yet their effect on total glucosinolate concentrations are under-studied. Therefore, our primary objective was to examine the independent and interactive effects of planting date and cultivar on total glucosinolate concentrations in cabbage. A second objective was to explore the reported link between total glucosinolate concentrations and pungency in cabbage.

3.3 MATERIALS AND METHODS

3.3.1 Plot establishment, maintenance and harvest

Hardened 'Blue Dynasty', 'Bronco', 'Cheers', 'HMX 0228', 'Matsumo' and 'Solid Blue 790' seedlings with two to four true leaves were planted to the field 10 May and 20 June, 2001 and 28 May and 25 June, 2002 at the OARDC Vegetable Crops Research Branch in Fremont, Ohio (latitude 40° 47' N, longitude 81° 55' W). Two-row plots were planted with a cone-type two-row transplanter (Holland Transplanter, Holland, MI). Plots were arranged in a randomized complete block design with four replications. Each replication contained both planting dates and all cultivars. Rows were 4.6 m long with 76 cm between rows and 28 cm between transplants. Soil type in each year was a Kibbie fine sandy loam (fine illitic mesic Mollic Ochraqualf). Preplant fertilizer applications were based on soil tests and local practices. For the 2001 planting,

31.4 kg · ha^{-1} of phosphorous (0N-20.1P-0K) and 256.4 kg · ha^{-1} of potassium (0N-0P-49.8K) were applied to the field on October 23, 2000 and 78 kg · ha^{-1} of nitrogen (45N-0P-0K) was applied on 1 May, 2001 and incorporated just prior to planting. For the 2002 planting, 140 kg · ha^{-1} of nitrogen (45N-0P-0K), 73.4 kg · ha^{-1} of phosphorous (0N-20.1P-0K) and 325.4 kg · ha^{-1} of potassium (0N-0P-49.8K) were applied to the field on 5 May, 2002 and incorporated just prior to planting. Each transplant was provided with approximately 150 ml of a dilute nutrient solution [(129 and 184 mg · L^{-1} of N and P, respectively (10N-14.8P-0K)] at planting. Standard pest management strategies, based on scouting, thresholds and application of labeled pesticides, were employed. Plots received 25 mm of irrigation on 28 June and 16 July in 2001 and 38 mm on 15 July 2002. Temperature data were collected hourly on-site by the OARDC Weather System (The Ohio State University, 2003).

3.3.2 Harvest and physical trait data collection

Plots were examined two to three times per week beginning 55 d after transplanting to assess harvest readiness. Harvest dates were selected based on estimated days to maturity from the seed source and visual examination of heads, following the practice of commercial growers in the area and that used in related research (Kleinhenz and Wszelaki, 2003; Wszelaki and Kleinhenz, 2003). All heads were collected from the center 3 m of both rows in each plot and scored as marketable or unmarketable as previously reported (Kleinhenz and Wszelaki, 2003). Heads were then randomly selected from the marketable group and trimmed (4 wrapper leaves

removed) prior to further evaluation. In 2001, individual weights were taken on 5 heads using an electronic scale (FV-60KWP, A and D Co., Ltd Tokyo, Japan or CW11-2EO, OHAUS, N.J.). Polar and equatorial head diameters were also measured. Three additional heads from each replication were selected for glucosinolate analysis and stored at 7 °C for < 48 h prior to processing. Also, in 2001, two heads from each replication were retained for pungency evaluation and stored 20 to 40 d at 7 °C until sensory panels were convened. In 2002, head weight and diameter were recorded on the three heads selected for glucosinolate analysis.

3.3.3 Pungency evaluation

In 2001, 12 (June-planted) or 14 (May-planted) untrained panelists familiar with the sensory evaluation process evaluated shredded samples of each cultivar for the attribute 'hot' as previously reported (Radovich et al., 2003, Chapter 2). Samples were homogenized composites of eight heads (two from each of the four field replications, with panelists serving as replications in the analysis of the sensory evaluation data (Radovich et al., 2003). The attribute 'hot' was described to panelists as the irritation (pungency) perceived in the mouth and nasal passages when consuming horseradish, a sensation familiar to all evaluators. Evaluations were made relative to a reference, on a continuous line scale, anchored on the left by "much less hot" and on the right by "much more hot" and centered with the reference (Radovich et al., 2003). Marks on the scale were converted to numerical values, and cultivar means generated with SAS for

Windows v.8 (Statistical Analysis System, Cary, N.C.). Data for May- and June-planted cabbage were analyzed separately due to differences in panel composition.

3.3.4 Glucosinolate analysis

A 1.5-cm thick longitudinal slice was taken from the center of each head and the core removed. The slice was halved longitudinally; one half was immediately frozen in liquid nitrogen and stored at –20 °C until lyophilized. Glucosinolates were extracted after the procedure of Rosa and Rodriques (1998). Ground, freeze-dried tissue (200 mg) was heated in 5 ml of 90% aqueous methanol in a capped test tube at 70 °C for 15 minutes, then vacuum filtered through coarse, qualitative filter paper (5.5 cm, Fisher Scientific, Pittsburgh, Pa.). Sinigrin (2-propenyl glucosinolate; 0.6 mmol, Sigma Chemical Co., St. Louis, Mo.) was added as an internal standard to duplicate tubes of ~25% of the samples to estimate recovery (mean = 82%, standard error = 1.5%, N=71) and confirm equality in recovery rates among treatments (data not shown). The residue was reheated twice in 5 ml of 70% aqueous methanol at 70 °C for 3 min and filtrates were collected in 500 ml Erlenmeyer flasks. The combined filtrate was evaporated under vacuum (Rotavapor VE 50 GD, Rinco Instrument Co. Inc., Greenville, Ill.) and the residue re-dissolved in 10 ml of 70% methanol. The extract was then centrifuged at 27,200 g_n for 10 minutes. The supernatant was collected in 10 ml vials and stored at 0 °C until analysis.

The glucose evolution procedure of Heaney and Fenwick (1981) was employed to quantify total glucosinolate concentrations in samples. Pasteur pipettes (14.6 cm)

plugged with glass wool were filled with 0.5 ml of Sephadex A-25 resin (Sigma Chemical Co., St. Louis, Mo.) previously swollen and degassed in 0.02 M pyridine acetic acid buffer, pH 5.0. Resin was rinsed twice with 2 ml distilled water, draining between rinses. Cabbage extract (1 ml) was added to columns and allowed to drain. The column was then washed with 0.3 ml of distilled water, allowed to drain, and washed again with 2 ml distilled water. Columns were then washed twice with 0.5 ml of 0.02 M pyridine acetic acid buffer. Glass collection vials (10 ml) were placed under the columns and 0.25 ml of thioglucosidase (Sigma Chemical Co., St. Louis, Mo.) solution (10 $mg \cdot ml^{-1}$ 0.02 M pyridine acetic acid, pH 5.0) was added to the column. Columns were left at ambient conditions (~25 °C) for 16 h, then eluted with two volumes of 0.5 ml distilled water. The glucose concentration of the eluate was determined colorimetrically at 340 nm employing glucose (HK) assay kits (Sigma Chemical Co., St. Louis, Mo.) and a Beckman spectrophotometer (Model DU 640, Beckman Instruments Inc., Fullerton, Calif.). Glucose values were converted to equimolar amounts of glucosinolate (the molecular weight of the internal standard sinigrin, 415.5, was used) and corrected for internal standard recovery. Data were analyzed with the General Linear Model and Correlation procedures of SAS for Windows v.8 (Statistical Analysis System, Cary, N.C.).

3.4 RESULTS

The main effects of year (Y), planting date (PD) and cultivar (C) on total glucosinolate concentrations were significant, as were the interactive effects of Y x PD and Y x C (Table 3.1). However, the PD x C interaction was not significant.

May planting resulted in higher cabbage glucosinolate concentrations compared to June planting (Fig.3.1). Also, the relative ranking of cultivars, as determined by Spearman's Rank Correlation procedure ($r_s = 0.82$, $P \leq 0.05$), was similar between planting dates in both years. Higher glucosinolate concentrations in May-planted cabbage coincided with higher air temperatures and a greater incidence of stress temperatures (>30 C) during head development in May- relative to June-planted cabbage (Fig. 3.2). May-planted heads were harvested 25 to 30 July, 2001 (76-81 DAP) and 26 August to 12 September, 2002 (90-107 DAP); June-planted heads were harvested 7 to 13 September 2001 (79-85 DAP) and 18 September to 17 October 2002 (85-114 DAP). Mean head weight and diameter were lower in May- compared to June planted cabbage in both years (data not shown). A greater incidence of stress temperatures in 2002 retarded head development compared to 2001, and differences among cultivars in the developmental response to stress temperatures contributed to the extended harvest period in 2002 relative to 2001.

Mean cultivar glucosinolate concentrations ranged between 17.1 and 29.0 $mmol \cdot kg^{-1}$ dry weight (Fig. 3.1). 'Cheers' and 'Solid Blue 790' contained the highest and lowest concentration of glucosinolates, respectively. 'Cheers' was also perceived to be the most pungent cultivar in evaluations of May- and June-planted cabbage

(Radovich et al., 2003, Chapter 2). Cultivar pungency scores were significantly (α = 0.10) correlated with mean glucosinolate concentrations in the evaluation of June-planted cabbage only (Fig. 3.3). The difference in glucosinolate concentrations between 'Cheers' and 'Solid Blue 790' was greater in 2002 (9.8 $mmol \cdot kg^{-1}$ dry weight) than in 2001 (6.0 $mmol \cdot kg^{-1}$ dry weight) (data not shown). Although mean total glucosinolate concentrations for all cultivars were greater in 2002 than 2001, cultivars varied in their magnitude of increase from 2001 (data not shown). Therefore, the range in glucosinolate concentrations among cultivars was greater in 2002 than 2001.

3.5 DISCUSSION

The importance of glucosinolates in crop sensory quality, human health and plant defense make them a primary target for horticultural manipulation during production. Therefore, it is important to note that both planting date and cultivar influenced total glucosinolate levels in the commercial genotypes employed here (Table 3.1). More importantly, the PD x C interaction was not significant and cultivar rank with regard to glucosinolate concentration was very similar between years and planting dates. Therefore, it appears that PD and C acted independently in influencing glucosinolate concentrations in this study. The absence of a significant PD x C effect on glucosinolate concentrations also simplifies interpretation of the planting date effect.

Temperatures of 30 °C or greater are considered supra-optimal for cabbage (Rubatzky and Yamaguchi, 1997). These temperatures were recorded more frequently during head development in May- than June-planted plots and, overall, more often in

2002 than 2001 (Figure 3.2). Perhaps as a result, mean glucosinolate concentrations were greater in 2002 compared to 2001 and the PD effect was less pronounced in 2002 than 2001 (data not shown). Glucosinolates are thought to act in plant defense, with environmentally-induced increases in glucosinolate concentrations regarded as a plant stress response (Rask et al., 2000; Siemens and Mitchell-Olds, 1996). Recent work demonstrating the induction of glucosinolate biosynthesis in *Arabidopsis* by jasmonic acid (Mikkelsen et al., 2003), a signaling molecule involved in plant defense responses, supports this hypothesis. Additional evidence that the PD effect observed here may have been a response to adverse temperature conditions during head development is provided by MacLeod and Nussbaum (1977) and Rosa et al. (1996), and is supported by the observation that May-planting tended to result in smaller, lighter heads compared to June-planting (Kleinhenz and Wszelaki, 2003; Wszelaki and Kleinhenz, 2003). Also, a trend for higher glucosinolate concentrations in smaller heads within cultivars has been observed (Bible et al., 1980; Van Etten et al., 1976). In controlled environment studies, both supra- and sub-optimal temperatures have been shown to increase glucosinolate concentrations in roots, shoots and reproductive tissues of various *Brassicacea* (Aksouth et al., 2001; Pereira, et al., 2002; Rosa and Rodrigues, 1998). However, temperature effects remain incompletely characterized and their potential interaction with soil moisture effects must be considered (Ciska et al., 2000). If the influence of air temperature on glucosinolate concentrations results primarily from greater evapotranspirative demand, then glucosinolate concentrations (and presumably pungency) in fresh cabbage may be minimized via irrigation during periods of high air temperatures, as suggested by Bible et al. (1980), who reported planting date effects on

cabbage head thiocyanate concentrations in unirrigated, but not irrigated, plots. Mitigation of low moisture stress via drip irrigation during head development was recently shown to increase cabbage head weight and size and affect flavor (Radovich et al., 2004, Chapter 4). Therefore, it is possible that the effects of air temperature on glucosinolate concentrations are not independent of soil moisture. Bible et al. (1980) attributed greater thiocyanate concentrations in June- compared to May-planted cabbage primarily to soil moisture deficits during the entire period of crop development. However, the timing of stress relative to plant-crop development and differences in compounds measured may explain the apparent contrast of their results with those reported here. Soil moisture levels were not recorded in the present study. However, it is reasonable to suspect that, given the similar amount of rainfall and irrigation received by May- and June-planted cabbage in both years (data not shown), soil moisture would have been lower under higher temperatures as a result of greater evapotranspiration. Seasonal differences in daylength, light quality, nutrient availability and pest incidence were not measured in this study, but may also have contributed to the planting date effect observed here (Rosa et al., 1997). However, elucidating the independent and interactive effects of air temperature and soil moisture on cabbage glucosinolate concentrations would contribute significantly to our understanding of the influence of abiotic environmental factors on the glucosinolate system, and our ability to manage it.

The cultivar means for total glucosinolate concentrations reported here generally agree with those reported previously (Ciska et al., 2000; Kushad et al., 1999; Rosa et al., 1996; Van Etten et al., 1976), although differences in analytical methods and reporting units complicate direct comparisons. Sinigrin is a major component of the

cabbage glucosinolate profile and the precursor of allyl isothiocyanate, the primary source of pungency in cabbage (Chin et al., 1996; MacLeod, 1976; Yano et al., 1987). Ball et al. (1999) reported a strong ($r^2 = 0.95$), positive correlation between total glucosinolate and sinigrin concentrations among 12 cabbage cultivars, which suggests that the cultivars in the current study differing in total glucosinolate concentrations may also differ similarly in their concentrations of the flavor compound sinigrin. Therefore, it is reasonable to conclude that the relationship between pungency and total glucosinolate levels for June-planted cabbage shown in Fig. 3.2 is consistent with earlier findings. Glucosinolate concentrations may change in storage (Rosa et al., 1997). However these changes are expected to be minimal and uniform across treatments under the storage conditions employed in the study (Ball et al., 1999, Radovich et al., 2004, Chapter 4). However, if changes did occur, they may have contributed to a reduction in fit between glucosinolate and pungency values. Differences in storage time (20 d) among cultivars may have contributed additional variability in sensory scores. However, Ball et al. (1999) reported no significant change in glucosinolate concentrations or pungency over a period of 60 days in cabbage heads stored at 1 °C and 90% relative humidity. Although our storage conditions differed slightly from Ball et al. (1999), we expect that minimal variability was introduced due to differences in storage time, and that any such variability would affect the fit but not direction of the relationship in Figure 3.3. Differences in panel composition, perhaps combined with much higher levels of total glucosinolates leading to panelist fatigue, may have also contributed to the lack of a significant relationship in May-planted cabbage.

Data reported here demonstrate that the planting date effect was consistent across the cultivars studied. Although few in number, the genotypes employed in this study are commercially important in the U.S. Higher glucosinolate concentrations in May-planted cabbage was attributed to the greater incidence of supra-optimal temperatures during head development in May- than June-planted cabbage. The data suggest that PD and C may act independently in influencing total glucosinolate concentrations in commercially available cabbage. Likewise, the data underscore the need to describe potential soil moisture effects (and their interaction with temperature) on crop glucosinolate concentrations; doing so will improve our understanding of the influence of abiotic environmental factors on the glucosinolate system and the ability to manage it for grower and consumer benefit.

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Source	df	Total glucosinolate concentration[z]
Year (Y)	1	***
Planting Date (PD)	1	***
Cultivar (C)	5	***
Y x PD	1	**
Y x C	5	*
PD x C	5	NS
Y x PD x C	5	NS

[z]NS, *, **, *** = Not significant or significant at $P \leq 0.05$, 0.01 or 0.001, respectively.

Table 3.1. Analysis of variance for the influence of year, planting date, and cultivar on total glucosinolate concentrations in six cultivars of fresh market cabbage planted in May and June of 2001 and 2002 at the OARDC Vegetable Crops Research Branch in Fremont, Ohio.

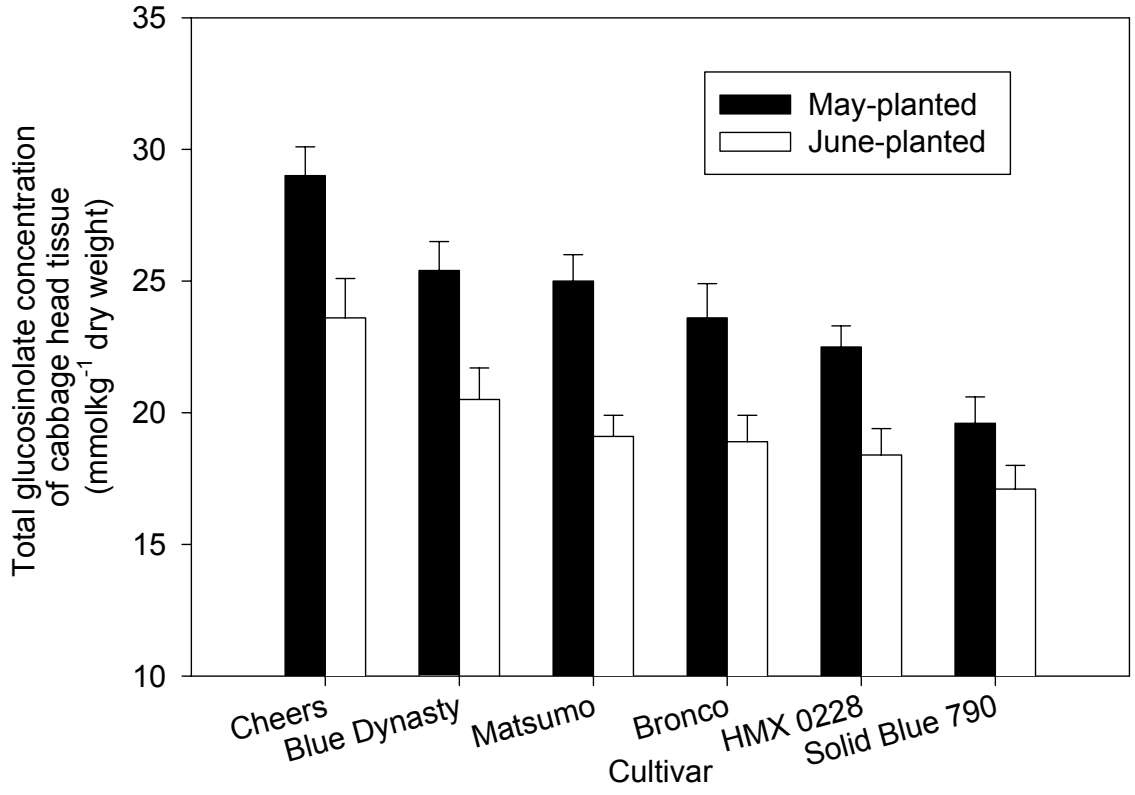

Figure 3.1. Mean total glucosinolate concentrations in head tissue of six cabbage cultivars planted in May and June of 2001 and 2002 at the OARDC Vegetable Crops Research Branch in Fremont, Ohio. Values are means of three sub-samples from each of four replications across years. Error bars are standard mean errors.

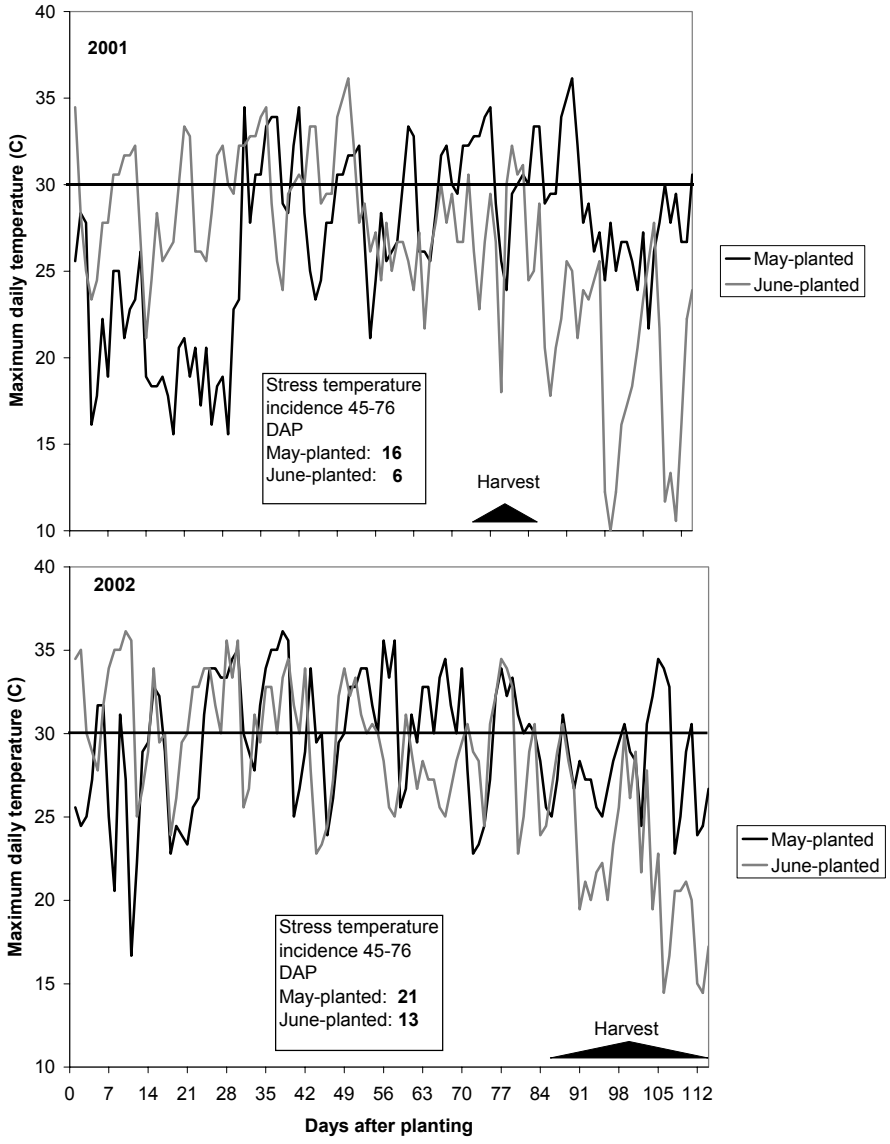

Figure 3.2. Maximum daily air temperatures during cabbage development in May and June of 2001 and 2002 at the OARDC North Central Agricultural Research Station in Fremont, Ohio. Within years, the difference between planting dates in days to harvest (DTH) was less than 7 d for all cultivars. The base of each solid triangle indicates the harvest period for both planting dates within years.

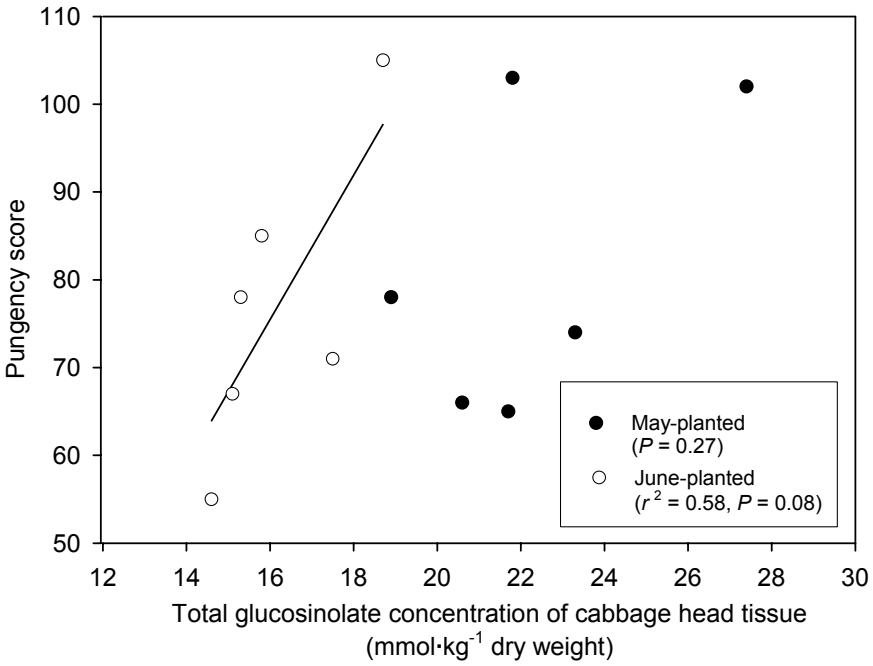

Fig. 3.3. Relationship between cultivar pungency scores and cultivar mean total glucosinolate concentrations in cabbage planted 10 May and 20 June, 2001 at the OARDC North Central Agricultural Research Station in Fremont, OH. Data from May- and June-planted plots are presented separately due to differences in panel composition between evaluations.

CHAPTER 4

TRIANGLE TESTS INDICATE THAT IRRIGATION TIMING AFFECTS FRESH CABBAGE SENSORY QUALITY

T.J.K. Radovich, M.D. Kleinhenz, J.F. Delwiche and R.E. Liggett

Food Quality and Preference 15:471-476

4.1 ABSTRACT

A replicated triangle test was employed to determine if judges could distinguish, by tasting, between shredded samples of fresh cabbage drip-irrigated during different periods of plant development. Irrigation was provided either: 1) throughout plant development (no stress, NS), 2) during frame development only (head stress, HS), or 3) during head development only (frame stress, FS). Control plants received no irrigation for the duration of plant development (frame and head stress, FHS). In a total of three sessions, fourteen judges evaluated two replications each of the six possible treatment comparisons in triangle tests. Results were analyzed using the beta-binomial model. Judges detected differences ($\alpha = 0.05$) between cabbage from NS plots and cabbage from the two plots that received no irrigation during head development (HS, FHS),

as well as between heads from FS and FHS plots. Physical traits of cabbage heads (e.g. weight, mean diameter, shape) at harvest were also affected by irrigation treatment. This is the first report to suggest that the timing of irrigation relative to crop development may influence the sensory characteristics of fresh cabbage. The data also suggest that cabbage head physical traits may respond more frequently to irrigation than cabbage sensory attributes.

4.2 INTRODUCTION

Vegetable crop sensory quality has received more attention in recent years as a result of efforts to secure and extend shares of increasingly competitive markets for fresh produce (Kuchenbuch, et al., 1999). Although the cultural requirements to maximize production of head cabbage (*Brassica oleracea* var. *capitata*) are well documented (Wien and Wurr, 1997), the effects of field management on cabbage sensory characteristics are less known. Variety, plant spacing and planting date are reported to affect a wide range of organic compounds associated with cabbage flavor (MacLeod and Nussbaum, 1977; Rosa, et al., 2001; Van Etten, et al., 1976). Some production factors may also play a role in human perception of cabbage quality. For example, panelists in triangle tests distinguished among cabbage grown under varying soil moisture and sulfur conditions (Freeman and Mossadeghi, 1972; Freeman and Mossadeghi, 1973). Also, in employing a three-point hedonic scale and 82 panelists, Yano, et al. (1990) detected differences in preference for shredded samples of five cabbage varieties and concluded that flavor and moisture content are highly important

in determining preference. It is clear that flavor may differ among cabbage varieties and that flavor has a strong effect on sensory quality (Martens, 1985; Radovich, et al., 2002, Chapter 2). However, although soil moisture availability strongly influences plant physiology, the effect of irrigation on cabbage flavor is under-studied. Freeman and Mossadeghi (1973) used a variety not currently of commercial importance and failed to account for the crop developmental stage at which irrigation was applied or corresponding effects on important physical head traits. Although utilized in other areas, irrigation is not regularly applied to cabbage grown in the Midwest, partly due to the relative scarcity of water resources and costs associated with irrigation (Swaider, et al., 2002). Recently, the potential to improve head quality through irrigation has increased interest in the use of irrigation in Midwest cabbage production. However, additional information is needed to determine if irrigation-related expenses are justified and, if so, how best to employ irrigation to optimize crop sensory quality. Therefore, the objectives of this work were to determine: 1) if irrigation leads to a discernable change in fresh cabbage quality and 2) whether the plant developmental period during which irrigation is applied influences human differentiation between samples. The study was also designed to help estimate the relative sensitivity of cabbage physical and sensory traits to irrigation.

4.3 MATERIALS AND METHODS

4.3.1 Cabbage production

Seeds of the commercially significant cabbage variety 'Bravo' were planted in late April 2002 and grown in the greenhouse for approximately six weeks. Seedlings were then transplanted to the field on 10 June, 2002 at the Ohio Agricultural Research and Development Center (OARDC) in Wooster, Ohio (latitude 40° 47' N, longitude 81° 55' W) using a single-row mechanical transplanter. Plant spacing was 0.31 m within single 18 m long rows with 1.5 m between treatment rows. Fertilizer was applied to the field prior to transplanting at the rates of 56, 49 and 93 kg ha^{-1} of nitrogen, phosphorous and potassium, respectively. The field experiment was arranged in a randomized complete block design with five replications. The three irrigation treatments were: irrigation throughout plant development (no stress, NS), irrigation during frame (non-heading leaves) development only (head stress, HS) and irrigation during head development only (frame stress, FS). Control plants (frame and head stress, FHS) received no irrigation for the duration of plant development. All plants were irrigated for 14 d after transplanting to aid in their establishment. Thereafter, irrigation treatments were imposed. Drip irrigation tape (3.4 l^{-1} h^{-1} m^{-1}, T-Systems International, San Diego, California) was laid within 8 cm of the base of seedlings in irrigated rows. Valved connectors allowed for watering of individual rows by turning valves on or off as necessary. During the treatment period, irrigation was applied when soil sampled from the top 18 cm of the soil profile was unable to maintain its shape when formed into a

ball in the hand (Klocke and Fischbach, 1984). Gypsum blocks (Delmhorst, Towaco, New Jersey) were installed within the crop root zone at 18 and 38 cm deep 28 d after planting to the field (DAP) to record soil water potential during the remainder of the study. The amount of water (irrigation + precipitation) received by all plots is shown in Figure 1. Gypsum block readings confirmed that soil moisture was lowest in non-irrigated treatments (data not shown). At 86 DAP, three adjacent heads were harvested from each of three randomly selected positions in each row. Physical characteristics were immediately recorded on two groups of three heads. Head weight was measured with a commercial field scale (model FV-60KWP, A and D Corp., Tokyo, Japan). Head diameter was measured in two directions; stem end to apex (polar) and perpendicular to the polar transect (equatorial). Head shape was calculated as the ratio of polar to equatorial diameter (1.0 = round). Percent moisture (PM) was calculated with the formula PM = 100 - (FW/DW), where FW = fresh weight (g) of a representative sample of head tissue (minus core) and DW = weight (g) of the same tissue dried for 7 d at 70°C. The third group of heads was held in darkened storage at 7°C in nylon mesh bags for 30 d prior to sensory evaluation. Storage of commercial fresh market cabbage for 30 d is not uncommon (Billingsley, 1994).

4.3.2 Sample preparation for sensory evaluation

Each day for three days (8-10 October, 2002), samples were prepared 1 hr prior to evaluation. One head from each replication was halved along its longitudinal axis to ensure that treatment samples were homogenized composites of heads from all field

replications. The core and damaged tissue were discarded, as was one half of the head. The remaining half was cut into smaller sections and shredded using a FoodPro2 food processor (Hamilton Beach/Proctor-Silex, Washington, North Carolina). Four-liter plastic containers with airtight lids (Rubbermaid, Wooster, Ohio) were filled with homogenized sample, sealed and held in the dark at 7°C until use. Percent moisture of three sub-samples from each treatment composite was determined as above (section 2.1). At the time of evaluation, approximately 35 g of sample was placed in 0.125 L polystyrene cups (Dart Container Corp., Mason, Michigan), assigned a random three-digit code and loosely covered with tinfoil.

4.3.3 Triangle test procedure

The testing protocol was approved by The Ohio State University Office of Research Risks Protection (ORRP). The sensory panel group consisted of 14 untrained volunteers: 6 males, 8 females, smokers and nonsmokers, aged 21-65 years. The triangle test was chosen as it allows one to distinguish between samples without having to specify the sensory characteristic(s) that differ. As it is a discrimination task, especially for untrained panelists, it is also better at detecting small differences between samples than are intensity ratings (Lawless and Heymann, 1998).

Panelists were not trained, but prior to sample evaluation, panelists received instruction regarding the evaluation procedure in both written and verbal formats. The following written instructions were placed on the ballot: “Taste samples from left to right. Two of the samples are identical. Determine which one is the **odd** sample. You

may re-taste samples. If no difference is apparent, you must guess." Verbal instruction prior to evaluation included reiteration of written instructions, as well as instructions to focus on flavor, evaluate samples one at a time, keep samples capped when not being tasted, proceed at own pace, and to cleanse the palate with bread and water between samples.

Panelists seated at partitioned booths were presented with three samples simultaneously, two from one irrigation treatment and one from another treatment. To minimize visual comparison of samples and eliminate side-by-side comparisons, panelists were instructed to keep samples capped until use, removing caps only to obtain the sample and to disregard visual cues. While it is not possible for panelists to "disregard" cues, it is possible to redirect their focus to other sensory characteristics. Panelists tasted samples at a self-determined pace with no time limit for completing the session, although sessions tended to last 20-30 minutes. To minimize adaptation, a 2-3 min break occurred between triads and panelists were instructed to take additional breaks as they desired. Panelists were provided with bottled spring water (White House Artesian Springs, Inc., Elyria, Ohio) and white bread (Beuhler's Fresh Food Market Bakery, Wooster, Ohio) for palate cleansing, which they used between samples and between triads. Samples were swallowed and re-tasting was permitted. Panelists evaluated two treatment pairs (replicated twice) per day during the 3-d evaluation period following a counter-balanced design. Replicates were employed to test for overdispersion and improve test power (Dacremont and Sauvageot, 1997; Ennis and Bi, 1998). The presentation order of treatment comparisons was counter-balanced across

panelists and sample presentation was randomized within triads. Evaluations were conducted each day between 10:00 A.M. and 1:00 P.M.

4.3.4 Data analysis

Overdispersion, symbolized by gamma, is a measure of panelist variability. Similar to the coefficient of determination, gamma varies from zero to one. A gamma of zero indicates that there is no overdispersion, panelist variability is minimal, and panelists are assessing products in an identical fashion. A gamma of one indicates that there is complete overdispersion, panelist variability is high, and each panelist is making product assessments in a unique fashion. An intermediate value indicates that panelist variability lies between these two extremes, and one can test to see if this intermediate value is significantly different from zero. To account for potential overdispersion in the sensory evaluation data, the beta-binomial model, which allows one to account for gamma (i.e., panelist variability), was used to determine whether there was a significant difference in cabbage sensory characteristics across treatment conditions and if panel overdispersion was significant (Ennis and Bi, 1998).

Head physical trait data were subjected to analysis of variance (ANOVA) using the General Linear Model procedure of SAS (Statistical Analysis System for Windows^{TM}, v. 8, Cary, North Carolina). Treatment means were compared using Fisher's Protected Least Significant Difference test ($\alpha = 0.05$) in SAS.

4.4 RESULTS

Analysis of the triangle test data with the beta-binomial model indicated that for all comparisons, gamma was less than 0.0001. Therefore, the simpler binomial model was used to evaluate differences between comparisons. Panelists detected differences ($\alpha = 0.05$) between heads irrigated throughout development (NS) and heads from the two plots that received no irrigation during head development (HS and FHS) (Table 1). Differences were also detected between heads irrigated only during head development and the control (FHS). No differences were detected in the NS vs. FS, FS vs. HS or HS vs. FHS comparisons.

Differential irrigation also affected physical traits of cabbage heads recorded at harvest, with head percent moisture (PM), weight and mean diameter greatest in the NS and FS treatments (Table 2). Differences among treatments in PM were also found at sample preparation, 30 d after harvest (Table 2). Freshly shredded head tissue from the HS and FHS plots was slightly discolored (i.e. brown) relative to head tissue from the NS and FS plots (data not shown).

4.5 DISCUSSION

4.5.1 Triangle test results

As the primary ingredient of coleslaw and other salads, the value of fresh, shredded cabbage depends on its sensory characteristics (Ball, et al., 1999; Martens,

1985; Yano et al., 1990). Data in Table 4.1 demonstrate that irrigation and its timing affected the sensory perception of fresh cabbage. Cabbage irrigated during head development (NS, FS) was identified as tasting different from cabbage receiving no irrigation (FHS). Panelists had greater difficulty distinguishing cabbage irrigated early in plant growth only (HS) from cabbage receiving no irrigation (FHS). Similarly, cabbage watered only during head development (FS) was difficult to distinguish from cabbage watered throughout development (NS). Therefore, these data suggest that to obtain large perceptible differences in cabbage sensory characteristics from a non-irrigated control group, water may need to be applied only during head development. Factors contributing to cabbage that consumers prefer remain to be determined. However, these data establish irrigation conditions which may lead to significant differences in cabbage sensory characteristics. Studies of the influence of cultural practices on fresh vegetable quality have been largely limited to effects on physical characteristics contributing to yield (Barber and Raine, 2002; Kuchenbuch et al., 1999; Sanchez, et al., 1994). This first report of an effect of irrigation timing on fresh cabbage sensory characteristics is a unique contribution to the expanding body of work demonstrating a direct link between field management and the perception of fresh vegetable sensory quality (Radovich, et al., 2000; Scheerens and Hosfield, 1976; Simonne, et al., 2001).

Triangle tests are unable to determine the magnitude or direction of perceived changes in sensory quality (Lawless and Heymann, 1998). In our study, the relatively small size, light weight, elongated shape and slightly discolored tissue of heads produced in HS and FHS plots during the relatively warm and dry 2002 growing season

reduced their potential commercial value. Percent moisture (PM) and the related attributes crispness and juiciness are thought to be important to the acceptability of fresh cabbage (Martens, 1985; Yano et al., 1990). The smallest perceptible change in PM of fresh cabbage is not known. However, earlier reports (Martens, 1985; Yano et al., 1990) suggest that preference ratings would favor cabbage irrigated during head development, which had significantly (~3%) higher PM values than treatments not irrigated during head development (Table 4.2). The lower PM of cabbage not irrigated during head development may have resulted in stronger flavor due to a higher concentration of dry matter, including organic flavor compounds. Freeman and Mossadeghi (1973) reported both stronger flavor and higher concentrations of volatile isothiocyanates (flavor compounds) in water-stressed cabbage relative to well-watered cabbage. Strong flavor generally corresponds to a decrease in acceptability of fresh cabbage (Ball et al., 1999; Yano et al., 1990). Therefore, we speculate that the flavor of cabbage from FHS plots would have been judged stronger and less desirable relative to cabbage from NS or FS plots.

4.5.2 Cabbage head characteristics

Differences in head physical traits were found among all irrigation treatments (Table 2). Heads from plants receiving irrigation throughout plant development were larger, heavier and more round than heads from other treatments. Withholding water during head development resulted in a more than 50% reduction in head fresh weight (Table 2). This would correspond to unacceptable commercial losses. In addition to

lower yields, a reduction in crop value would be expected to result from deviations in head shape and size from the optimum for packing, shipping and processing, as well as from the browning observed in shredded tissue from cabbage not irrigated during head development.

4.5.3 Head physical traits vs. sensory quality

Fewer significant differences among treatment comparisons were found in the sensory data compared to the physical trait data (Table 2). Three of six comparisons were significantly different in the triangle test. However, four comparisons were significantly different in percent moisture data, and all six comparisons were significantly different with regard to head weight, mean diameter and shape. Therefore, as a group, the physical traits measured here responded more frequently to irrigation than cabbage flavor attributes. This may be due in part to the relatively low power of the triangle test to detect differences in the non-significant comparisons (Table 1). Irrigation applied only during head development resulted in an 18% decrease in head weight relative to irrigation throughout development. Yet, panelists perceived little difference in sensory properties between the two treatments. If some decrease in head weight is justified by reduced water cost and resource conservation, cabbage growers may need to irrigate only during head development to achieve crop flavor quality goals. However, testing this hypothesis in additional, commercially significant varieties is important.

4.6 CONCLUSIONS

These data demonstrate that irrigation and its timing relative to plant development stage can influence cabbage sensory quality. The data also suggest that physical head traits are affected by soil moisture availability, perhaps more frequently than sensory characteristics. Provided some decrease in head weight is acceptable, we conclude that, relative to irrigating throughout development, irrigating only during head development may help reduce irrigation costs and conserve water resources while maintaining sensory quality.

4.7 ACKNOWLEDGMENTS

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Treatment Comparison	P-value	Power
NS vs. FS	0.1138	0.394
NS vs. HS	**0.0003**	0.989
NS vs. FHS	**0.0132**	0.733
FS vs. HS	0.0827	0.423
FS vs. FHS	**<0.0001**	1.000
HS vs. FHS	0.4470	0.036

Table 4.1. Results of the triangle test data analysis. The p-values and power were generated with the binomial model and are based on the responses of 14 panelists over two replications with gamma <0.0001. Treatments were irrigation throughout plant development (NS, no stress), irrigation during head development only (FS, frame stress), irrigation during frame development only (HS, head stress) and no irrigation for the duration of plant development (FHS, frame and head stress).

Treatment	Weight (kg)	Mean Diameter (cm)	Shape	Moisture content at harvest (%)	Moisture content of evaluated samples (%)	Perceived Sensory Differences
NS	1.6 a	15.5 a	1.00 d	91.3 a	91.6 a	a
FS	1.3 b	14.8 b	1.06 c	91.4 a	92.1 a	ab
HS	0.7 c	11.6 c	1.12 b	88.1 b	89.7 b	bc
FHS	0.5 d	10.7 d	1.23 a	88.7 b	89.7 b	c

Table 4.2. The effect of irrigation on physical and sensory characteristics of cabbage heads. Physical trait values are means of five replications. Values within columns followed by the same letter are not significantly different ($\alpha = 0.05$) according to Fisher's Protected Least Significant Difference (LSD) test. Shape value is the ratio of head polar to headequatorial diameter (1.0 = round). Treatments were irrigation throughout plant development (NS, no stress), irrigation during head development only (FS, frame stress), irrigation during frame development only (HS, head stress) and no irrigation for the duration of plant development (FHS, frame and head stress). Sensory treatment comparisons containing the same letter are not significantly different ($\alpha = 0.05$) from each other as determined by the binomial model.

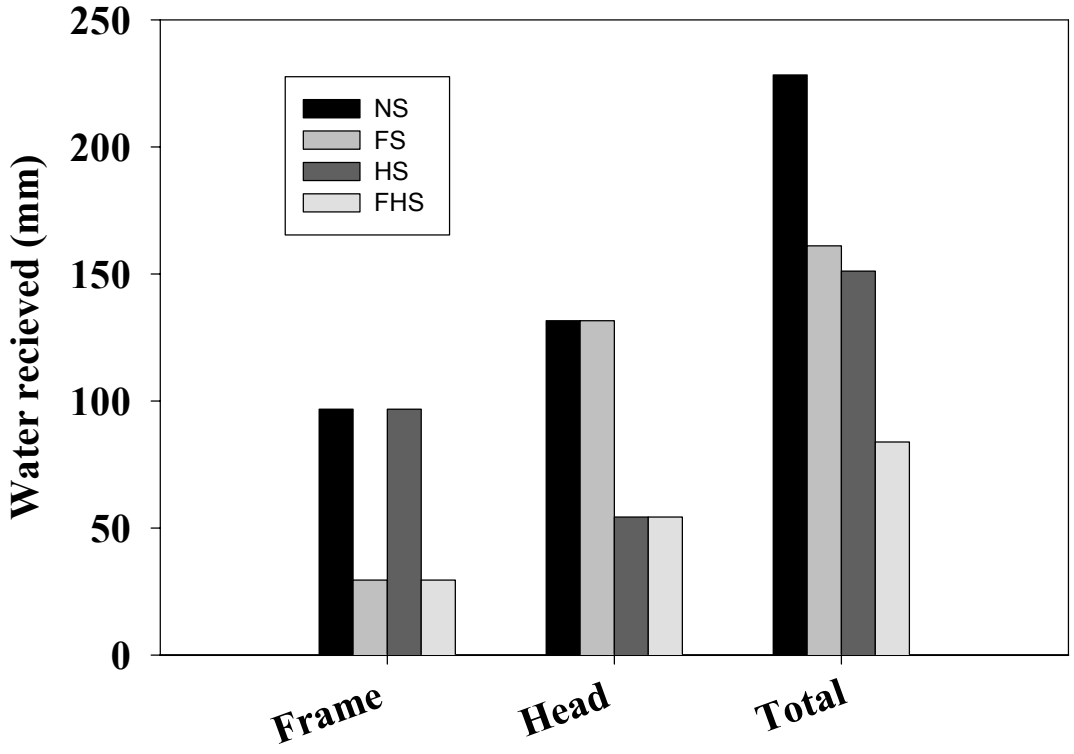

Figure 4.1. Water (mm) received by the crop as irrigation and rainfall after establishment. Frame development occurred 14-50 days after planting in the field (DAP) and head development occurred 51-86 DAP.

CHAPTER 5

IRRIGATION TIMING RELATIVE TO HEAD DEVELOPMENT INFLUENCES YIELD COMPONENTS, SUGAR LEVELS AND GLUCOSINOLATE CONCENTRATIONS IN CABBAGE (*BRASSICA OLERACEA* GROUP CAPITATA)

T.J.K. Radovich, M.D. Kleinhenz and J.G. Streeter

Journal of the American Society for Horticultural Science (in process)

5.1 ABSTRACT

To better understand the influence of environmental factors on crop yield and potential health value, the fresh-market cabbage 'Bravo' was irrigated at different stages of head development in a field study conducted in 2002 and 2003 at the Ohio Agricultural Research and Development Center in Wooster, OH. Irrigation was provided either: 1) from planting to maturity, 2) during frame development only, or 3) during head development only. Control plants received no irrigation after plant establishment. Irrigation timing relative to crop stage significantly affected all head characteristics with the greatest differences between cabbage receiving irrigation during head development and cabbage not irrigated during head development. On average,

heads from cabbage irrigated during head development were heavier, larger, less pointed, and had less volume occupied by the core than heads from cabbage not irrigated during head development. A positive, linear relationship (r^2 = 0.89) was found between head volume and head weight. Combined head fructose and glucose concentrations were significantly greater in cabbage receiving irrigation during head development than in cabbage not irrigated during head development. Sucrose concentrations were significantly greater in cabbage not irrigated during head development than cabbage receiving irrigation during head development. Total and individual glucosinolate levels were greater in cabbage not irrigated during head development relative to cabbage receiving irrigation during head development. Head weight, fructose and glucose were positively related to the proportion of estimated crop evapotranspiration replaced by irrigation during head development, while the opposite response was observed in head sucrose and total and indole glucosinolate concentrations.

5.2 INTRODUCTION

The dependence of metabolic processes on environmental factors may be exploited to manipulate crop yield, flavor and nutritional quality (Hochmuth, 2003; Lester and Crosby, 2002; Rosenfeld, 1999). In cabbage, planting date influences yield components and glucosinolate concentrations and may affect fresh cabbage flavor (Kleinhenz and Wszelaki, 2003; Radovich et al, 2004a, Chapter 3). The planting date effect is thought to result from separate and interactive effects of canopy temperature

and plant water status during head development (Bible et al., 1980; Radovich et al., 2004a, Chapter 3; Rosa et al., 1996). Applying irrigation to mitigate plant stress, specifically during head development may assist in management of multiple aspects of cabbage quality, including yield and flavor (Radovich et al., 2004b, Chapter 4). However, most studies regarding the influence of irrigation on cabbage quality focused on a limited number of variables, primarily those contributing to yield (Singh and Alderfer, 1966; Swaider et al., 2002), while not accounting for effects on other important variables.

Soil tensions < 25 kPa and soil moisture > 80% of field capacity, especially during head development, maximize yield and individual head weight (Mamman and Haque, 1999, Singh and Alderfer, 1966; Smittle et al., 1994). Similarly, replacing 100% of estimated crop consumptive use is reported to optimize yield and cabbage head weight (Sammis and Wu, 1989; Sanchez et al., 1994; Tiwari et al., 2003). Despite the emphasis on yield and its components, there are still gaps in the literature with regard to irrigation effects on physical head quality. For example, the relationship between head volume and weight is important because it allows for the prediction of yield based on individual head size. A strong, positive relationship appears to be stable across a range of genotypes, environments and development stages (Kleinhenz, 2003; Kleinhenz and Wszelaki, 2003; Radovich and Kleinhenz, 2004; Radovich et al., 2004c, Chapter 6; Wszelaki and Kleinhenz, 2003). However, the influence of irrigation on this relationship has not been reported.

Levels of production are generally adequate to meet demands for fresh-market cabbage. As a result, focus has shifted to head characteristics influencing buyer- and

consumer-oriented assessments of crop quality (Kuchenbach et al., 1999; Radovich et al., 2003, Chapter 1). Sensory quality is important because cabbage is the primary ingredient in cole-slaw and other salads (Martens, 1985; Radovich, 2004b). Most important among fresh cabbage sensory characteristics are the flavor attributes pungency and sweetness, which are determined in large part by concentrations of sugar and glucosinolates (Yano et al., 1987, van Doorn et al., 1998).

Fructose, glucose and sucrose comprise the majority of the sugar present in cabbage and account for approximately 20-40% of the total mass of cabbage heads on a dry weight basis (Janes 1950; Rosa et al., 2001). Yet, the factors influencing sugar concentrations in cabbage are poorly understood. Rosa et al. (2001) reported that planting date influences fructose, glucose and sucrose concentrations in cabbage and broccoli, with higher sugars in heads of plants grown in Portugal in the fall. Janes (1950) reported that greater irrigation frequency increased the concentrations of total reducing sugars in heads of a single cabbage cultivar, and that the effect of irrigation depended on the season of growth.

Glucosinolates are amino acid-derived secondary plant metabolites of considerable scientific and practical interest. The hydrolysis products of glucosinolates may exhibit antibiotic, goitrogenic, anti-carcinogenic or organoleptic activity (Fahey et al., 2001). Sinigrin and progoitrin are the most important compounds with regard to flavor, being the primary determinants of pungency, bitterness and sulfurous aroma in cabbage (Buttery et al., 1976; van Doorn et al., 1998).

Glucosinolate responses to planting date indicate that their levels in cabbage are influenced by abiotic growth factors (Bible et al., 1980; Radovich et al., 2004c Chapter

3; Rosa et al. 1996). Supra-optimal temperatures increase the concentrations of glucosinolates in tissues of *Brassica* species (Charron and Sams, 2004; Pereira et al., 2002) and frequent, high temperature stress during head development is associated with high glucosinolate concentrations in heads of field-grown cabbage (Radovich et al., 2004c, Chapter 3; Rosa et al., 1996).

Maximizing plant capacity for transpiration-dependent leaf cooling through irrigation has been identified as a potentially effective method to mitigate heat related plant stress (Hermann et al., 1990; Jiang and Huang, 2001). Bible et al. (1980) reported that irrigation can eliminate seasonal variation in head concentrations of thiocyanate (a glucobrassicin hydrolysis product), while Freeman and Mossadeghi (1973) showed that soil moisture stress increased the concentrations of allyl-isothiocyanate (a product of sinigrin hydrolysis) four-fold relative to well watered plants. However, as with sugars, the relative response of individual glucosinolates in cabbage heads to supplemental irrigation is not known.

The objective of this study was to document the influence of irrigation timing, particularly in relation to head development, on important indicators of cabbage head physical and chemical quality, including yield components, sugar levels and glucosinolate concentrations.

5.3 MATERIALS AND METHODS

5.3.1 Plant material, experimental site and design

Greenhouse-grown seedlings of the commercial cabbage variety 'Bravo' with approximately 4 true leaves were transplanted to the field 10 June, 2002 and 17 June, 2003 at the Ohio Agricultural Research and Development Center (OARDC) in Wooster, Ohio (latitude 40° 47' N, longitude 81° 55' W) using a single-row mechanical transplanter. Soil at the site was a Fine-loamy, Mixed, Mesic, Typic Fragiuldalf. Plant spacing in both years was 0.31 m within single 18 m long rows with 1.5 m between treatment rows. In 2002, pre-plant fertilizer applications were at the rate of 560 kg ha^{-1} of a 10N-8.7P-16.6K. In 2003, fertilizer was applied prior to planting at the rate of 448 kg ha^{-1} of a 19N-8.3P-15.8K fertilizer. Standard pest management strategies, based on scouting, thresholds and application of labeled pesticides, were employed. The field experiment was arranged in a randomized complete block design with five replications. The irrigation treatments were: irrigation throughout plant development (no stress, NS), irrigation during frame development only (head stress, HS) and irrigation during head development only (frame stress, FS). Control plants (frame and head stress, FHS) received no irrigation for the duration of plant development after a two week establishment period in the field. Drip irrigation tape (340 l^{-1} h^{-1} m^{-1}, T-Systems International, San Diego, California) was laid within 8 cm of the base of seedlings in irrigated rows. Valved connectors allowed for watering of individual rows by turning

valves on or off as necessary. Line pressure was regulated to 70 kPa. Irrigation treatments were initiated following an establishment period (2 weeks) during which all plants were irrigated. During the treatment period, irrigation was applied when soil sampled at a depth of 18 cm was unable to maintain its shape when formed into a ball in hand, a method of scheduling chosen for its simplicity and its popularity among producers (Heermann et al., 1990; Klocke and Fischbach, 1984). Irrigation was run from 3-5 h per application. Soil moisture was monitored occasionally at 18 cm using gypsum blocks (Delmhorst, Towaco, New Jersey), time domain reflectometry (TDR-300, Spectrum Technologies, Plainfield, IL) or gravimetrically.

5.3.2 Evapotranspiration calculation

Temperature, light intensity, wind speed and relative humidity data were collected hourly at 400 m from the experimental site by the OARDC Weather System (The Ohio State University, 2003). Reference evapotranspiration (Et_o)was calculated with the Daily Reference Evapotranspiration Calculator (University of California, Davis, Ca.) employing the FAO modified Penman-Monteith equation (Allen et al., 1998; Snyder and Etching, 2003). Crop evapotranspiration (ET_c) was calculated using the equation $ET_c = K_c * Et_o$, where K_c is the crop coefficient. An adjusting K_c was used during frame development, beginning at 0.7 and increasing 8.75×10^{-3} per day until head development. Thereafter, transpiration rate stabilizes and a constant K_c of 1.05 was used (Allen et al., 1998; Nelson and Hwang, 1976).

5.3.3 Harvest and evaluation of yield components

At 86 days after planting (DAP), six adjacent heads were harvested from a randomly selected position in each row. Physical characteristics were immediately recorded on one group of three heads. Head weight was taken with a commercial field scale (A and D Corp., Japan). Head diameter was measured in two directions; stem end to apex (polar) and perpendicular to the polar transect (equatorial). Head shape was expressed as the ratio of the polar diameter to equatorial diameter, where 1.0 = round. Volume was calculated from mean head diameter values as previously reported (Radovich and Kleinhenz, 2004, Chapter 7). Dry matter concentration was calculated as a percent of total fresh tissue weight by dividing the dry weight of a section (core removed) of three heads by the fresh weight (dried for seven days at 70 °C) and multiplying by 100. The second group of heads was held in darkened storage at 7 °C in net bags for < 48 h, after which individual head weight and diameter were recorded and head tissue was processed for chemical analysis as previously described (Radovich et al., 2004c, Chapter 3, Appendix A).

5.3.4 Sugar analysis

Head tissue sugar concentrations were determined by gas-liquid chromatography using the method of Streeter and Strimbu (1998). Ground, freeze-dried cabbage tissue (4 mg) was placed in 2 ml vials, to which was added 125 µl pure pyridine and 125 µl

STOX reagent (25 mg/ml hydroxylamine hydrochloride and 6 mg/ml phenyl-D-glucopyranaside). The mixtures were vortexed and heated at 70 °C for 40 min, during which the mixtures were vortexed several more times. The mixture was allowed to cool, after which 200 µl hexamethyldisalazane (HMDS) plus 20 µl trifluoroacetic acid (TFA) were added, thoroughly mixed and allowed to incubate for 60 min at ambient temperature. Injection volume was 1 ml and TMS-oxime derivatives were separated on a packed column of 3% OV-17 on Chromsorb WHP using a Hewlett-Packard 5890 Series II gas chromatograph. Peak areas were quantified using a Hewlett-Packard 3396A integrator. Sugar concentrations were quantified from peak areas using previously established standard curves.

5.3.5 Glucosinolate analysis

Total glucosinolate concentrations in freeze-dried cabbage head tissue were determined using the glucose evolution procedure of Heaney and Fenwick (1981), with some modifications as previously described (Radovich et al., 2004c, Chapter 3, Appendix A). Individual glucosinolate concentrations in methanol extracts used for the glucose evolution procedure were analyzed by HPLC employing the method of the International Organization for Standards (ISO, 1992), with some modifications. Sample extract (1 ml) was applied to 0.5 ml of swollen Sephadex A-25 resin (Sigma Chemical Co., St. Louis, Mo.) and washed with water and pyridine-acetate buffer (Radovich et al., 2004c, Chapter 3, Appendix A). Sulfatase (1.25 U in 250 µl of 0.02 M pyridine-acetate buffer) from *Helix pomatia* (Sigma Chemical Co., St. Louis, Mo.) was applied to the

column and allowed to incubate for 16 h at ambient temperature. Desulphoglucosinolates were eluted with 1 ml of water and the eluate (1.25 ml total volume) transferred to 2 ml vials. Samples were stored at < 0 °C for < 24 h prior to analysis. Desulphoglucosinolates in 5 µl of sample extract were separated on a 250 x 2.1 mm Supelcosil LC-18 5 µm column (Supelco, Bellefonte, PA) using a Waters (Waters, Milford, Mass.) LC/MS system and a Waters 996 Photo Array Detector at a scanning wavelength range of 210 - 400 nm. The flow rate was 0.27 ml · sec^{-1}. The mobile phase was H_2O (A) and 20% aqueous acetonitrile (B) at 99% A for 1 min, followed by a 20 min linear gradient to 99% B, then a linear gradient over 2 min to 99% A that was held for 18 min. Column temperature was maintained at 30 °C. Desulfoglucosinolates were identified and quantified using authentic 2-propenyl (sinigrin), 3-methylsulfinylpropyl (glucoiberin), 2(R)-2-hydroxy-3-butenyl (progoitrin) and benzyl glucosinolate standards (KVL, Frederiksberg, Denmark) and published response factors (ISO, 1992).

5.3.6 Statistical analysis

Data were analyzed with the General Linear Model procedure of SAS for Windows v.8 (Statistical Analysis System, Cary, N.C.) and with the Regression Wizard of SigmaPlot 2000 for Windows v. 6.0 (SPSS Inc., Chicago, Il.).

5.4 RESULTS

5.4.1 Climatic data

Estimated ET_c, average daily temperature, and the amount of water received by treatment plots for 7 d periods from planting to harvest are shown in Fig. 1. Gravimetric and volumetric soil moisture across treatments ranged between 8-21 and 14-35%, respectively, depending on the amount of water received. In 2002, temperatures and ET_c were higher and water received was less than in 2003. This resulted in a smaller proportion of the estimated crop water requirement being supplied as rainfall and irrigation in 2002 than 2003 during both frame and head development.

5.4.2 Physical head traits

The main and interactive effects of year (Y) and irrigation treatment (I) were significant for all but one (density) of the physical traits measured, with density influenced only by year (Table 1). Overall, treatment (I) differences were greater in 2002 than 2003.

In both years, head size and weight were greatest in treatments receiving irrigation during head development (NS and FS, Table 2). In 2002, heads from NS plots were only 2% larger, but were 20% heavier than heads from FS plots (Table 2). There was no

difference in head size or weight between NS and FS treatments in 2003, although heads from those treatments were 15% heavier than heads from HS and FHS plots. Heads were, on average, 35% heavier and 45% larger in 2003 than 2002. Across Y and I there was a strong ($r^2 = 0.88$) relationship between head size (volume) and weight described by the linear equation W = (0.0007 * V) + 0.12, where W = head weight (kg) and V = head volume (cm^3).

Across years, P: E diameter ratios and dry matter concentrations were lowest in heads from plants irrigated during head development (NS, FS). In 2002, these differences followed the order NS < FS < HS < FHS for diameter ratio and NS = FS < HS = FHS for moisture content. In 2003, there was no difference between NS and FS plots in diameter ratio, and only NS differed in diameter ratio from HS and FHS. Also, in 2003, there was no clear treatment effect on percent dry matter. Across treatments, diameter ratio and percent dry matter were 20 and 40% lower, respectively in 2003 than in 2002.

5.4.3 Sugar concentrations

The main and interactive effects of Y and I were significant for fructose, glucose and sucrose (Table. 3), and the three sugars collectively accounted for ~30-60% of sample dry weight (data not shown). Across years, heads from plants receiving irrigation during head development were higher in fructose and glucose, and lower in sucrose, than plants not receiving irrigation during head development (Table 4). This tendency was markedly more pronounced in 2002 than 2003, when heads from NS and

FS treatments were ~30% higher in fructose and glucose and 33% lower in sucrose. The opposite relationship of responses between the mono- and disaccharides to irrigation treatment resulted in a lower ratio of fructose + glucose to sucrose in head-stressed treatments HS and FHS relative to NS and FS. On average, fructose and glucose levels in 2003 were twice those in 2002, and sucrose levels were 28% lower in 2003 than in 2002.

5.4.4 Glucosinolate concentrations

The glucosinolates identified in our samples were the methionine-derived sinigrin, glucoiberin and progoitrin, and the tryptophan-derived indol-3ymethyl glucosinolate (glucobrassicin) (Table 5). Irrespective of Y and I, the relative contribution of individual to total glucosinolate concentrations in cabbage heads was in the order: glucoiberin > sinigrin > glucobrassicin > progoitrin. Small peaks of what was tentatively identified as 4-methoxyglucobrassicin followed glucobrassicin concentrations closely but are not reported here. The main and interactive effects of Y and I were significant for total and all individual glucosinolate concentrations. The exceptions were sinigrin, which was unaffected by Y, and glucoiberin for which there was no significant Y x I interaction (Table 3). Generally, heads from NS and FS plots were lower in total and individual glucosinolates than those from HS and FHS plots. Although influential in both years, irrigation during head development was most effective in lowering glucosinolates relative to the control (FHS) in 2002, when glucosinolates were, on average, ~ 40 % lower in NS and FS compared to FHS plots

(Table 5). This decrease was most notable in glucobrassicin (60 %). Also, in 2002, and in contrast to 2003, there were significant differences between NS and FS treatments. Specifically, methionine-derived glucosinolate concentrations in FS heads were 10-18% lower than those in NS heads (Table 5). Glucoiberin, progoitrin and glucobrassicin were 19, 63 and 152% higher in 2002 than 2003, respectively.

5.5 DISCUSSION

5.5.1 Physical head traits

Ranges in the size, weight and diameter ratio recorded here are consistent with those found in previous work, including in experimental and commercial fields (Kleinhenz, 2003; Kleinhenz and Wszelaki, 2003). Irrigation during head development resulted in larger, heavier heads, with relatively low P:E diameter ratios and dry matter content. Head expansion occurs preferentially in the equatorial direction (Radovich et al., 2004a). Lower P:E diameter ratios, along with overall increases in volume, indicate greater head leaf expansion in the NS and FS plots. Restrictions in head leaf expansion in response to drought stress are likely the main reason that withholding water during head development reduces yield. Supplying irrigation to provide ~100% of ET_c is reported to maximize potential yields in cabbage (Bucks et al., 1974; Sanchez et al., 1994; Tiwari et al., 2003). Our data suggest that replacing daily consumptive use is most important during head development (Table 2, Fig. 2). Although there were no differences among head traits between NS and FS plots in 2003, heads were larger and

heavier in NS than FS plots in 2002 (Table 2). ET_c deficits were greater in FS (12% of ET_c) than in NS plots (39% of ET_c) during frame development in 2002, and there was little difference in the amount of water received between the two treatments during frame development in 2003. This suggests that drought during frame development and the earliest stages of head development may influence yield by reducing frame size and restricting growth in the outermost head leaves.

The positive relationship between head size and weight across treatments is explained by the consistency in head density (Table 2). Marketable yield in cabbage can be predicted based on the mean head size in a given production area (Kleinhenz, 2003). The data here suggest that the strong, positive relationship between head size and weight is stable across irrigation regime, an observation not previously reported.

5.5.2 Sugars

Allowing for differences in methodology and genotype, the concentrations of sugars reported here agree with previous reports (Janes, 1950; Rosa et al., 2001). Across years, the influence of irrigation during head development on sugar concentration was attributed to the proportion of ET_c supplied to the crop during that period (Fig. 5.3). Our data agree with Janes (1950), who reported lower reducing sugars in heads of rain-fed cabbage in Florida compared to plants receiving supplemental irrigation. Higher fructose and glucose concentrations in heads from NS and FS plots likely contributed to the flavor differences previously reported between those treatments and the control (FHS) (Radovich et al, 2004b, Chapter 4). Additionally, the treatment response of

fructose and glucose were negatively related to that of sucrose, resulting in lower monomer:dimer ratios in plants not receiving irrigation during head development. This change in sugar ratio may significant, and reflect a modification of sink strength and/or cell osmotic adjustment in head tissues, possibly through regulation of enzymes involved in sucrose metabolism (Gonzalez et al., 1995; Roitsch, 1999).

5.5.3 Glucosinolates

The absolute and relative concentrations of glucosinolates reported here agree with others in the literature (Rosa et al., 1996; Van Etten et al., 1980). All of the glucosinolates measured were influenced by irrigation, with glucosinolate levels lowest in heads receiving irrigation during head development. Although previously observed in the reproductive tissues of *Brassica napus* (Champilivier and Merrien, 1996; Mailer and Cornish, 1997), a response of glucosinolate concentrations to stress relative to developmental stage has not been reported in head cabbage. Glucobrassicin concentrations were most responsive to Y and I, and mean total glucosinolate and glucobrassicin concentrations across years were lower in treatments receiving a greater proportion of ET_c during head development (Fig. 4). The limited influence of year on the methionine-derived glucosinolates resulted in the relationship between aliphatic glucosinolates and ET_c being less clear (data not shown).

The differential response of aliphatic and indole glucosinolates to treatments and ET_c implies a measure of independence between the mechanisms responsible for the increase in methionine- and tryptophan-derived compounds under stress conditions. The

differential response to irrigation may be related to differences between the biosynthetic pathways (indole glucosinolates and IAA share a common intermediate) and/or metabolic function of the two groups of glucosinolates (Mikkelsen et al., 2000; Rosa et al., 1997).

In contrast to glucobrassicin, methionine-derived glucosinolates were lower in FS than in NS plots in 2002 (Table 5). In 2002, plants in FS plots were exposed to greater ET_c deficits during frame development (12% of required) than plants in NS plots (39% of required) (Fig. 1). This previous exposure to stress may have resulted in a lower sensitivity of FS plants to high temperatures and ET_c deficits than both FS and NS treatments experienced during head development in 2002 (Fig.1). Also, plants were exposed to higher temperatures and greater ET_c deficits during frame development in 2002 than in 2003. Possibly as a result, aliphatic glucosinolate concentrations in NS and FS plots in 2002 were similar to those in HS and FHS plots in 2003, despite greater ET_c deficits during head development in 2002. This suggests a pre-conditioning of plants exposed to relatively severe ET_c deficits during frame development. Preconditioning of plants to drought reduces their sensitivity to heat and drought stress later in development (Chaves et al., 2003; Ladjal and Ducrey, 2000). Preconditioning during frame development may be responsible for the weak link between aliphatic glucosinolates and environmental differences between years.

5.5.4 Implications for cabbage quality

These data represent a comprehensive investigation of irrigation influence on cabbage head physiology as it directly relates to grower- and consumer-oriented indicators of quality. Irrigation as applied in this study was ineffective in consistently meeting 100% of estimated plant ET_c, but did succeed in generating a range of ET_c deficits. The data strongly suggest that heading is the developmental stage during which irrigation has the most influence on all variables. Head size and weight were greatest in plants irrigated during head development, but the relationship between these two variables was not greatly influenced by irrigation. The effect on yield components observed here suggests, for the first time, that head weight may be predicted across irrigation regimes based on its relationship to head size.

Sugar and glucosinolate concentrations were differentially influenced by irrigation and may have influenced head flavor, particularly in 2002. In 2002, the difference in total glucosinolate concentrations between treatments receiving irrigation during head development (NS and FS) and the control (FHS) was 15 $mmol \cdot kg^{-1}$. Radovich et al. (2004c, Chapter 3) reported an increase in the perception of pungency in cabbage differing in glucosinolate concentrations by 6 $mmol \cdot kg^{-1}$. Increases in the perceived pungency of drought-stressed cabbage would be enhanced by lower sugar, particularly fructose, concentrations (Table 1). Therefore, Figs. 3 and 4 suggest that supplying at least 50% of Et_c may be adequate to minimize pungency in cabbage, a proportion of Et_c lower than that (>80 %) suggested for maximizing yield (Fig. 2). This

supports the previous suggestion that head physical traits may be more responsive to irrigation than flavor characteristics (Radovich et al., 2004b, Chapter 4).

We conclude that supplying irrigation to achieve maximum cabbage yield will also optimize sensory quality by minimizing the compounds responsible for pungency and likely increase the perception of sweetness. We also suggest that irrigation may be withheld during head development to increase glucosinolate concentrations to maximize vegetable chemo-protection potential, provided that the value added exceeds that lost to yield reduction.

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Source	df	Weight (kg)	Mean diameter	Diameter ratio	Density	Dry matter (%)
Year (Y)	1	[z]***	***	***	**	***
Irrigation (I)	3	***	***	***	NS	***
Y x I	3	*	***	***	NS	***

[z]NS, *, **, *** = Not significant or significant at $P \leq 0.05$, 0.01 or 0.001, respectively.

Table 5.1. Analysis of variance for the influence of year and irrigation on head traits in 'Bravo' cabbage planted 10 June, 2002 and 17 June, 2003 at the Ohio Agricultural Research and Development Center in Wooster, Ohio.

Year	Irrigation treatment	Weight (kg)	Mean diameter (cm)	Diameter ratio	Density ($g \cdot cm^3$)	Dry matter (%)
2002	[Z]NS	1.61 ± 0.10	15.5 ± 0.2	1.00 ± 0.02	0.81 ± 0.04	8.6 ± 0.2
	FS	1.34 ± 0.06	14.7 ± 0.2	1.06 ± 0.02	0.80 ± 0.01	8.6 ± 0.2
	HS	0.71 ± 0.05	11.6 ± 0.3	1.13 ± 0.02	0.82 ± 0.02	11.9 ± 0.4
	FHS	0.51 ± 0.03	10.7 ± 0.3	1.23 ± 0.02	0.76 ± 0.02	11.3 ± 0.2
2003	NS	3.04 ± 0.14	19.8 ± 0.3	0.86 ± 0.01	0.74 ± 0.02	6.6 ± 0.1
	FS	3.08 ± 0.16	19.7 ± 0.4	0.87 ± 0.02	0.76 ± 0.03	6.3 ± 0.2
	HS	2.78 ± 0.15	18.9 ± 0.3	0.90 ± 0.01	0.78 ± 0.01	5.7 ± 0.3
	FHS	2.51 ± 0.17	18.6 ± 0.3	0.89 ± 0.01	0.72 ± 0.04	6.1 ± 0.3

[Z]NS = irrigation provided throughout plant development, HS = irrigation provided during frame development only, FS = irrigation provided during head development only, FHS = no irrigation provided from establishment to harvest.

Table 5.2. Head characteristics in 'Bravo' cabbage planted 10 June, 2002 and 17 June, 2003 at the Ohio Agricultural Research and Development Center in Wooster, Ohio. Values are means of five replications. Means are ± standard errors.

Source	df	Fructose	Glucose	Sucrose	Total GS[Y]	Glucoiberin	Progoitrin	Sinigrin	Glucobrassicin
Year (Y)	1	***	***	***	***	***	***	NS	***
Irrigation (I)	3	*	**	***	***	***	***	***	***
Y x I	3	*	*	***	***	NS	***	**	**

[z]NS, *, **, *** = Not significant or significant at $P \leq 0.05$, 0.01 or 0.001, respectively.

[y]Total glucosinolate.

Table 5.3. Analysis of variance for the influence of year and irrigation on sugars and glucosinolates in 'Bravo' cabbage planted 10 June, 2002 and 17 June, 2003 at the Ohio Agricultural Research and Development Center in Wooster, Ohio.

Year	Irrigation treatment	Fructose	Glucose	Sucrose	Total	[z]Ratio
		----------Mg ·g $^{-1}$ dwt----------				
2002	[y]NS	128.4 ± 3.1	181.7 ± 3.9	71.3 ± 7.0	381.5 ± 8.7	4.9 ± 0.4
	FS	131.4 ± 4.5	184.5 ± 5.6	60.7 ± 3.7	376.3 ± 11.2	5.4 ± 0.4
	HS	98.9 ± 5.2	143.9 ± 7.4	115.9 ± 11.1	358.7 ± 13.9	2.3 ± 0.2
	FHS	99.5 ± 3.8	140.9 ± 5.2	90.3 ± 5.6	330.8 ± 11.1	2.8 ± 0.3
2003	NS	242.6 ± 8.9	340.7 ± 13.0	61.9 ± 4.4	645.3 ± 25.3	9.8 ± 0.4
	FS	227.5 ± 6.1	323.0 ± 8.2	53.9 ± 2.7	604.6 ± 14.7	10.5 ± 0.5
	HS	229.4 ± 10.0	319.7 ± 14.0	57.7 ± 6.0	606.7 ± 28.2	10.3 ± 0.6
	FHS	241.3 ± 8.6	334.4 ± 12.5	67.8 ± 4.9	643.5 ± 20.6	9.0 ± 0.6

[z]Ratio of glucose + fructose : sucrose.

[y]NS = irrigation provided throughout plant development, HS = irrigation provided during frame development only, FS = irrigation provided during head development only, FHS = no irrigation provided from establishment to harvest.

Table 5.4. Fructose, glucose and sucrose concentrations in head tissue of 'Bravo' cabbage planted 10 June, 2002 and 17 June, 2003 at the Ohio Agricultural Research and Development Center in Wooster, Ohio. Values are means of three sub-samples from each of five replications. Means are ± standard errors.

Year	Irrigation treatment	Total GS	Glucoiberin	Progoitrin	Sinigrin	Glucobrassicin
		----------mmol·kg^{-1} dwt----------				
2002	[z]NS	24.8 ± 3.2	9.8 ± 0.4	1.8 ± 0.2	6.4 ± 0.4	3.0 ± 0.2
	FS	20.4 ± 0.8	9.0 ± 0.3	1.6 ± 0.1	5.3 ± 0.4	3.6 ± 0.4
	HS	33.7 ± 3.1	12.5 ± 0.5	3.6 ± 0.3	9.3 ± 0.4	8.0 ± 1.6
	FHS	38.2 ± 2.9	12.6 ± 0.6	3.7 ± 0.3	8.4 ± 0.4	8.7 ± 0.9
2003	NS	21.1 ± 0.5	8.5 ± 0.4	1.5 ± 0.1	7.1 ± 0.7	1.8 ± 0.2
	FS	21.0 ± 0.7	8.4 ± 0.4	1.5 ± 0.1	6.8 ± 0.3	2.0 ± 0.3
	HS	22.1 ± 1.0	10.0 ± 0.7	1.8 ± 0.2	7.9 ± 0.5	2.9 ± 0.4
	FHS	24.6 ± 1.2	10.0 ± 0.6	2.1 ± 0.2	8.8 ± 0.5	2.7± 0.4

[z]NS = irrigation provided throughout plant development, HS = irrigation provided during frame development only, FS = irrigation provided during head development only, FHS = no irrigation provided for the duration from establishment to harvest.

Table 5.5. Total and individual glucosinolate (GS) concentrations in head tissue of 'Bravo' cabbage planted 10 June, 2002 and 17 June, 2003 at the Ohio Agricultural Research and Development Center in Wooster, Ohio. Values are means of three sub-samples from each of five replications. Means are ± standard errors.

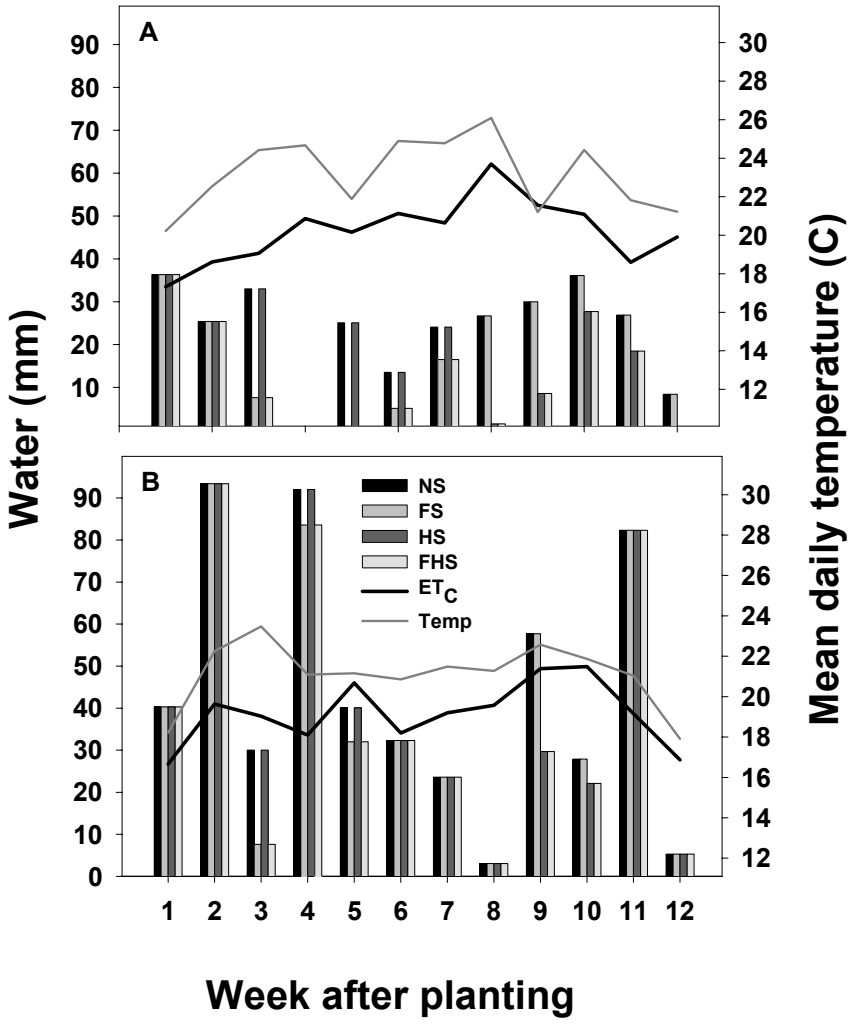

Figure 5.1. Total water received, crop evapotranspiration (ET_c) and mean daily temperature during the development of 'Bravo' cabbage planted 10 June, 2002 (A), and 17 June, 2003 (B) at the Ohio Agricultural Research and Development Center in Wooster, Ohio. FHS treatment bars indicate the amount of water received as rainfall during each period.

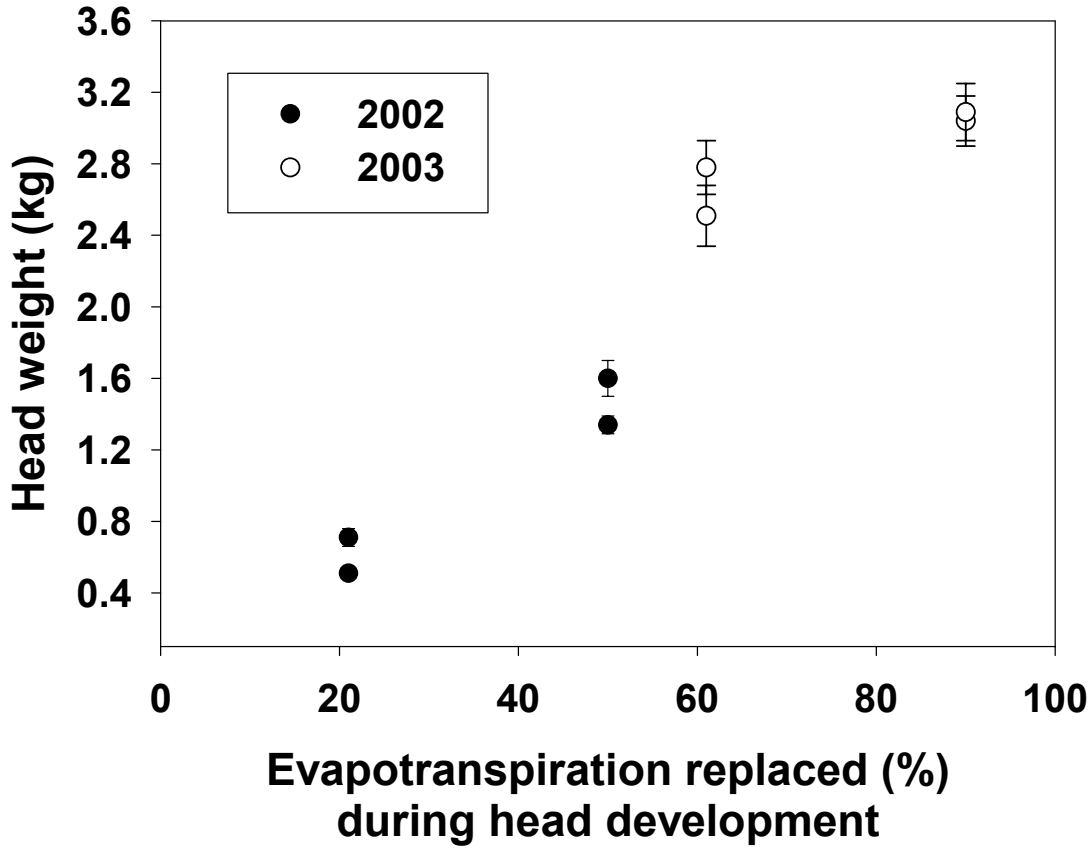

Figure 5.2. Mean head weight across treatments and years relative to the proportion of calculated crop evapotranspiration requirement received as rainfall and irrigation during head development in 'Bravo' cabbage planted 10 June, 2002 and 17 June, 2003 at the Ohio Agricultural Research and Development Center in Wooster, Ohio. Data points are yearly treatment means of 15 values. Error bars are standard errors.

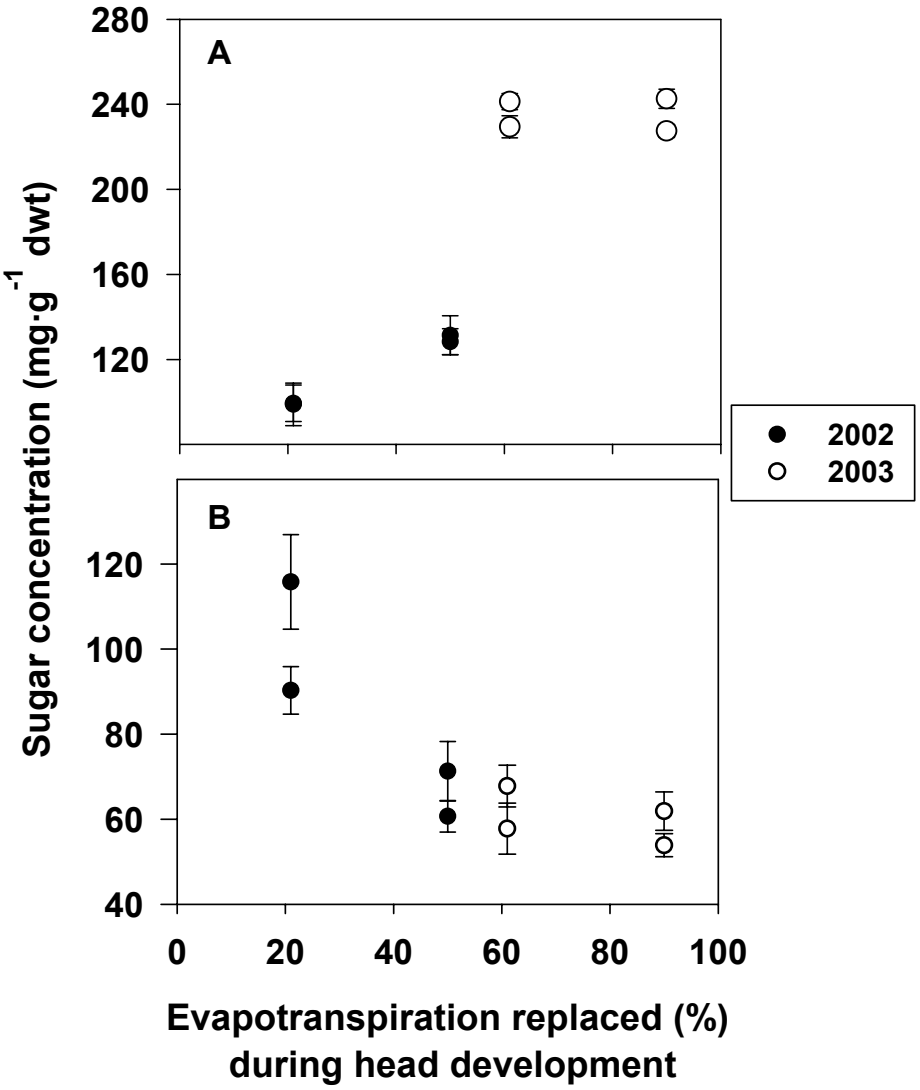

Figure 5.3. Mean head fructose (A) and sucrose (B) concentrations across treatments and years relative to the proportion of calculated crop evapotranspiration requirement received as rainfall and irrigation during head development in 'Bravo' cabbage planted 10 June, 2002 and 17 June, 2003 at the OARDC Ohio. Data points are treatment means of 15 values. Error bars are standard errors.

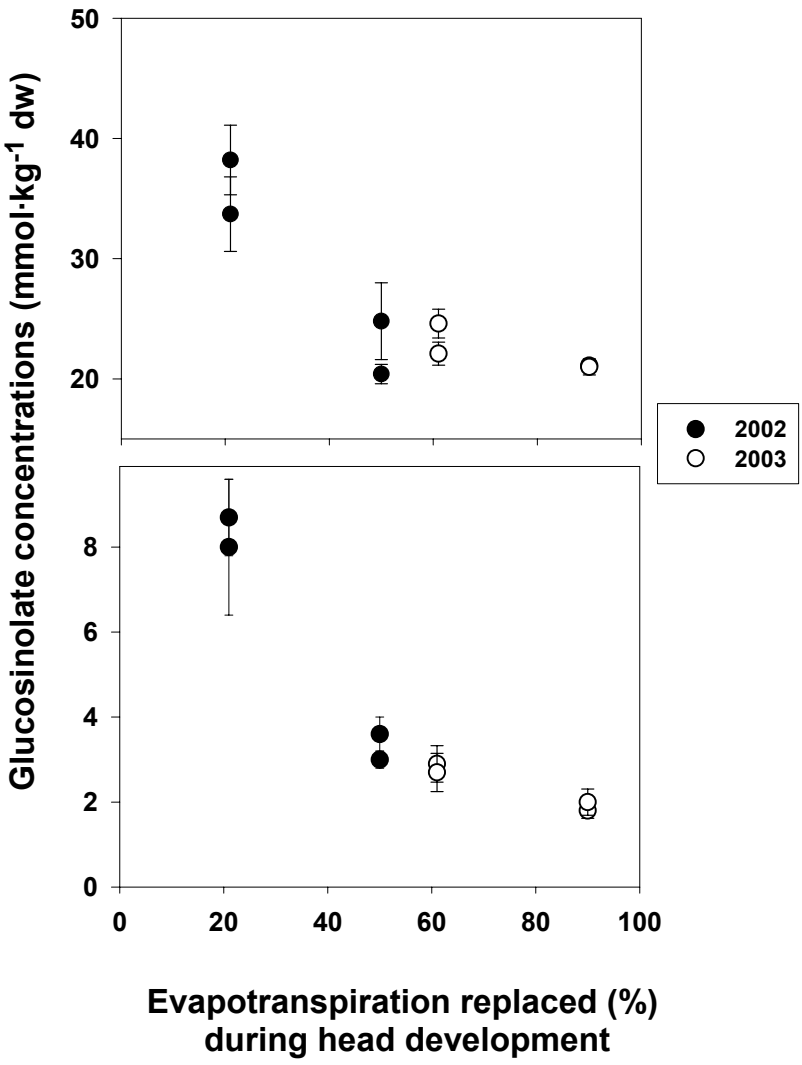

Figure 5.4. Mean head total glucosinolate (A) and glucobrassicin (B) concentrations across treatments and years relative to the proportion of calculated crop evapotranspiration requirement received as rainfall and irrigation during head development in 'Bravo' cabbage planted 10 June, 2002 and 17 June, 2003 at the OARDC, Wooster, Ohio. Data points are yearly treatment means of 15 values. Error bars are standard errors.

CHAPTER 6

IMPORTANT CABBAGE HEAD TRAITS AND THEIR RELATIONSHIPS AT FIVE POINTS IN DEVELOPMENT

T.J.K. Radovich, M.D. Kleinhenz and N.J. Honeck

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6.1 ABSTRACT

We set out to document events and relationships among key traits throughout cabbage head formation, particularly in early stages, in order to help develop and implement efficient strategies to increase crop yield and quality. Head traits used as indicators of horticultural maturity and crop quality were documented at five stages of development in 3 commercial fresh market/slaw and processing cabbage cultivars grown in 2001 and 2002 at The Ohio State University Ohio Agricultural and Development Center in Wooster, Ohio. Seedlings containing 2-4 true leaves were planted in June of both years. Trait measurement began 35 days prior to the estimated

market maturity date for each cultivar and continued weekly for five weeks. Harvest timing affected all head traits evaluated. Head weight, diameter, volume, and density and core volume generally increased with harvest date, while the ratio of head polar to equatorial diameter and the percent of head volume occupied by the core decreased. A strong curvilinear relationship between head mean diameter and head weight was found. Developmental changes in head density, in contrast to weight and size, were found to be largely independent of thermal time. Information gained in this study adds to our understanding of cabbage crop development. It also strongly suggests that accurate assessments of developmental stage during the scheduling of harvest are required to maximize head quality. The results also indicate that head growth and maturation should be viewed as separate and distinct concepts in discussions of head development.

6.2 INTRODUCTION

In head cabbage, the formation of true leaves is followed by a three-stage developmental sequence culminating in horticultural maturity: frame development, cupping and head initiation, and head development (Rubatzky and Yamaguchi, 1997). While head development is a continuum, major head traits and their relationships have been more thoroughly studied at stages associated with market readiness. Cabbage heads are considered to be horticulturally mature once they have reached a minimum size, weight and/or density. Density is a measure of solidity and the most frequently employed indicator of maturity for specific cultivar, environment and market combinations (Day, 1986; Isenberg et al., 1975; Reid, 1992; Swaider and Ware, 2002;

Wien and Wurr, 1997). Minimal head density values are cultivar- and market-specific, but generally exceed 0.70 $g \cdot cm^{-3}$ (Day, 1986, Isenberg et al., 1975; Kleinhenz and Wszelaki, 2003; Stofella and Fleming, 1990). Nevertheless, head development or enlargement may continue after horticultural maturity has been reached, a critical fact since environmental, labor and equipment, or market factors may postpone harvest (Kleinhenz, 2003). With head development ongoing, harvest timing is likely to affect head size, weight, shape, density and other traits important to crop yield and quality. Hara and Sonoda (1979) reported a sigmoidal increase in the dry weight of head leaves 60-120 days after planting. Isenberg et al. (1975) recorded increases in weight and density over a 20-30 day period beginning approximately 100 days after planting and found that the changes were cultivar dependent. Although not well documented, and studied principally at horticultural maturity, major head traits and their relationships are thought to change throughout development (de Moel and Evaraarts, 1990; Wszelaki and Kleinhenz, 2003). What remains unknown about events and relationships among key traits early in head formation restricts our fundamental understanding of cabbage crop development and lowers our ability to develop and implement efficient strategies to increase crop yield and quality. Therefore, our goal was to document key cabbage head traits, and relationships among them, beginning early in head formation.

6.3 MATERIALS AND METHODS

A factorial set of treatments (3 cultivars and 5 harvest dates) was established in a randomized complete block design with four replications at the Ohio Agricultural Research and Development Center in Wooster, Ohio (latitude 40° 47' N, longitude 81° 55' W). One processing ('Transam') and two fresh market ('Bravo' and 'Bronco') cultivars of commercial importance were started in the greenhouse. Hardened seedlings with 2 to 4 true leaves were planted to the field 28 June 2001 and 20 June 2002 in single-row plots established with a cone-type transplanter. Soil type in each year was a Wooster silt loam (Fine-loamy, Mixed, Mesic Typic Fragiudalf). No preplant fertilizer application was made in 2001. This decision was based on a lack of equipment availability in time for planting, a history of moderate to heavy fertilizer applications to the site, and soil tests that indicated no major nutrient deficiencies. In 2002, 56N-49P-93K $kg \cdot ha^{-1}$ was applied to the field and incorporated one month prior to planting, to more closely follow standard commercial practices. A soybean-winter wheat rotation immediately preceded cabbage in both years. In 2001, rows were 6 m long with 1.2 m between rows and 28 cm between transplants. In 2002, rows were 4.8 m long, with between and within row spacing the same as in 2001. Standard pest management strategies, based on scouting, thresholds and application of labeled pesticides, were employed. Rainfall and irrigation maintained adequate soil moisture.

Plots were harvested weekly beginning 35 days prior to and at horticultural maturity (H1, H2, H3, H4, and H5, respectively), with horticultural maturity (H5)

serving as the control. In 2001, H1 was set when heads reached 10 cm in diameter. In the same year, H5 corresponded strongly with published days to maturity (Kleinhenz and Wszelaki, 2003; Wszelaki and Kleinhenz, 2003) information for the cultivars used. Therefore, in 2002, H1 was set at 35 d prior to published days to maturity. At harvest, all heads were collected from the center 4.8 m (2001) or 3 m (2002) of each plot. Heads were trimmed (3-4 wrapper leaves removed) prior to further evaluation. Individual head weights were taken using an electronic scale (FV-60KWP, A and D Co., Ltd Tokyo, Japan or CW11-2EO, OHAUS, N.J.). Heads were split longitudinally and core height and base width, and head polar (radial) and equatorial (transverse) diameters measured. Head and core volumes were calculated as previously reported (Kleinhenz and Wszelaki, 2003) using the formula for sphere and cone volume, respectively. Growing degree-days (GDD) were calculated using upper and lower threshold temperatures (21 and 0° C, respectively) selected based on work detailing cabbage metabolism and growth response to temperature (Criddle et al., 1997; Hara and Sonoda, 1982). Two formulae were used to calculate GDD, which were then summed over the course of development (planting to harvest) for each cultivar and year. If the daily maximum temperature (T_{max}) fell below the upper threshold, then GDD = $(T_{min} + T_{max})/2) – B$, where T_{min} = daily minimum temperature and B = the base temperature (0° C). If T_{max} exceeded 21° C, then an intermediate cutoff method (University of California, 2003) was employed, where GDD = $[(T_{min} + 21)/2) – B] – [(T_{max} – 21) * 2]$. Using this cutoff method, GDD = 0 when $T_{max} \geq 30$ C. Temperatures ≥ 30 C are reported as stressful to cabbage (Criddle et al., 1997; Rubatzky and Yamaguchi, 1997). Data were analyzed

with the General Linear Model procedure of SAS for Windows v.8 (Statistical Analysis System, Cary, N.C.) and with the Regression Wizard of SigmaPlot 2000 for Windows v. 6.0 (SPSS Inc., Chicago, Il.).

6.4 RESULTS

Year (Y), cultivar (C), harvest date (HD) and their interactions affected all head traits evaluated (Table 6.1). Although significant, the HD x C and HD x Y interactions resulted from changes in magnitude, not direction. Differences between H1 and the H5 ranged from 100-800% for head weight, 20-100% for mean diameter, 7-30% for density, and 4-230% for core volume, with differences greater in 2002 than in 2001 (data not shown). Head weight, diameter and density generally increased with harvest date, although the rate of increase varied with cultivar and was greatest between H1 and H3 (Fig. 6.1). Percent dry matter of heads was recorded in 2002 only, and showed no apparent relationship to plant developmental stage (data not shown). In plotting treatment means for head weight x mean diameter, a linear relationship between the two was apparent, although the slope of the lines differed between relatively early (H1 and H2) and late (H3-H5) stages of development (data not shown). However, when plotted on an individual head basis, the head weight x mean diameter relationship was strong across all treatments ($R^2 = 0.96$, $P < 0.0001$), curvilinear and described by a power equation ($y = 0.0004 * x^{3.05}$), where y = head weight (kg) and x = mean head diameter (cm) (Fig. 6.2). Head polar and equatorial diameter increased with harvest date, with

greater gains in equatorial relative to polar diameter values between H1 and H3 (Figs. 6.3A, 6.3B). Decreases in head diameter ratios resulted in noticeable changes in head shape between H1 and H3 (Figs. 6.3C, 6.4). Core dimensions also changed, but more slowly than head diameter (Figs. 6.5A, 6.5B). This resulted in a decline in the percent of head volume occupied by the core during head development (Fig. 6.5C). Thermal time as calculated was more strongly related to cabbage head growth across treatments and years than thermal time calculated with formulae lacking either an upper threshold or cutoff procedure (data not shown). Accumulated growing degree-days explained year-to-year variability in head weight and mean diameter, but not density (Fig. 6.6).

6.5 DISCUSSION

6.5.1 Head weight, mean diameter and density

Increases in head weight, size and density were relatively rapid after initiation but slowed as heads reached horticultural maturity. These results are similar to those reported by de Moel and Everaarts (1990), Hara and Sonoda (1979) and Isenberg et al. (1975). Increases in density suggest that gains in head weight outpaced increases in head volume throughout much of head development, perhaps due to the expansion of internal head leaves (head fill). Although higher density may also result from increases in percent dry matter, Strandberg and White (1979) reported no increase in the percent dry matter of heads as they matured. Although previously proposed (North, 1957), the relationship between head diameter and weight has only recently been described in cabbage at horticultural maturity (Kleinhenz and Wszelaki, 2003; Wszelaki and

Kleinhenz, 2003). However, work described here is the first to document similar relationships across a wide range of developmental stage and head size.

6.5.2 Head polar and equatorial diameter and shape

Interestingly, head expansion was greatest in the equatorial direction, as indicated by a decrease in the ratio of polar:equatorial diameter during development. This phenomenon is not explained by a slowing of stem growth, since the most pronounced period of preferential equatorial expansion (H1 to H3) coincides with the period of most rapid stem elongation. Rather, it is likely that asymmetric head expansion resulted from rapid extension of older head leaves, attached at near right angles to the core, combined with thickening of the petiole of newer leaves arising more vertically from the stem. These concurrent events would result in head growth occurring primarily perpendicular to the core, and would explain preferential equatorial expansion of heads, which was evident as a change in head shape.

6.5.3 Core characteristics

Like other key traits, core height and base width changed most rapidly between H1 and H3. Although stem elongation slowed between H3 and H5 in this study, Hara and Sonoda (1979) reported only a slight decrease in the number of leaves produced late in head development. This suggests that internode length decreases markedly in later stages of head maturation and that the continuous production of leaves, though with

minimal expansion, contributes to head fill. Overall, changes in core traits were less pronounced than in other traits, particularly mean head diameter. This is also evident in the decreased percent of head volume occupied by the core.

6.5.4 Effect of thermal time

Variation in the influence of harvest date on head traits was greater between years than among cultivars, suggesting that, in this study, environment had a greater influence on treatment differences than did genetics. Environmental differences between years included higher air temperatures in 2002 compared to 2001 (data not shown), and a lack of fertilizer applications in 2001. Despite the absence of fertilizer applications in 2001, heads produced in that year met or exceeded characteristic size and weight values for the cultivars (Kleinhenz and Wszelaki, 2003, Wszelaki and Kleinhenz, 2003), and were larger and slightly denser than heads grown with fertilizer in 2002. Therefore, temperature appears to be the factor contributing most to the variation in growth observed between years. Because growth rate is influenced by temperature, plant development is frequently discussed relative to thermal time (Allen, 1976). Growing degree-day accumulation, adjusted for the negative effect of high temperatures on cabbage growth, explains much of the year-to-year variation in head weight and mean diameter (Fig. 6.6). However, head density, a primary indicator of horticultural maturity, appeared to be influenced less by thermal time. These observations may be significant since they suggest that air temperatures, while influencing the terminal size and weight of mature heads, have a minimal effect on the

rate of head maturation. While changes in density may be "genetically hardwired" (i.e., largely dependent on chronological time), photoperiod or other environmental factors may also be involved (Wurr et al., 1996). Based on the results of this and previous work (de Moel and Everaarts, 1990; Hara and Sonoda, 1979; Isenberg et al., 1975; Wurr et al., 1996) we propose that major aspects of cabbage head development follow a sigmoidal pattern as a function of time. However, although changes in head size and weight correlate strongly with thermal time, additional work is necessary to determine the most appropriate measure of time (e.g., chronological, photo-thermal) relative to changes in density as heads mature.

6.5.5 Practical implications

Beyond increasing our basic understanding of cabbage crop development, information gained in this study may help lead to improvements in the management of cabbage yield and quality. Cabbage may be harvested at various points during the period of development studied here in order to meet the head size requirements of specific markets (Day, 1986; Senior and Whitwell, 1989) or because of labor, equipment or climatic concerns (Kleinhenz, 2003). Head weight, size, shape, density and core dimensions, as well as relationships among them, are also critical indicators of quality in the development, evaluation and selection of cabbage germplasm (Kleinhenz and Wszelaki, 2003; Stofella and Fleming, 1990; Wszelaki and Kleinhenz, 2003). For example, the relationship between head size and weight reported here is noteworthy because it suggests an ability to predict head weight (and, therefore, crop yield) across a

wider range of head size and maturity than previously reported (Kleinhenz, 2003). Also, head shape was observed to flatten during development. Since optimal head shape for most markets is represented by a polar:equatorial diameter ratio of 1.0 (round), changes in head shape during development may affect the relative marketability of heads harvested at different maturities. They may also affect the efficacy of tools designed to assist in the prediction of yield, which assume a constant head shape (Kleinhenz, 2003). Finally, the relationship between head and core volume is a key indicator of crop quality, since the core is removed prior to fresh-market consumption or processing. As heads develop, a decrease in the percent head volume occupied by the core results in more usable product available to processors and consumers.

This study documents the status of a comprehensive list of cabbage head traits at five points in head development ending with horticultural maturity. Although fertilizer applications and plant spacing employed in this study deviate slightly from standard production practices, the lack of an environmental influence on the direction of the HD effect indicates that similar trends would be found in commercial settings. Head weight, volume, density and core size increased, while head polar:equatorial diameter ratio and head volume occupied by the core decreased as heads developed and expanded. The rate of change in all traits slowed with time, a trend more pronounced beginning approximately two weeks prior to expected horticultural maturity. Early in development, head expansion was greater in the equatorial than in the polar direction and this was attributed to the elongation of leaves in a direction perpendicular to the stem and the thickening of petioles in leaves arising more parallel to the stem. Head weight increased at a greater rate than head volume, perhaps due to thickening of older

leaves and a marked shortening of internodes in the production of new leaves. The economically important relationships between head size and weight, polar and equatorial diameter, and core and head volume were also affected by harvest date, and strongly suggest that accurate assessments of developmental stage are required to establish harvest schedules intended to maximize head quality. With head density apparently unrelated to thermal time, it may be appropriate to view head growth and maturation as separate and distinct concepts in discussions of head development. It also underscores the need to determine the genetic and/or environmental factors that influence head density.

6.6 ACKNOWLEDGMENTS

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Source	Weight	Head diameter: polar (P)	Head diameter: equatorial (E)	Head diameter: mean	P:E ratio	Volume	Density	Core height	Core width	Core volume	Volume core/head
Year (Y)	***	***	***	***	***	***	**	***	***	***	***
Cultivar (C)	*	***	***	***	***	***	***	***	***	***	***
Harvest date (HD)	***	***	***	***	***	***	***	***	***	***	***
Y x C	***	***	***	***	NS	***	**	*	***	*	***
Yx HD	***	***	***	***	***	***	NS	**	***	**	***
C x HD	***	***	***	***	***	***	NS	***	***	***	***

[z] NS, *, **, *** = Not significant or significant at $P \leq 0.05$, 0.01 or 0.001, respectively.

TABLE 6.1. Analysis of Variance for the influence of year, cultivar and harvest date on physical characteristics of heads from three cabbage cultivars planted in June of 2001 and 2002.

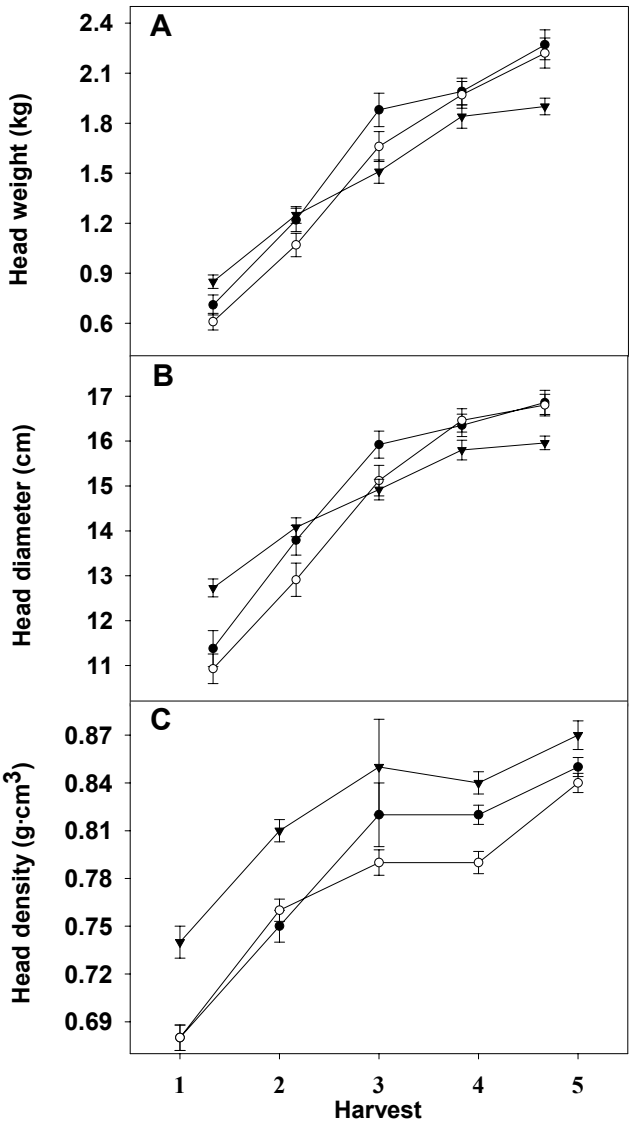

Figure 6.1. Mean head weight (A), head diameter (B) and head density (C) at 5 harvest dates on weekly intervals, beginning 35 d prior to, and ending with, horticultural maturity. 'Bravo' = ●, 'Bronco' = ○ and 'Transam' = ▲. Values are means of 8 to 16 heads from each of four replications across two years. Error bars are standard errors.

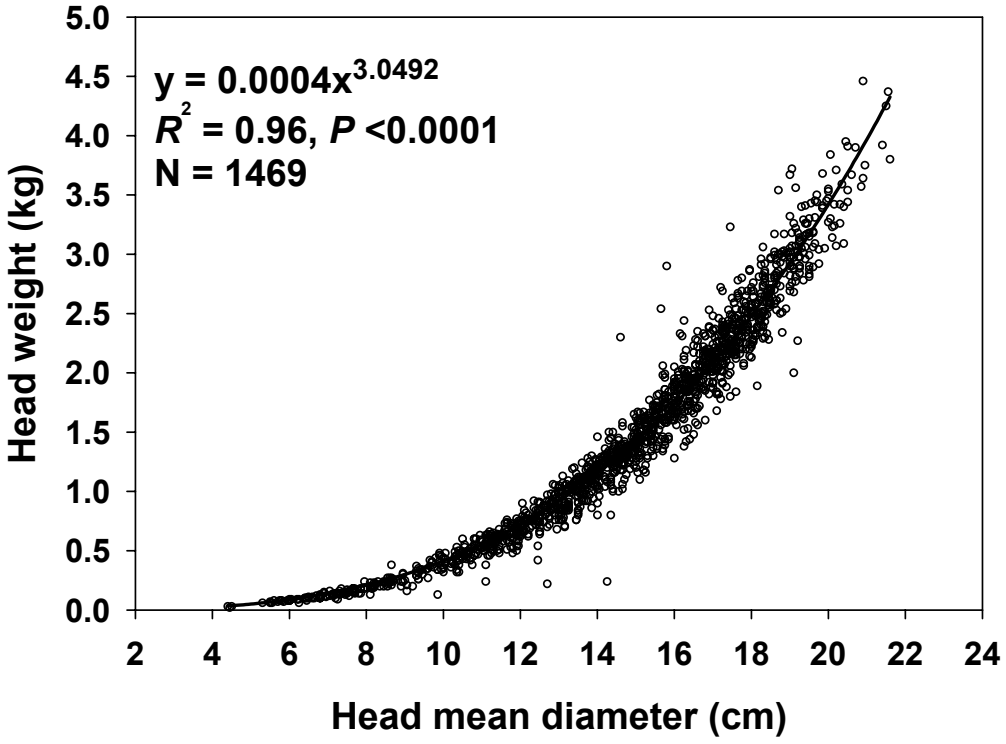

Figure 6.2. Relationship between mean head diameter and head weight of cabbage planted in June 2001 and 2002 at the Ohio Agricultural Research and Development Center. Relationship is across cultivars ('Bravo', 'Bronco' and 'Transam') and 5 harvest dates on weekly intervals, beginning 35 d prior to, and ending with, horticultural maturity. N = 1469.

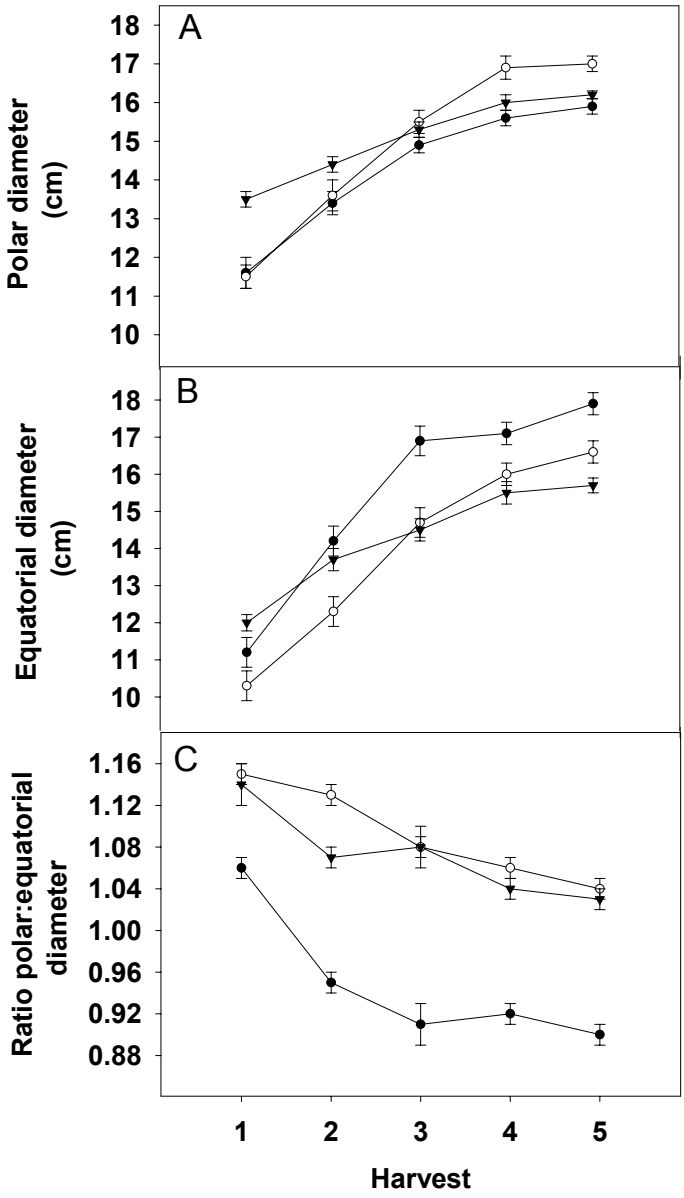

Figure 6.3. Mean polar (A) and equatorial (B) diameter, and ratio of polar:equatorial diameter (C) of cultivars at five harvest dates on weekly intervals, beginning 35 d prior to, and ending with, horticultural maturity. 'Bravo' = ●, 'Bronco' = ○ and 'Transam' = ▲. Values are means of 8 to 16 heads from each of four replications across two years. Error bars are standard errors.

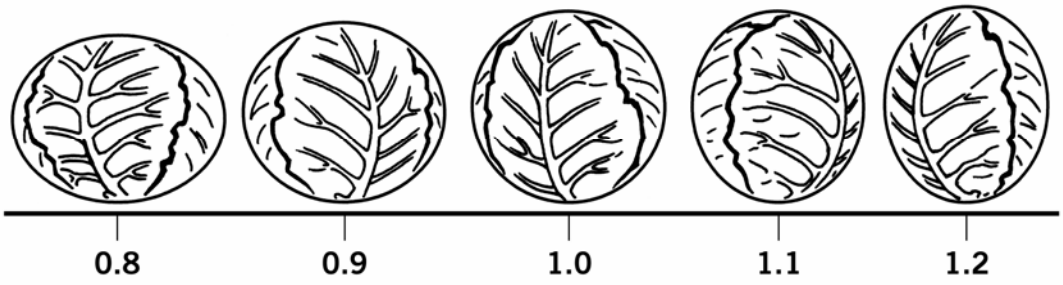

Figure 6.4. Idealized cabbage head shapes corresponding to polar:equatorial diameter ratios of 0.8, 0.9, 1.0, 1.1 and 1.2.

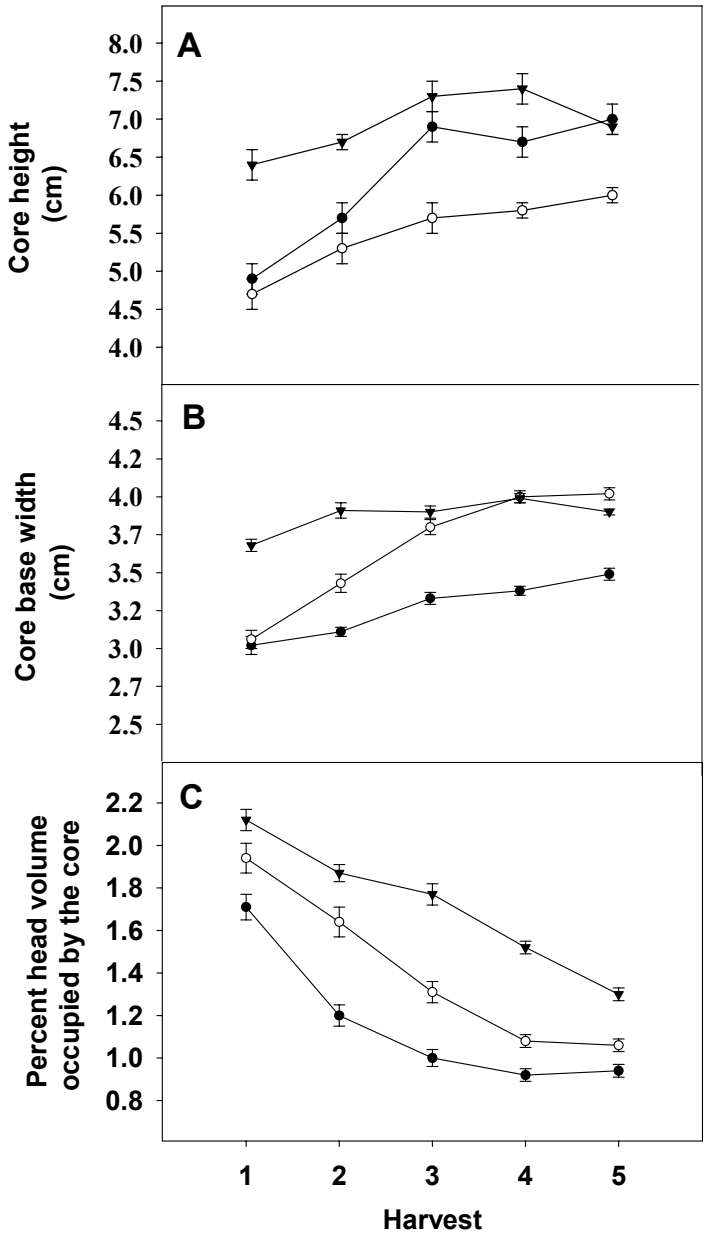

Figure 6.5. Mean core length (A), base width (B) and core volume (C) of cultivars at five harvest dates on weekly intervals, beginning 35 d prior to, and ending with, horticultural maturity. 'Bravo' = ●, 'Bronco' = ○ and 'Transam' = ▲. Values are means of 8 to 16 heads from each of four replications across two years. Error bars are standard errors.

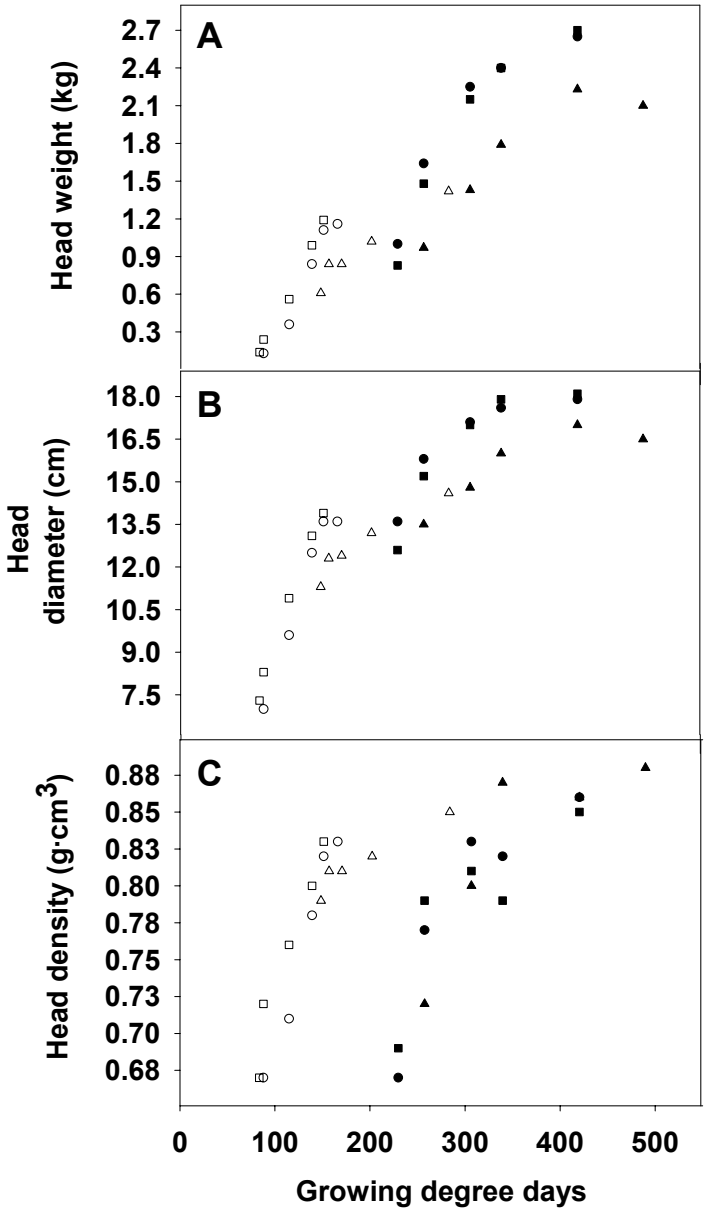

Figure 6.6. Relationship between growing degree-days (GDD) and head traits of cabbage planted in June 2001 and 2002 at the Ohio Agricultural Research and Development Center. Treatment means of 'Bravo', 'Bronco' and 'Transam' are represented by circles, squares, and triangles, respectively. Filled and open symbols represent 2001 and 2002, respectively.

CHAPTER 7

RAPID ESTIMATION OF CABBAGE HEAD VOLUME ACROSS A POPULATION VARYING IN HEAD SHAPE: A TEST OF TWO GEOMETRIC FORMULAE

T.J.K. Radovich and M.D. Kleinhenz

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7.1 ABSTRACT

Volume measurements are useful in crop quality management because they offer three-dimensional estimates of commodity size, which is often closely related to commodity weight and density. The objective of this study was to compare volume estimates calculated with the sphere and spherical ellipsoid volume formulae with direct measures of volume via water displacement across a population of cabbage (*Brassica oleracea* Capitata Group) heads varying widely in shape. A total of 157 heads with polar (P):equatorial (E) diameter ratios ranging between 0.5 (flat) to 2.1(tall) were harvested at horticultural maturity from plants grown in 2002 and 2003 at the Ohio Agricultural Research and Development Center (OARDC) in Wooster, Ohio. The sphere formula underestimated volume in heads with P:E ratios < 1 and overestimated

volume in heads with P:E ratios >1. Use of the spherical ellipsoid formula reduced the shape-dependency of volume estimates and was determined to be a valuable tool for the accurate, precise and rapid measurement of head volume.

7.2 INTRODUCTION

Vegetable crop quality depends on many traits, including chemical composition, color, size, shape, weight and density (Rubatzky and Yamaguchi, 1997). Measures of commodity volume are particularly valuable because they provide three-dimensional estimates of commodity size and allow for calculations of density (Beyer, 1985). Volume is also an important component of yield in horticultural crops (Kalloo and Bergh, 1993). Direct measures of volume via water displacement are time consuming and commercially impractical; therefore, formulae to calculate volume in a range of crops from measures in two dimensions are preferred (Currence et al., 1944; Jenni et al., 1996; Marcelis, 1992; Mutschler et al., 1986; Ngouajio et al., 2003). Generally, these formulae correct for irregularities in commodity shape. For example, a modified formula for spheroid volume may be used to estimate the volume of mature muskmelon (*Cucumis melo*) fruits (Currence et al., 1944). The formula includes a correction factor for shape (i.e., fruit length:width ratio) based on dozens of measurements on each of eighty individual muskmelon fruit. This formula has been employed to estimate the volume of immature muskmelon ovaries and bell pepper (*Capiscum annuum*) fruits, with varying success (Jenni et al., 1996; Ngouajio et al., 2003). Volume formulae not including a correction factor may also be used to estimate commodity volume. For

example, predicted and actual (water displacement) volumes of cucumber (*Cucumis sativus*) fruits may be well correlated when fruit volume is estimated from fruit length and average circumference values using the formula for the volume of a cylinder (Marcelis, 1992). In tomato (*Lycopersicon esculentum*), a strong relationship has been observed between actual fruit volume and volume predicted from fruit height and width using the formula for the volume of a spherical ellipsoid (Mutschler et al., 1986). In cucumber and tomato, geometrically derived volumes may also correlate well to individual fruit weight (Marcelis, 1992; Mutschler et al., 1986). Similar formulae may also be applied to cabbage (*Brassica oleracea* Capitata Group). For example, most commercially important cabbage cultivars produce spherical, or nearly spherical heads, and the formula for sphere volume can be employed to estimate head volume and predict crop yield from mean head diameters (Kleinhenz, 2003; Wszelaki and Kleinhenz, 2003). Volume is also used to calculate density, an important indicator of cabbage head maturity (Isenberg et al., 1975; Radovich et al., 2004a, Chapter 6). However, direct comparisons of head volume estimates from the sphere formula and water displacement have not been reported. Also, although spherical heads are preferred in most markets, much genetic diversity exists for head shape, and unusually shaped cabbage heads have potential niche market value (Kleinhenz and Wszelaki, 2003; Swaider and Ware, 2002). Environmental and developmental factors are also important, with the head shape of specific cultivars depending on planting date, plant population, soil moisture and developmental stage (Kleinhenz and Wszelaki, 2003; Radovich et al., 2004a, Chapter 6; Radovich et al., 2004b, Chapter 3; Stofella and Fleming, 1990;

Sundstrom and Story, 1984). Since the estimation of cabbage yield and maturity depends on head volume, a rapid and reliable method of volume measurement is integral to the management of cabbage quality. Therefore, the objective of this study was to compare estimates of head volume using the sphere and spherical ellipsoid formulae with measures taken using water displacement. Heads of four test groups comprising a large population containing a wide range in head shape were used for this purpose.

7.3 MATERIALS AND METHODS

7.3.1 Plant materials

A total of 157 mature cabbage heads were harvested from four test groups grown at the Ohio Agricultural Research and Development Center in Wooster, Ohio (latitude 40° 47' N, longitude 81° 55' W) in 2002 and 2003. For all test groups, soil type was a Wooster silt loam (fine-loamy, Mixed, Mesic Typic Fragiudalf), with 4.3% organic matter and pH = 6.6. Heads were selected to ensure a broad range in head shape within the population tested. The cultivars and management practices used to produce the source populations varied and are described below.

In 2002, 6-week-old seedlings of ‘Bravo’ (BRV02) were planted to the field 10 June using a single-row mechanical transplanter. Plant spacing was 0.3 m (1 ft) within single 18.3 m (60 ft) long rows with 1.2 m (4 ft) between treatment rows. Fertilizer was

applied to the field prior to transplanting at the rate of 560.4 kg ha^{-1} (500 lb/acre) of 10-20-20 fertilizer (10N-8.7P-16.6K). Irrigation was applied as previously reported (Radovich et al., 2004a) so that plants experienced moisture stress either during frame development (FS), during head development (HS), throughout development (FHS), or not at all (NS). Standard pest management strategies, based on scouting, thresholds and application of labeled pesticides, were employed (Kleinhenz and Wszelaki, 2003). At 86 days after planting (DAP), three heads from each of four replicates were harvested from each treatment, for a total of 48 heads. Heads were stored at 7.2 °C (45 °F) for 7 d prior to volume estimation.

In 2003, 6-week-old seedlings of 'Early Jersey Wakefield' (EJW) and 'Early Flat Dutch' (EFD) were planted to the field 17 June. Fertilizer was applied one month prior to planting at the rate of 448.3 kg ha^{-1} (400lb/acre) of 19-19-19 fertilizer (19N-8.3P-15.8K). Row length, plant spacing and pest management practices were as in BRV02. Irrigation was applied during times of rainfall deficit to mitigate stress throughout plant development. Sixty-one (EJW) or twenty-nine (EFD) heads with a shape characteristic for each cultivar (i.e., tall or flat) were harvested at horticultural maturity, which corresponded to 65 and 86 DAP for EJW and EFD, respectively. Heads were stored at 7.2 °C for up to 21 d prior to volume estimation.

In 2003, 6-week-old seedlings of 'Bravo' (BRV03) were grown as EJW and EFD with the exception of irrigation, which was applied as in BRV02. Nineteen heads were harvested at 86 DAP. Heads were stored at 7.2 °C for up to 21 d prior to volume estimation.

7.3.2 Volume estimation

Heads were prepared for measurement by removing all wrapper leaves not tightly associated with the head (~3-4) and weighed using a commercial field scale (A and D Weighing, Milpitas, Calif.). Mean head diameter (MD) was calculated from measures of head polar and equatorial diameters (PD and ED, respectively) taken with a 50 cm (19.7 inch) sapling caliper (Haglof Inc., Madison, Miss.). The volume of each head was then estimated using both the sphere and spherical ellipsoid formulae [**4/3*pi*(1/2 MD)^3** and **4/3*pi*(1/2 PD)*(1/2 ED)^2**, respectively](Beyer, 1985). The sphere and spherical ellipsoid formulae may be simplified to $MD^3/6$ and $(PD*ED^2)/6$, respectively (Ngouajio et al., 2003). The spherical ellipsoid formula has both a prolate form giving greater weight to the major axis $(PD^2*ED)/6$), and an oblate form in which the minor axis is emphasized $(PD*ED^2)/6$)(Beyer, 1985). Variation in ED is thought to contribute more than variation in PD to variability in head volume (Radovich et al., 2004b), and ED explained more variation ($r^2 = 0.82$) in head displacement volume than PD ($r^2 = 0.62$) in these data. Therefore, the oblate form was used here. Displacement volume of each head was recorded concurrently using the procedure reported by Isenberg et al. (1975), with some modifications. A 18.9 L (5 gal) plastic bucket was modified by drilling a 3 cm (1.2 inch) diameter hole approximately 5 cm (2.0 inch) from the rim, and fitting a PVC elbow joint through the hole to form a downward-facing spout to allow the collection of water displaced into a 6 L (1.6 gal) plastic tub. To

initiate measures of head volume, water was added to the bucket until it began to run from the spout, with excess water allowed to drain from the bucket to the tub, from which it was emptied. Thereafter, a sharpened metal rod (18 x 0.5 cm; 7.1 x 0.20 inch) was inserted approximately 5 cm (2.0 inch) into the core and the head was placed into a thin plastic produce bag (Crown Poly Inc., Los Angeles, Calif.) to prevent the intrusion of water into the submersed head. One end of a 1 m (3.3 ft) length of 8 x 0.6 mm (0.3 x 0.02 in) rubber tubing (Saint-Gobain Performance Plastics, Akron, Ohio) was attached to the metal rod and placed in contact with the head inside the bag. A vacuum generated with an aspirator at the other end of the tubing secured the bag tightly around the head. The head was then slowly submersed until displaced water stopped running from the spout. Displaced water volume was determined with a 1 L (0.3 gal) graduated cylinder, scored in 10 mL (0.34 fl oz) increments. Analysis of variance and means separation were conducted with SAS for Windows v.8 (Statistical Analysis System, Cary, N. C.) and with the Regression Wizard of SigmaPlot 2000 for Windows v. 6.0 (SPSS Inc., Chicago, Ill.).

7.4 RESULTS AND DISCUSSION

Mean polar:equatorial (P:E) diameter values varied widely among heads from the different test groups (Table 7.1, Figure 7.1). The relatively wide range observed within and across BRV02 and BRV03 was attributed to environmental factors, such as irrigation regime and annual variation in precipitation, air temperature and relative humidity (Radovich et al. 2004b, data not shown). However, this variation represents

the range of values commonly found among commercial cabbage cultivars (Kleinhenz and Wszelaki, 2003; Sundstrom and Story, 1984). In contrast, head shapes observed in EFD and EJW fell outside the range of current commercial types and represent the extreme range of head shape in conventional markets. Therefore, heads from the four test groups collectively comprised a population spanning a large range in head shape (Swaider and Ware, 2002). Across the population, values for volume by displacement were more strongly correlated with estimates of volume based on the spherical ellipsoid rather than the sphere formula. A closer fit of displacement-spherical ellipsoid formula data for EJW, in particular, may have been responsible for this overall effect (Table 7.1, Fig. 7.2).

Plotting the ratio of volume predicted by the sphere formula to displacement volume against head P:E values revealed that the sphere formula under- and over-estimated volume in heads with P:E ratios < 1 and >1, respectively (Table 1). Moreover, predicted:actual volume values for EFD and EJW were closer to 1 (i.e., most accurate) when the spherical ellipsoid formula was used (Table 7.1). It is also important to note that there was little difference in r^2 and no significant difference in accuracy between the two estimates for BRV02 or BRV03, suggesting that either formula may be used for heads with average P:E ratios of 0.8-1.2, which are common in commercial settings. However, the spherical ellipsoid formula may allow for the most accurate and precise estimates of cabbage head volume and, therefore, density across a wider range in head shape. As in previous studies, a proportional relationship between individual head volume and weight was also observed in this study, ($r^2 = 0.92$, $P \leq 0.001$). In

experimental and commercial fields, the average head volume of a sampled area has been successfully employed to predict the yield of that area (Kleinhenz, 2003). The potential application of head volume to predict crop yield reinforces the importance of accurate and precise measures of volume. With this in mind, we suspect that the measurement of diameter along a third axis and calculation of volume using the formula for a triaxial ellipsoid (Beyer, 1985) would further improve the accuracy of volume estimates of EJW and similarly shaped (i.e., tall-headed) cultivars. However, it is unclear whether improvements in the accuracy of volume estimates in these cases would offset the additional time required to measure diameter along a third axis.

Finally, formulae-based estimates of volume required less than one minute, compared to measures of volume by displacement, which required approximately five minutes. Therefore, we conclude that formulae-based estimates of cabbage head volume, especially those employing the spherical ellipsoid formula, are applicable in commercial and research settings when rapid, accurate and precise measurements of head size, weight, density and maturity are needed.

7.5 ACKNOWLEDGMENTS

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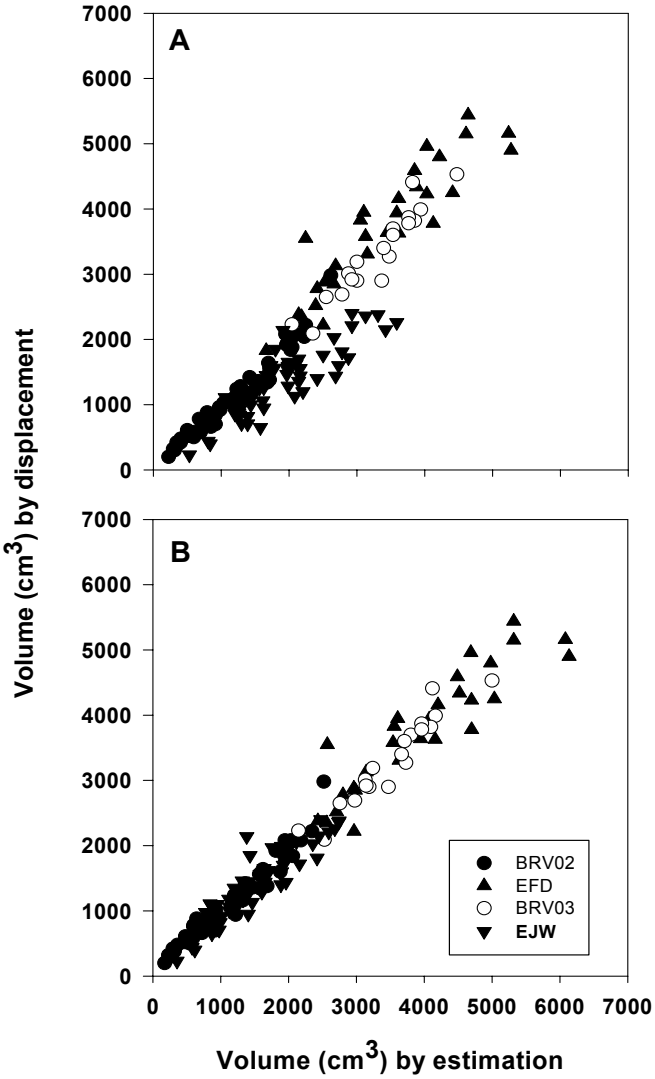
	Polar: equatorial diameter		[Z]Coefficient of determination		Predicted:actual volume	
Test group	Mean	Standard deviation	Sphere	Spherical ellipsoid	Sphere	Spherical ellipsoid
EJW	1.46	0.13	0.73	0.80	1.41 a[Y]	1.10 b
BRV02	1.15	0.22	0.99	0.95	1.06 bc	0.98 cd
BRV03	0.86	0.05	0.90	0.91	1.00 bcd	1.07 bc
EFD	0.65	0.04	0.84	0.84	0.91 d	1.05 bc

[Z] r^2 values of relationships between predicted volume using either the sphere or spherical ellipsoid formula. N= 61 (EJW), 48 (BRV02), 19 (BRV03) or 29 (EFD).

[Y] Predicted:actual volume values followed by the same letter are not significantly different from each other according to Duncan's Multiple Range Test ($P \leq 0.05$).

Table 7.1. Differences among test groups of 'Early Jersey Wakefield' (EJW), 'Bravo' 2002 (BRV02), 'Bravo' 2003 (BRV03), and 'Early Flat Dutch' (EFD) in head shape, and relationships between predicted and actual (displacement) head volume values.

Figure 7.1. Representative heads of cultivars Early Flat Dutch (left), Bravo (center) and Early Jersey Wakefield (right).



7.2. The relationship between cabbage head volume estimated using the formula for a sphere (A) or spherical ellipsoid (B) and measured by displacement.

Across test groups (N=157), $r^2 = 0.88$ (A) and 0.96 (B). 1 $cm^3 = 0.06$ $inch^3$.

CHAPTER 8

SUMMARY

8.1 INTRODUCTION

The overall objective of this project was to elucidate the influence of key abiotic environmental factors on indicators of fresh cabbage quality and to assess the extent to which these influences may be affected by crop factors such as genotype and developmental stage. Quality is subjective and multi-dimensional. This work, conducted from 2001-2004 in Fremont and Wooster, OH, explored treatment effects on three important groups of cabbage quality indicators: glucosinolate concentrations, sensory attributes, and yield components. The major findings of the project were:

1) Glucosinolate concentrations were higher in May- relative to June-planted cabbage, and this difference was associated with higher air temperatures during head development.
2) Withholding irrigation during head development increased head concentrations of all glucosinolates in mature cabbage heads.
3) The magnitude of response to irrigation was greater in tryptophan- than in methionine-derived glucosinolates.
4) Leaf tissue glucosinolate concentrations were negatively correlated with the

amount of estimated crop evapotranspiration (ET_c) replaced during head development (cabbage) and the percent moisture in the growing media (radish).

5) A positive relationship between glucosinolate concentrations and myrosinase activity was observed in leaves of radish.

6) Flavor was more important than texture, aroma or color in determining the acceptability of fresh cabbage, and panelists could detect differences in flavor among genotypes.

7) Judges detected flavor differences between cabbage irrigated from establishment to harvest and cabbage that received no irrigation during head development. Panelists failed to distinguish between treatments receiving irrigation during head development.

8) Accumulated heat units (HU) explained much of the variability in head size and weight, but not density, across multiple maturities, cultivars and years.

9) A strong relationship between cabbage head size and weight was observed across multiple levels of plant water status, air temperature, developmental stage and genotype.

8.2 GLUCOSINOLATES

Effects of cultivar and planting date on glucosinolate concentrations in Ohio were suggested by the literature (Chapter 1) and differences in sample flavor in previous studies (Chapter 2), although direct evidence was lacking. In order to

determine the independent and interactive effects of planting date (PD) and cultivar (C) on total glucosinolate concentrations in cabbage, six commercial fresh market cabbage cultivars were planted in May and June of 2001 and 2002 at the Ohio Agricultural Research and Development Center (OARDC) Vegetable Crops Research Branch in Fremont, Ohio. Total glucosinolate concentrations in horticulturally mature heads were determined using a glucose evolution procedure. Planting date effects on glucosinolate concentrations in mature cabbage heads were confirmed in six commercial cabbage cultivars (Table 3.1). Higher glucosinolate concentrations in May- relative to June-planted cabbage were associated with higher air temperatures during head development, a trend noted in all years and cultivars (Figs. 3.1 and 3.2, Table 3.1). A greater incidence of supra-optimal air temperatures during head development in May-planted cabbage may have contributed to it having higher glucosinolate concentrations relative to June-planted cabbage.

In the Midwest, differences in cabbage planting date (e.g., May vs. June) typically expose crops to different temperature profiles during development. Plant responses to temperature may result from associated changes in plant water status and applying or withholding irrigation relative to head development is hypothesized to be an effective strategy to manage glucosinolate concentrations in heads. To test this hypothesis, the fresh-market cabbage 'Bravo' was irrigated at different stages of head development in a field study conducted at the Ohio Agricultural Research and Development Center in 2002 and 2003. Irrigation was provided either: 1) from planting to maturity, 2) during frame development only, or 3) during head development only.

Control plants received no irrigation after plant establishment. Withholding irrigation during head development increased head concentrations of all glucosinolates detected (Table 5.5). The differential response of individual glucosinolates to irrigation was largely one of magnitude, suggesting that providing optimum environmental conditions for the crop during head development may reduce the concentrations of all glucosinolates in cabbage. Preconditioning young plants to stress may further minimize concentrations of the methionine-derived glucosinolates in mature heads (Section 5.5.3). However, the level or duration of stress required for a pre-conditioning or whether the irrigation effects on glucosinolate levels observed in 2002 are biologically relevant is unknown. The absence of a similar effect in the tryptophan-derived glucosinolate glucobrassicin suggests that the response of the two groups to stress differs. The mechanism behind the observed differences in response are unknown, but may be related to differences in biosynthetic pathways and/or metabolic function between the two classes of glucosinolates.

Although treatment effects were similar in both years, the method of irrigation employed was inadequate to replace 100% of estimated ET_c (Fig. 5.1). Differences among treatments in the proportion of ET_c applied during head development appear to explain much of the main effect of year and its interaction with treatment on glucosinolate levels (Fig. 5.4). Certainly, the stability of the relationship in Fig. 5.4 requires testing over multiple years by applying, in a single year, the levels of ET_c spanning the range reported here (20-100% ET_c replaced during head development).

Results from the radish study conducted at 40% RH demonstrated that a

negative relationship between soil moisture and glucosinolate concentrations in leaves (Fig. B.6) and a positive relationship between glucosinolate concentrations and myrosinase activity (Fig. B.7). The relationship between glucosinolate concentrations and soil moisture indicates that, as with Et_c, irrigation scheduling based on measures of soil moisture may be used to manage levels of glucosinolate in crop leaves. Correlations between myrosinase activity and glucosinolate concentrations suggest that increases in glucosinolate levels in response to stress may be biologically relevant (e.g., increase pungency), since the products of glucosinolate hydrolysis are more bioactive than glucosinolates themselves. We also suspect that changes in leaf glucosinolates are opposite those in other organs (Figs. B.3 and B.8). So, it is unlikely that management practices designed to optimize glucosinolate levels in leafy vegetables like cabbage would be appropriate for crops consisting of different tissues, such as radish.

8.3 SENSORY QUALITY

Glucosinolates play an important role in determining cabbage flavor, but there is little information available on the contribution of flavor to the overall sensory quality of cabbage or the degree to which genotype affects flavor (Chapter 1). To address this gap in the literature, twenty-one untrained but experienced panelists were asked to evaluate samples of 26 cultivars planted in May and June at the OARDC Vegetable Crops Research Branch in Fremont, Ohio in 2001 (Chapter 2). Panelists scored the overall desirability of samples and their acceptability based on flavor, aroma, texture, and color.

Linear scales were also used to quantitatively describe flavor and texture components (hot, sweet, bitter, crisp) relative to a known reference (cv. Bravo) which was also included as a sample. Flavor was more important than texture, aroma or color in determining the acceptability of fresh cabbage (Table 2.1), and panelists could detect differences in flavor among genotypes (Fig. 2.1 and 2.2), indicating that cultivar selection is an important step in managing quality. Weak but significant relationships were observed between flavor acceptability and hot (positive), sweet (positive), and bitter (negative) scores (Table 2.1) demonstrating that the industry preference for low pungency stems from a need for product consistency, rather than consumer preference. In fact, 'Cheers' had highest desirability, pungency and glucosinolate values in the evaluation of May-planted cabbage (Figs. 2.1, 2.2 and 3.1).

In addition to genotype selection, irrigation may also be employed to manage flavor. A replicated triangle test was employed to determine if judges could distinguish, by tasting, between shredded samples of fresh cabbage drip-irrigated during different periods of plant development at the Ohio Agricultural Research and Development Center in 2002. Judges detected differences between cabbage irrigated from establishment to harvest and cabbage from plots that received no irrigation during head development. However, panelists failed to distinguish between treatments receiving irrigation during head development. Evidence developed in this study, then, strongly suggests that flavor was more responsive to environmental conditions (i.e. soil moisture) during head development than to conditions during frame development (Table 4.1).

8.4 YIELD COMPONENTS

Yield and its components determine the market availability of a commodity, which can influence its price, and, subsequently, perceptions of its quality. Therefore, yield should be considered when investigating environmental effects on crop quality. Head weight, the most important component of yield, was closely related to both the proportion of ET_c replaced during head development (Fig 5.2) and accumulated heat units (HU) (Fig 6.6). The relationship between yield and ET_c was more linear than that between ET_c and either flavor or glucosinolate and sugar concentrations, suggesting that once a minimum threshold ET_c (e.g., 60%) is met during head development, increases in the proportion of ET_c replaced will have a greater effect on yield than other quality factors (Figs. 5.2-4). HU explain much of the variability in head size and weight, but not density, across multiple maturities, cultivars and years (Fig 6.6). A strong relationship between cabbage head size and weight was observed across multiple levels of plant water status, air temperature, developmental stage and genotype (Sections 5.5.1 and 7.4, Fig. 6.2), indicating that yield prediction tools based on this relationship may be applied across a wide range of conditions.

APPENDIX A

LABORATORY PROTOCOLS

A.1 GLUCOSINOLATE ANALYSIS OF CABBAGE

A.1.1 Sample preparation

A 1.5 cm thick slice was cut longitudinally from the center of a head and the core removed. Half of the slice was immediately frozen in liquid nitrogen. Frozen samples were placed in cloth bags, broken into small pieces by hitting the bag on the benchtop and stored at < -20 ºC. Breaking the sample into small pieces reduced the time required for lyophilization. Samples were lyophilized for approximately 5 d and finely ground with a coffee grinder. After lyophilization, samples were stored in air-tight containers to prevent re-hydration.

A.1.2 Glucosinolate extraction

A protocol after Rosa and Rodrigues (1997) was used for glucosinolate extraction. Added to labeled, capped test tubes were 200 mg of ground sample and 5.50 ml of 90% aqueous methanol. To test for glucosinolate recovery, 2.5 mg of sinigrin

(Sigma, St. Louis, MO) (500 µl of 5 mg·ml^{-1} in 90% aqueous methanol) and 5.0 ml of 90% aqueous methanol were added to tubes with 200 mg of duplicate sample. A duplicate sample was run for approximately every 10 unknown samples. Tubes were vortexed and heated for 10 min at 70 ºC in a circulating water bath. Tubes were removed from the bath, vortexed and allowed to cool. Samples were then vacuum filtered using a 5.5 cm Buchner funnel and 250 ml Erlenmeyer flasks, labeled with the sample identification. With the vacuum maintained to hold the filter paper (no. 1, 5.5 cm, VMR, West Chester, PA) in place, the sample residue was removed with a steel spatula and returned to the test tube. Next, 5 ml of 70% aqueous methanol was added to the tube, which was returned to the bath and reheated at 70 ºC for 5 min. The filtering process was repeated using the original paper, residue returned to the tube with 5 ml of 70% methanol added, and the sample reheated for a final time at 70 ºC for 5 min. When filtering the final time, the sample test tube and sides of the buchner funnel were washed with approximately 5 ml of 70% methanol to remove sample residue. Sample extracts contained in Erlenmeyer flasks were poured into labeled, 250 ml rotary evaporator flasks. Erlenmeyer flasks were rinsed with approximately 5 ml of 70% methanol and the rinsate added to the evaporator flask. Samples were evaporated at 40 ºC until dry (~30 min). Dry samples were reconstituted by adding 5 ml of 70% methanol to the flask and agitating with a Pasteur pipet. The solution was poured into a 50 ml centrifuge tube. Flasks were then rinsed with an additional 5 ml of 70% methanol, and the rinsate added to the centrifuge tube. Flasks were allowed to sit for 5 min. Thereafter, the remaining solution at the bottom of the flask was collected with a

Pasteur pipet and added to the centrifuge tube. Balance tubes were prepared with water and samples were centrifuged at 27,000 g*n* for 10 min at 10 ºC**.** The supernatant was carefully poured into capped labeled vials and stored at < 0 ºC prior to analysis.

A.1.3 Determination of total glucosinolates

A protocol after Heaney and Fenwick (1981) was used for the determination of total glucosinolate concentrations. DEAE sephadex A-25 dry resin (approximately 125 mg per sample) was suspended in an excess of 0.5 M pyridine acetate buffer and vacuum filtered to remove excess buffer (i.e., not to complete dryness) using a Buchner funnel and no. 1 filter paper (5.5 cm, VMR, West Chester, PA). The process was completed twice consecutively. Resin was then suspended in 0.02 M pyridine acetate so that the settled volume of hydrated resin equaled half of the total suspension volume. Resin was allowed to hydrate for at least 3 h before use and degassed before being poured into glass columns. Pyridine acetic acid buffer (0.5 M) was prepared by mixing 930 ml triple deionized water (TDI) with 30 ml acetic acid and 40 ml pyridine (Fisher Scientific, Fair Lawn, N.J.). Diluting 8 ml of this buffer to 200 ml with TDI gives a final concentration of 0.02 M. Columns were prepared by inserting a small plug of glass wool into a Pasteur pipet (15 cm). Pipets were placed in a rack with large test tubes below them to collect effluent. Columns were washed twice with TDI water by filling the columns and allowing to drain between washes. Resin was then mixed thoroughly and columns were half filled with water, followed immediately with 1 ml of the resin

suspension, for a final bed volume of approximately 0.5 ml. Columns were checked to ensure that air bubbles did not exist and that bed volumes were equal. Columns were washed twice with TDI water and allowed to drain (~3 min) between washes. Then, columns were half filled with TDI water, 1.0 ml of sample extract was added to the column, and the columns were allowed to drain (~3 min). Columns were washed with 300 µl of TDI water, allowed to drain (~3 min), then filled with water and allowed to drain (~3 min). Columns were then washed twice with 500 µl of 0.02 M pyridine buffer, draining (~ 1 min) between washes. Collection test tubes were placed under the columns and 0.8 U of myrosinase in 250 µl 0.02 M pyridine buffer was added. Columns incubated for 16 h at 25 ºC. In preliminary studies, sinigrin recovery was greater with 16 h incubation than with 8 or 20 h incubation. After incubation, columns were eluted with two, 500 µl volumes of TDI water. Total eluate volume was ~1.15 ml, 100 µl generally being lost to evaporation overnight. The eluate was analyzed for glucose concentration either immediately, or stored at -20 ºC for <24 hrs before analysis. To determine the concentration of glucose in the eluate, 200 µl was added to duplicate test tubes, to which 1 ml of aqueous hexokinase (HK) solution (product number G2020, Sigma, St. Louis, MO), prepared per manufacturer instructions, was then added. For a blank tube, 200 µl of water instead of eluate was used. To confirm the slope of the standard curve for each analysis, 50 µl of glucose solution (1 $mg \cdot ml^{-1}$) + 150 µl water + 1 ml HK solution was added to dulplicate test tubes. All tubes were vortexed and incubated for 30 min at 30ºC in a circulating water bath. Next, 1 ml of each reaction mixture was added to a cuvette, and A_{340} recorded. Glucosinolate content of 200 mg of

sample was calculated with the formula: **(sample A_{340} * slope * 2.3 * 5.8 * 10) / R,** where slope = micrograms of glucose per unit A_{340}, 2.3 = molecular weight ratio of sinigrin:glucose, 5.8 = the dilution factor of eluate aliquot (0.20 ml aliquot * 1.15 ml total volume), 10 = dilution factor of sample aliquot (1.0 ml aliquot * 10 ml total sample volume), and R = average recovery of internal standard, calculated by subtracting the amount of glucosinolate in samples from that of duplicate samples containing 2.5 mg of internal standard and dividing by 2.5.

A.2 HPLC ANALYSIS OF INDIVIDUAL GLUCOSINOLATES

A.2.1 Analysis of desulfo-glucosinolates

Cabbage extracts prepared as in A.1.1-A.1.2 were used for HPLC analysis. Benzyl glucosinolate (140 μg) was added to samples by drying 10 μl of solution (14 $mg \cdot ml^{-1}$) in small test tubes, adding 1 ml of sample extract and vortexing several times. To identify peaks and establish relative factors, 0.3 mmol solutions were made of authentic 2-propenyl (sinigrin, 125 $mg \cdot ml^{-1}$), 3-methylsulfinylpropyl (glucoiberin, 145 $\mu g \cdot ml^{-1}$), 2(R)-2-hydroxy-3-butenyl (progoitrin, 132 $\mu g \cdot ml^{-1}$) and benzyl glucosinolate (140 $\mu g \cdot ml^{-1}$). Authentic standards were obtained from KVL (Frederiksberg, Denmark).

Samples and standards (1 ml) were then applied to columns and washed as described in A.1.3. Instead of adding myrosinase, sulfatase (1.25 U in 250 μl of 0.02 M pyridine-acetate buffer) from *Helix pomatia* (Sigma Chemical Co., St. Louis, Mo.) was

applied to the column and allowed to incubate for 16 h at ambient temperature (25 °C). Desulfoglucosinolates were eluted with 1 ml of water and the eluate transferred to 2 ml vials. Samples were stored at < 0 °C for less than 24 h prior to analysis. Desulphoglucosinolates in 5 µl of sample extract were separated on a 250 x 2.1 mm Supelcosil LC-18 5 µm column (Supelco, Bellefonte, PA) using a Waters (Milford, Mass.) LC/MS system and a Waters 996 Photo Array Detector at a scanning wavelength range of 210 - 400 nm. The flow rate was 0.27 ml · sec^{-1}. Eluant A was distilled water. Eluant B was acetonitrile, HPLC grade, 20% v/v in water. The mobile phase was 99% A for 1 min, followed by a 20 min linear gradient to 99% B, then a linear gradient over 2 min to 99% A that was held for 10 min. Column temperature was maintained at 30 °C. A single desulfatation and analysis was conducted per sample. The four standard solutions were desulfated in triplicate and eluates were analyzed in triplicate. Standard error in peak area between columns and injections was 3.0 and 0.5% of the mean, respectively (N = 12).

A.2.2 Identification and quantification of desulfo-glucosinolates

Desulfo-glucoiberin, -progoitrin and -sinigrin were identified based on chromatogams of authentic standards. Despite the unavailability of an authentic standard, desulfo-glucobrassicin was confidently identified based on its position relative to the internal standard benzyl glucosinolate in published chromatograms (ISO, 1992; Spinks, 1984) and the quantity present; glucobrassicin is frequently reported as one of

the three most abundant glucosinolates in cabbage heads (Rosa et al., 1996; Van Etten et al., 1980). A representative chromatogram is presented in Fig.1. Our response factors relative to sinigrin for glucoiberin (1.64), progoitrin (1.69) and benzyl glucosinolate (1.25) were directly proportional ($r^2 = 0.99$) to those previously reported (ISO, 1992), and the linear relationship ($y = 0.31x + 0.57$) was used to calculate a response factor for glucobrassicin (0.65). Glucosinolates were then quantified based on the formula: $((\mathbf{A_g/A_s}) * (\boldsymbol{n/m}) * \mathbf{K_g})/ \mathbf{R}$, where: A_g = peak area of desulfoglucosinolate, A_s = mean peak area of desulfo-sinigrin standard, n = micromoles of desulfo-sinigrin in standard, m = tissue mass (g) in test portion, and K_g = response factor. Recovery of benzyl glucosinolate added to samples after extraction but prior to desulfating was 93 ± 1.2% (N = 60).

A.3 DETERMINATION OF MYROSINASE ACTIVITY IN PLANT TISSUE

A.3.1 Column preparation and storage

Sephadex G-25 (super fine grade, Sigma, St. Louis, MO.) was hydrated in an excess volume of eluting buffer (20 mM Morpholinepropanesulfonic acid (MOPS) + 1 mM Ethylenediaminetetraacetic acid (EDTA), pH 7.0) for at least three hours. Tubing was put on the bottom tip of plastic columns (12.0 cm x 1.5 cm, Bio Rad Laboratories, Hercules, CA), to which clamps were attached to adjust flow rate (note: tubing should be kept as short as possible, ca. 3 cm, to minimize dilution of eluate). Collection

beakers were placed below the columns. The swollen resin was degassed and poured in to give a final bed height of 6 cm. Flow rate was restricted to 0.5 ml·s^{-1} (one drop every 15 seconds) during pouring. Once poured, columns were stored at 5 °C with 2 ml of either distilled water or elution buffer on the gel surface. If columns were not being used regularly, they would be washed occasionally (every 7-14 d) with water.

A.3.2 Enzyme extraction

Extraction was conducted at 5 °C. Frozen (-80 °C) samples were allowed to thaw slightly, then partially cut up or broken into small pieces and ground in extraction buffer (200 mM MOPS + 10 mM EDTA, pH 7.0) using a mortar and pestle. Approximately 4 ml of buffer was used per gram of tissue. The exception was root tissue, with 1 g ground in 10 ml of buffer. Tissue was ground to a fine slurry in 75% of total buffer volume with ~ 1 gram of glass sand. The amount of extraction buffer used was recorded. The slurry was poured into a large centrifuge tube through a funnel lined with cheesecloth. The mortar and pestle were rinsed with the remaining buffer and the rinsate added to the centrifuge tube. Excess buffer was removed from the sample by squeezing the cheesecloth, with the eluate dispensed to the centrifuge tube and the dry cheesecloth discarded. A balance tube was filled with water to equal the weight of the sample tube. Tubes were centrifuged for 4 min at 17,400 g_n at 4 °C. Two ml of the centrifuged extract was mixed in a labeled test tube with 2 drops of concentrated aqueous blue dextran. Blue dextran absorbs light at the wavelength used (340 nm), so

care should be taken that the amount added is consistent across tubes and mixed well with the sample.

A.3.3 Gel filtration

The column was pre-conditioned with approximately 5 ml of eluting buffer and allowed to drain. A small amount of eluting buffer was added to the gel surface, to which was then added the 2 ml aliquot containing blue dextran. The aliquot was allowed to fully infiltrate the column and then washed in with ~1 ml of eluting buffer. Once washed in, an excess of eluting buffer was applied to the column to elute the blue band (protein). As it flows through the column, the protein-containing blue band moved faster than, and separated from, other pigments. This was easier to see in hypocotyl tissue with high quantities of anthocyanins, than in roots, which had little pigmentation. A small amount of eluate was allowed to come off the column before collecting 1.5 ml of eluate in 10 ml graduated cylinders. Preliminary work indicated that enzyme activity decreased more quickly in crude extracts (~10% in 3 h) than in gel filtered preparations (~10% in 24 h), so enzyme extraction and filtration were conducted without prolonged interruptions. The eluate was considered to be more stable than unfiltered extracts due to the removal of phenolics from the preparations during filtering.

A.3.4 Activity measurement

A protocol after Wilkinson et al. (1984) was used to measure myrosinase activity. An aliquot (300 µl) of the eluate was heated at 100 °C in a water bath for use in the blank cuvette. Two cuvettes (1 blank, 1 live) per sample were prepared. All constituents except the live enzyme were allowed to warm to room temperature (25 °C) before use. Cuvettes received 100 µl of ascorbate solution (1.98 $mg \cdot ml^{-1}$ in eluting buffer), 400 µl of hexokinase (HK) solution (product number G2020, Sigma, St. Louis, MO) and 200 µl of either boiled (blank) or live crude extract. Prepared cuvettes were placed next to the spectrophotometer prior to adding substrate (sinigrin). The kinetics/time function was set to run for 30 min, with A_{340} recorded every 30 s and a read time of 30 s. The reaction was started by adding 300 µl of sinigrin solution (10 $mg \cdot ml^{-1}$ eluting buffer) to each cuvette. Cuvettes were inverted vigorously several times and inserted into the spectrophometer, blanked and the kinetics/time function started. Total volume (TV) of the reaction mixture was 1 ml and final concentrations of the reation mixture constituents were: 1.0 mM ascorbate; 0.4 U HK; 0.4 mM ATP; 0.4 U glucose-6-phosphate dehydrogenase; 0.6 mM NAD; 7.2 mM sinigrin. The slope of a 3-5 minute linear portion of the reaction curve was used to obtain the ΔA_{340}, later used in calculating myrosinase activity in the sample. Myrosinase activity on a fresh weight and protein basis was calculated as follows: $(m\Delta A_{340} * TV)/ (\varepsilon * x)$, where TV = 1.0, ε = millimolar extinction coefficient for NADH at 340 nm (6.22), and x = the amount of tissue or protein represented in the reaction mixture.

A.3.5 Determination of total protein in samples

Protein concentration were determined using the DC protein assay (Bio-Rad, Hercules, CA). Standard curves were developed on each day of analysis using bovine serum albimum (BSA). To prepare standard curve tubes, 0, 20, 40, or 60 µl of BSA solution was added to single test tubes, followed by water to bring final volume of standard to 200 µl. For sample analysis, 200 µl of eluate was added to duplicate test tubes, followed by 125 µl of Reagent A (Catalog # 500-0113, Bio-Rad, Hercules, CA), and vortexing. Next, **to one tube at a time**, 1 ml of Reagent B (Catalog # 500-0114, Bio-Rad, Hercules, CA) was added to each tube and vortexed. It is important to mix each tube after adding Reagent B before moving to the next one for proper color development. Finally, all tubes were vortexed again and Abs 740 nm was read within 30 min. Protein concentrations in samples were determined using the slope of the standard curve line, 165 µg · A_{740}^{-1} in a total volume of 1.32 ml.

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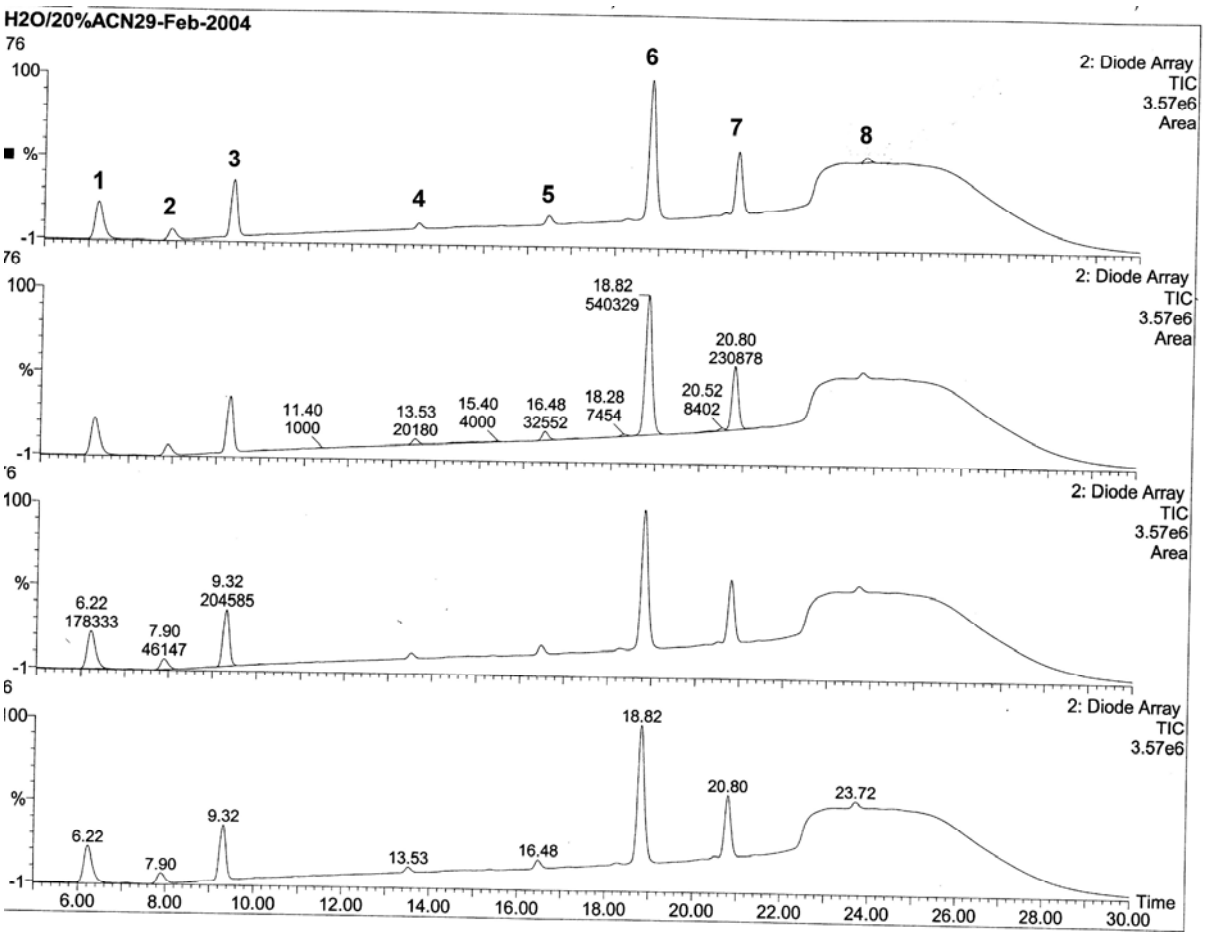

Figure A.1. HPLC chromatogram of a representative cabbage sample. Peak identification: 1. Glucoiberin, 2. Progoitrin, 3. Sinigrin, 4. Gluconapin (tentative), 5. Glucoiberverin (tentative) 6. Benzyl (internal standard), 7. Glucobrassicin, 8. 4-methoxy-3-indolylmethyl (tentative).

APPENDIX B

SOIL MOISTURE EFFECTS ON THE GLUCOSINOLATE – MYROSINASE COMPLEX IN A RADISH MODEL SYSTEM

T.J.K. Radovich, J.G. Streeter, P.P. Ling and M.D. Kleinhenz

B.1 ABSTRACT

Radish (*Raphanus sativus* 'Belle Glade') was grown in controlled environments at the Ohio Agricultural Research and Development Center to examine the influence of soil moisture on glucosinolate concentrations and myrosinase activity in root, hypocotyl and leaf tissue. Soil moisture treatments were: volumetric soil moisture maintained at 40-60% (50%), 20-30% (25%), and 10-20% (15%). The experiment was conducted twice under each of two relative humidity levels (15 and 40%) at 30 °C. Leaf stomatal conductance and plant size among soil moisture treatments followed the order 50%>25%>15%%, while canopy temperatures followed the order 15% > 25% > 50%. Soil moisture significantly influenced glucosinolate concentrations, especially at 40% relative humidity, where glucosinolate concentrations (fwt basis) in leaves and hypocotyls followed the order 15% > 25% > 50%. Although not significantly affected by treatment, myrosinase activity was positively correlated with glucosinolate concentrations in leaves and roots, while total protein was negatively correlated with

glucosinolate concentrations in leaves. On a dry weight basis, a trend for high glucosinolate concentrations in 15% was preserved in leaves, while the trend 50% > 25% > 15% in hypocotyl glucosinolate concentrations (dwt) was in contrast to that observed in hypocotyls on a fresh weight basis. The data suggest that increases in leaf glucosinolate concentrations under low moisture stress conditions may result from their translocation from roots to shoots or from their synthesis in leaves and either may coincide with a decrease in hypocotyl glucosinolate concentrations mediated by higher myrosinase activity. In this study, higher leaf myrosinase activity corresponded with increases in leaf glucosinolate levels, possibly to maximize the potential bioactivity of the substrate. In contrast to leaves, the trend for higher glucosinolate concentrations (fwt) in drought-stressed hypocotyls may be a passive response resulting from lower tissue moisture levels.

B.2 INTRODUCTION

Fluctuations in crop glucosinolate levels with changes in environmental conditions suggest that the flavor, pest resistance and potential health value of Brassicacious crops may be manipulated through production phase management. For example, the ability to employ irrigation in managing indicators of chemical, physical and sensory quality in commercial cabbage has been documented (Radovich et al., 2004, Chapters 4 and 5). Nevertheless, a greater understanding of the influence of root zone moisture on the glucosinolate myrosinase system is required to employ irrigation in managing a broader range of quality indicators related to the system.

It is also important to note that measures of glucosinolate levels are more common than measures of myrosinase activity. Even fewer studies include measures of both system components. Therefore, the literature is mostly silent on the relationship between glucosinolates and myrosinase activity under a range of environmental conditions, although the levels and biological activity of glucosinolates are tied to myrosinase activity (Rosa et al., 1997). Futhermore, irrigation is often scheduled based on soil moisture measurements made with instruments such as time domain reflectometry (TDR) sensors and tensiometers (Paschold et al., 1997). Nevertheless, studies correlating glucosinolate levels to soil moisture measured with these instruments are lacking (Bible et al., 1980; Bourchereau et al., 1996; Freeman and Mossadeghi, 1973). Finally, glucosinolates and myrosinase may move in the plant via the symplastic network and transpiration stream (Brudenell et al., 1999; Chen and Andreasson, 2001; Hoglund et al., 1991). Still, changes in the glucosinolate-myrosinase system among tissues of plants exposed to differential root zone moisture treatment are poorly characterized. Therefore, this study was designed to quantify differences in glucosinolate levels and myrosinase activity among radish plants grown under varying levels of root-zone moisture. A unique controlled environment chamber coupled with measures of glucosinolate and myrosinase activity in different tissues of the same plants was used for this purpose.

B.3 MATERIALS AND METHODS

B.3.1 Plant material and experimental design

'Belle Glade' radish was seeded to 288 cell seedling flats and germinated in the greenhouse at ~25 °C with a 12 h photoperiod. Flats were moved to acclimatize in the growth chamber 7-9 d after seeding. Temperature was maintained at a constant 30 °C with a 12 h photoperiod and a photon flux density approximating 560 $\mu mol \cdot m^{-2} \cdot s^{-1}$. After 3-5 d of acclimatization, seedlings of uniform size with one true leaf emerging were planted to standard 15 cm nursery pots filled with a rooting medium of 2:1 (v/v) of peat-based potting mix (Pro-Mix BX, Premier Horticulture Inc., Quakertown, PA) and sand into which fertilizer had been incorporated. The medium was prepared by screening 0.02 m^3 of mix to 1 cm and mixing with 0.01 m^3 of sand and 3.9 $kg \cdot m^{-3}$ of a 14.0N-6.1P-11.6K slow-release fertilizer (Osmacote, Scotts Co., Marysville, OH) in a cement mixer. Four seedlings were planted to each of 6 pots approximately 2.5 cm from the pot edge and 7.0 cm from each other. The six pots modified with internal TDR probes were placed on lysimeters attached to a circular rotating turntable as previously described (Kacira and Ling, 2001). Multiple measures of volumetric soil moisture content (VMC) and pot weight were averaged and recorded every 10 min. Each of the

three soil moisture treatments were randomly assigned to 2 pots each in a completely randomized design. The treatments were: VMC maintained at 40-60% (50%), 20-30% (25%), and 10-20% (15%). All pots were maintained at 40-60% VMC for 2 d, after which they were allowed to dry down to target VMC levels. After reaching target moisture levels (~2 d), treatments were maintained for 17 d by applying 110% of the amount of recorded evapotranspiration (ET) lost between watering periods. ET (ml) was calculated as the difference between pot weight (g) after the previous watering and pot weight just prior to the next watering. ET was replaced by hand 1-3 times daily: larger plants required more frequent watering to maintain soil moisture within the treatment range. The experiment was run twice at 40% RH and twice at 15% RH.

B.3.2 Harvest and sample analysis

Immediately before harvest (2-3 h into the photoperiod), stomatal conductance and temperature readings were taken on the most recently expanded leaf from three representative plants in each pot using a LI-1600 Porometer (LICOR Environmental, Lincoln, Nebraska). Thereafter, the same intact plants were collected and separated into leaves, hypocotyls and roots, which were removed and stored on ice for no more than 6 h prior to processing. Hypocotyl polar and equatorial diameters were recorded. Roots were cleaned of medium with pressurized water and wire mesh (1 mm). To ensure that similar tissue was analyszed in the glucosinolate and myrosinase assays, leaves were split with a razor through the mid-rib and petiole, and hypocotyls and roots were also

split longitudinally. Therefore, tissue was separated into two groups and weighed, myrosinase samples being placed into labeled vials and immediately stored at -80 °C and glucosinolate samples being placed into cloth bags and immediately lyophilized. Lyophyilized tissue was ground to pass through a 1 mm screen and 50-100 mg was placed into capped test tubes for extraction. The exception was root tissue, all of which was ground after recording the weight (20-50 mg), and placed into capped test tubes for extraction. Glucosinolate, myrosinase and protein analyses were conducted as previously reported (Chapter 3 and Appendix A).

B.3.3 Statistical analysis

Data were analyzed with the Mixed Model procedure of SAS for Windows v.8 (Statistical Analysis System, Cary, N.C.) and with the Regression Wizard of SigmaPlot 2000 for Windows v. 6.0 (SPSS Inc., Chicago, Il.).

B.4 RESULTS

VMC significantly affected the fresh weight, temperature, stomatal conductance and glucosinolate concentrations (fwt basis) in leaves (Table B.1). VMC also significantly affected hypocotyl fresh weight, percent moisture, mean diameter and protein concentration (Table B.2). VMC did not significantly influence any variable in roots (data not shown), or myrosinase activity, regardless of tissue (Tables B.1 and B.2).

Leaf stomatal conductance and fresh weight and hypocotyl fresh weight, percent moisture and mean diameter were highest and lowest in the 50 and 15% treatments, respectively, while canopy temperature and glucosinolate concentrations (fwt) followed the order 15% > 25% > 50%. Relative humidity (RH) had little influence on the variables studied, but the VMC x RH interaction was significant for leaf temperature, stomatal conductance and leaf glucosinolate concentrations (fwt), and for hypocotyl fresh weight. With respect to plant and tissue physical traits (e.g., weight and stomatal conductance), the VMC x RH interaction was one of magnitude, not direction (Table B.3). Conversely, the direction of treatment effects on leaf glucosinolate concentrations (fwt) varied with RH, as did trends in root glucosinolate concentrations (fwt) (Fig. B.1).

Leaf glucosinolate concentrations (fwt) increased with decreasing soil moisture at 40% RH; however, at 15% RH, leaf glucosinolate concentrations were greatest at 50% VMC (Fig. B.1). A trend for increased glucosinolate concentrations (fwt) with decreasing soil moisture at 15%, but not 40% RH was also noted in root tissue.

Although not statistically different, treatment means for glucosinolate concentrations (dwt), and myrosinase activity (fwt and dwt) are presented here for additional information. Also, relationships among tissue glucosinolate and myrosinase levels may provide insight into the mechanisms underlying the environmental control of glucosinolate levels observed in cabbage (see Discussion). Trends in leaf glucosinolate concentrations (dwt) were similar to treatment effects observed on a fresh weight basis, while trends in hypocotyls and roots were often different (Fig. B.2). Within RH levels, trends in leaf glucosinolate concentrations (dwt) were opposite those in roots (Figs. B.2

and B.3). Myrosinase activity on both a fresh and dry weight basis was greater at 15% than at 50% for leaf and hypocotyl tissue, regardless of RH (Figs. B.4 and B.5). Also, myrosinase activity (fwt) in roots tended to be higher at 25% at both levels of RH. This trend was also observed in activity on a dry weight basis at 15, but not 40% RH (Figs. B.4 and B.5).

B.5 DISCUSSION

The significant influence of soil moisture treatment on glucosinolate concentrations and myrosinase activities was most evident under conditions of 40% RH. Therefore, results from this component of the project will be emphasized.

VMC significantly affected leaf glucosinolate concentration (fwt). This suggests that modifications in soil water availability may be used to achieve target levels of glucosinolate concentrations in leafy crops, including cabbage and mustard (Fig. B.6). On a fresh weight basis, treatment mean myrosinase activity values paralleled mean glucosinolate concentrations in all tissues (Figs. B.1 and B.4), although the relationship was strongly evident in leaves (Fig. B.7). An increase in myrosinase activity proportional to glucosinolate concentrations may help make increases in the substrate biologically relevant. Glucosinolate concentrations reported here agree with those of Yen and Wei (1993), while myrosinase activity values (fwt) are within the range reported for seedlings and mature radish plants by Hara et al. (2000). Myrosinase activity values on a dry weight basis agree with the 13 $\mu mol \cdot min^{-1} \cdot g^{-1}$ dry weight

reported for radish by Wilkinson et al. (1984).

On a dry weight basis, trends in glucosinolate concentrations and myrosinase activity in leaves were opposite those in hypocotyls (Figs. B.2 and B.5). Charron and Sams (2004) reported an inverse relationship between leaf and root glucosinolate concentrations in rapid cycling *Brassica oleracea* grown under varying temperatures and suggested that glucosinolates may be translocated from storage to photosynthetic organs during periods of stress. An alternative hypothesis is that under conditions of low soil moisture, *in situ* synthesis of glucosinolates increases in leaves and coincides with a decrease in hypocotyl glucosinolate concentrations mediated by higher myrosinase activity in the hypocotyl tissue (Figure B.8). Higher leaf myrosinase activity corresponded with increases in glucosinolate levels, possibly maximizing the potential bioactivity of the substrate. In contrast to leaves, a trend for higher glucosinolate concentrations on a fresh weight basis in drought-stressed hypocotyls is likely a passive response resulting from lower tissue moisture levels.

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Source	df	Fresh weight	% Moisture	Temperature	Stomatal conductance	Glucosinolate concentration (fw)	Glucosinolate concentration (dw)	Myrosinase activity (fw)	Myrosinase activity (dw)	Protein
Relative humidity (RH)	1	0.58	0.26	0.92	0.52	0.21	0.22	**0.07**	0.12	0.42
Soil moisture (S)	2	**0.04**	0.36	**0.10**	**<0.0001**	**0.03**	0.26	0.16	0.23	0.32
RH x S	2	0.24	0.66	**0.04**	**0.05**	**0.004**	**0.02**	0.20	0.29	0.43

Table B.1. *P* values from an analysis of variance for the influence of soil moisture and relative humidity on leaf variables of radish grown in controlled environments in 2003 and 2004 at the Ohio Agricultural Research and Development Center in Wooster, Ohio. *P* values ≤ 0.10 are in bold type.

Source	df	Fresh weight	% Moisture	Mean diameter	Diameter ratio	Glucosinolate concentration (fw)	Glucosinolate concentration (dw)	Myrosinase activity (fw)	Myrosinase activity (dw)	Protein
Relative humidity (RH)	1	0.21	0.35	0.48	0.54	0.20	0.98	0.26	0.19	0.68
Soil moisture (S)	2	**0.0004**	**0.04**	**<0.0001**	0.35	0.38	0.53	0.26	0.52	**0.03**
RH x S	2	**0.03**	0.42	0.43	0.34	0.73	0.95	0.35	0.64	0.97

Table B.2. *P* values from an analysis of variance for the influence of soil moisture and relative humidity on hypocotyl variables of radish grown in controlled environments in 2003 and 2004 at the Ohio Agricultural Research and Development Center in Wooster, Ohio. *P* values ≤ 0.10 are in bold type.

		Leaf			Hypocotyl			
Relative Humidity	% VMC	Fresh weight (g)	Temperature (°C)	Stomatal conductance ($mol \cdot m^{2} \cdot s^{-1}$)	Fresh weight (g)	Moisture content (%)	Mean diameter (cm)	Total Protein ($\mu g \cdot g^{-1}$ fw)
40%	50	8.0 ± 0.8	23.2 ± 0.4	0.31 ± 0.03	6.9 ± 1.0	92.3 ± 0.3	2.7 ± 0.21	8.9 ± 1.2
	25	5.6 ± 0.6	24.0 ± 0.5	0.32 ± 0.06	6.8 ± 1.1	93.1 ± 0.6	2.5 ± 0.14	8.3 ± 1.3
	15	5.8 ± 1.6	26.8 ± 0.4	0.05 ± 0.01	4.9 ± 1.3	89.2 ± 1.5	1.9 ± 0.18	6.8 ± 1.7
15%	50	8.2 ± 0.5	22.8 ± 0.6	0.20 ± 0.02	16.0 ± 1.0	93.2 ± 0.2	3.0 ± 0.06	10.7 ± 0.6
	25	8.5 ± 0.6	23.2 ± 0.5	0.22 ± 0.03	15.9 ± 1.9	93.6 ± 0.4	2.9 ± 0.09	10.6 ± 1.0
	15	5.6 ± 0.4	23.9 ± 0.5	0.13 ± 0.02	9.2 ± 0.8	92.2 ± 0.2	2.4 ± 0.07	8.8 ± 0.5

Table B.3. Physical characteristics of leaves and hypocotyls of radish grown in controlled environments in 2003 and 2004 at the Ohio Agricultural Research and Development Center in Wooster, Ohio. Values are means ± standard errors.

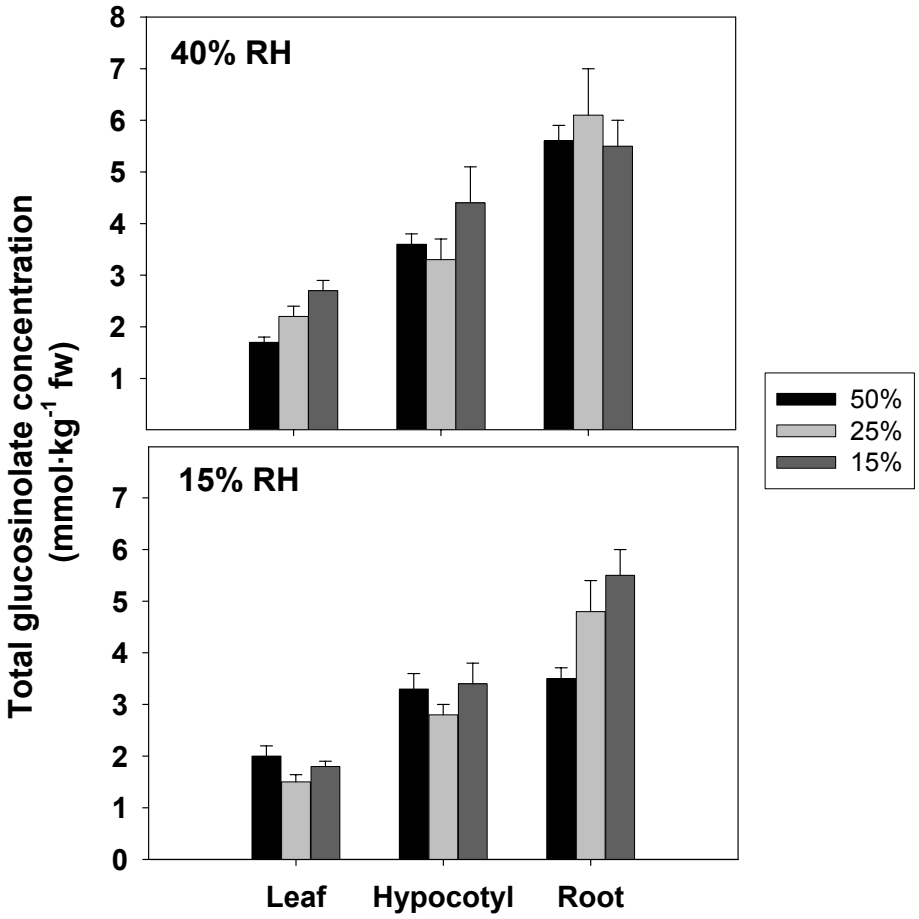

Figure B.1. Total glucosinolate concentrations (fwt) in radish grown in controlled environments in 2003 and 2004 at the Ohio Agricultural Research and Development Center in Wooster, Ohio. Values are treatment means across two runs of the experiment at each of 40 and 15% RH. Error bars are standard error values. Treatments 50, 25 and 15% correspond to volumetric soil moisture ranges of 40-60%, 20-30% and 10-20%, respectively.

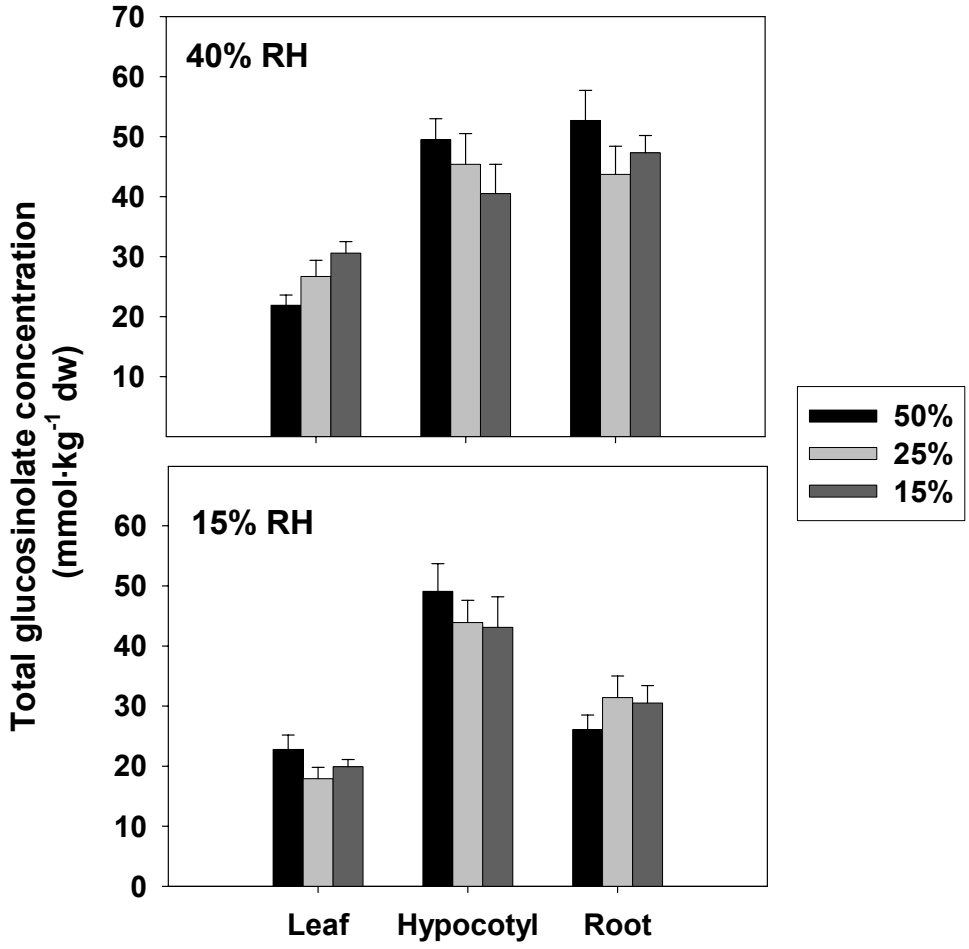

Figure B.2. Total glucosinolate concentrations (dwt) in radish grown in controlled environments in 2003 and 2004 at the Ohio Agricultural Research and Development Center in Wooster, Ohio. Values are treatment means across two runs of the experiment at each of 40 and 15% RH. Error bars are standard error values. Treatments 50, 25 and 15% correspond to volumetric soil moisture ranges of 40-60%, 20-30% and 10-20%, respectively.

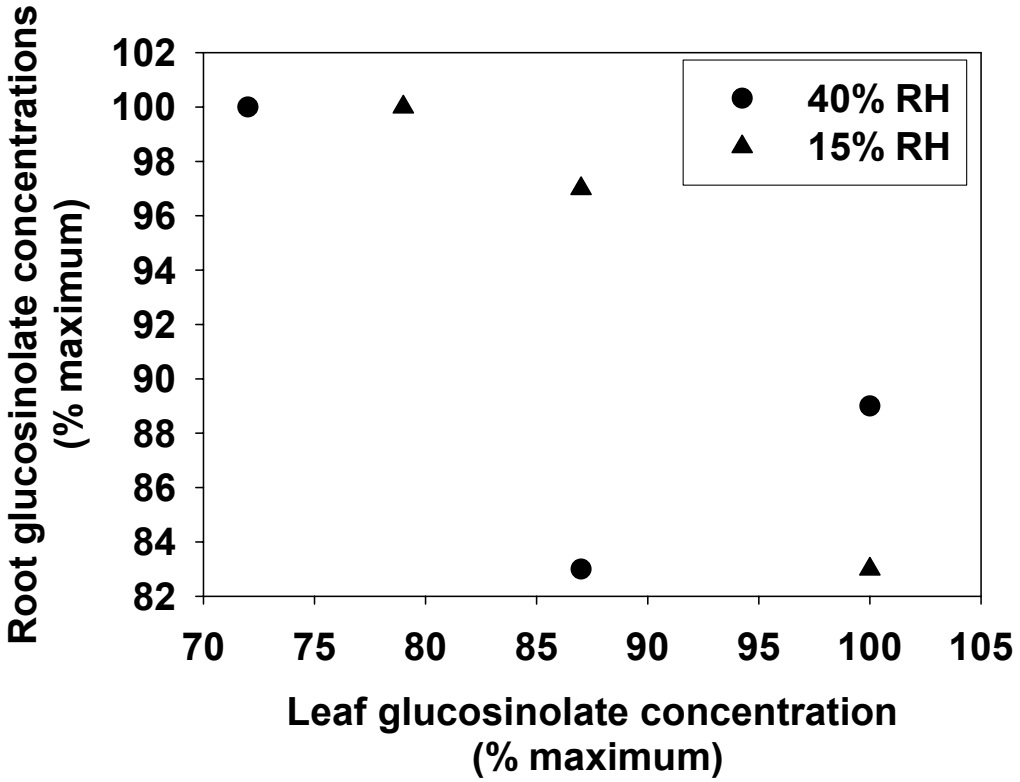

Figure B.3. Relationship between mean leaf and root glucosinolate concentrations of radish grown in controlled environments in 2003 and 2004 at the Ohio Agricultural Research and Development Center in Wooster, Ohio. Values are treatment means across two runs of the experiment at each of 40 and 15% RH.

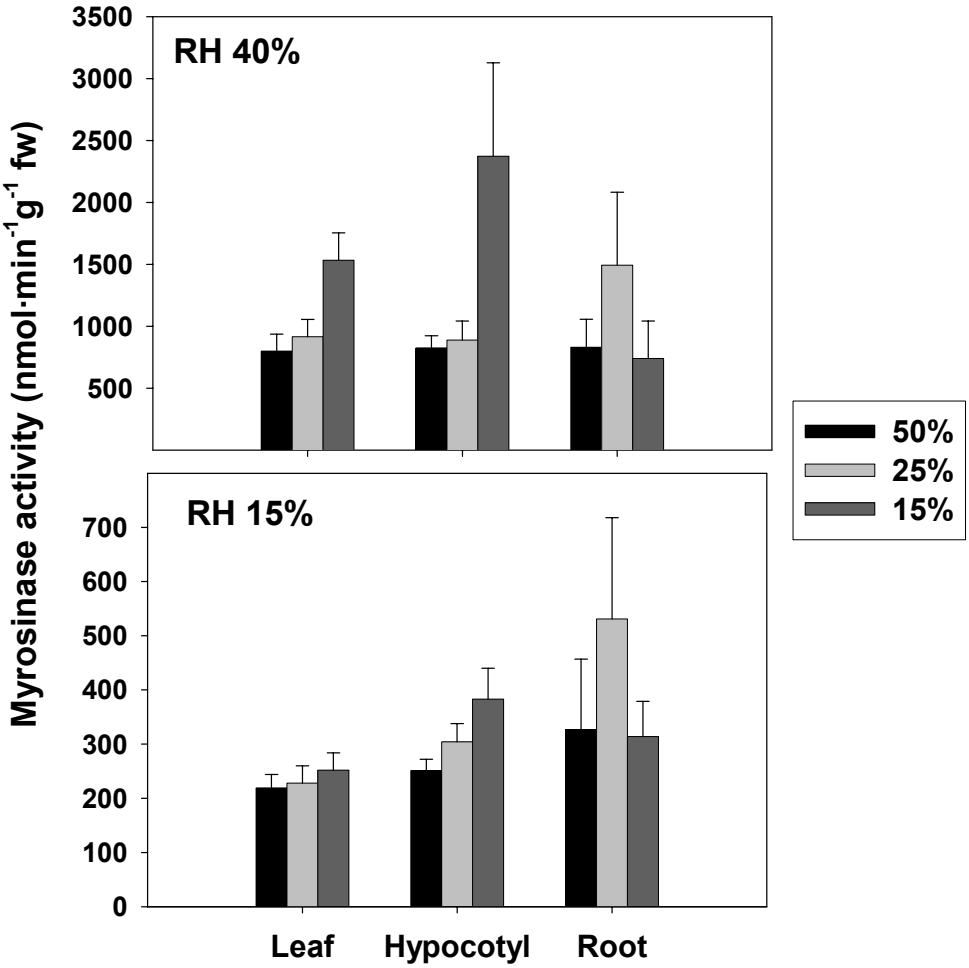

Figure B.4. Myrosinase activity (fwt) in radish grown in controlled environments in 2003 and 2004 at the Ohio Agricultural Research and Development Center in Wooster, Ohio. Values are treatment means across two runs of the experiment at each of 40 and 15% RH. Error bars are standard error values. Treatments 50, 25 and 15% correspond to volumetric soil moisture ranges of 40-60%, 20-30% and 10-20%, respectively.

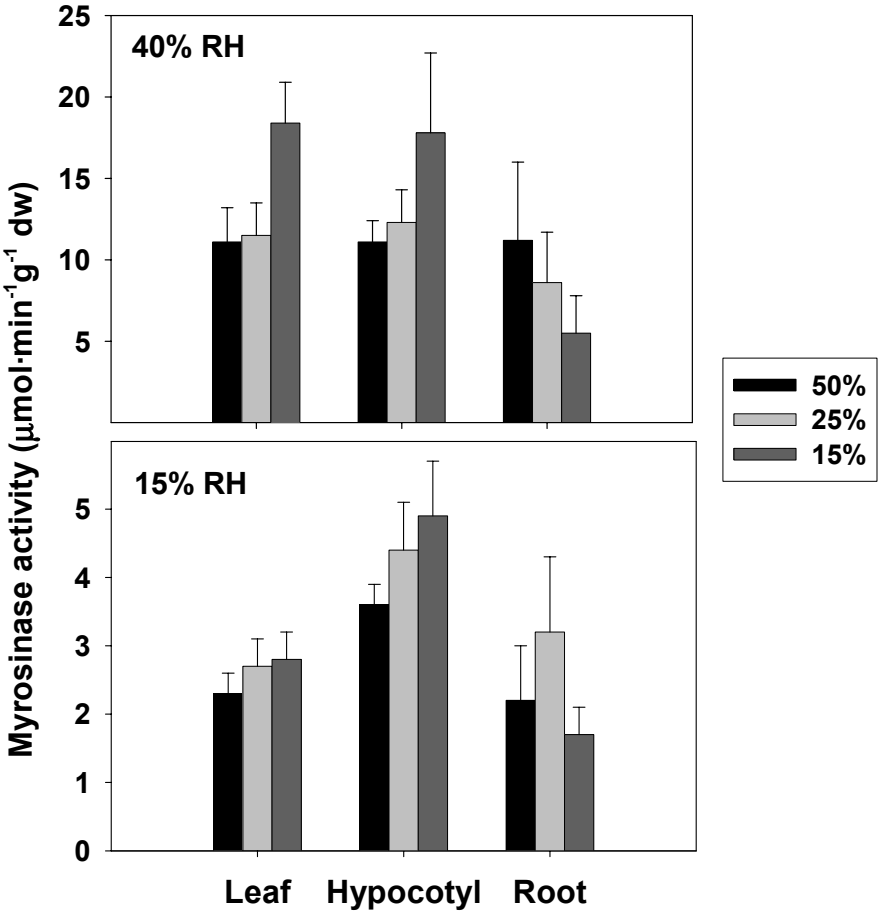

Figure B.5. Myrosinase activity (fwt) in radish grown in controlled environments in 2003 and 2004 at the Ohio Agricultural Research and Development Center in Wooster, Ohio. Values are treatment means across two runs of the experiment at each of 40 and 15% RH. Error bars are standard error values. Treatments 50, 25 and 15% correspond to volumetric soil moisture ranges of 40-60%, 20-30% and 10-20%, respectively.

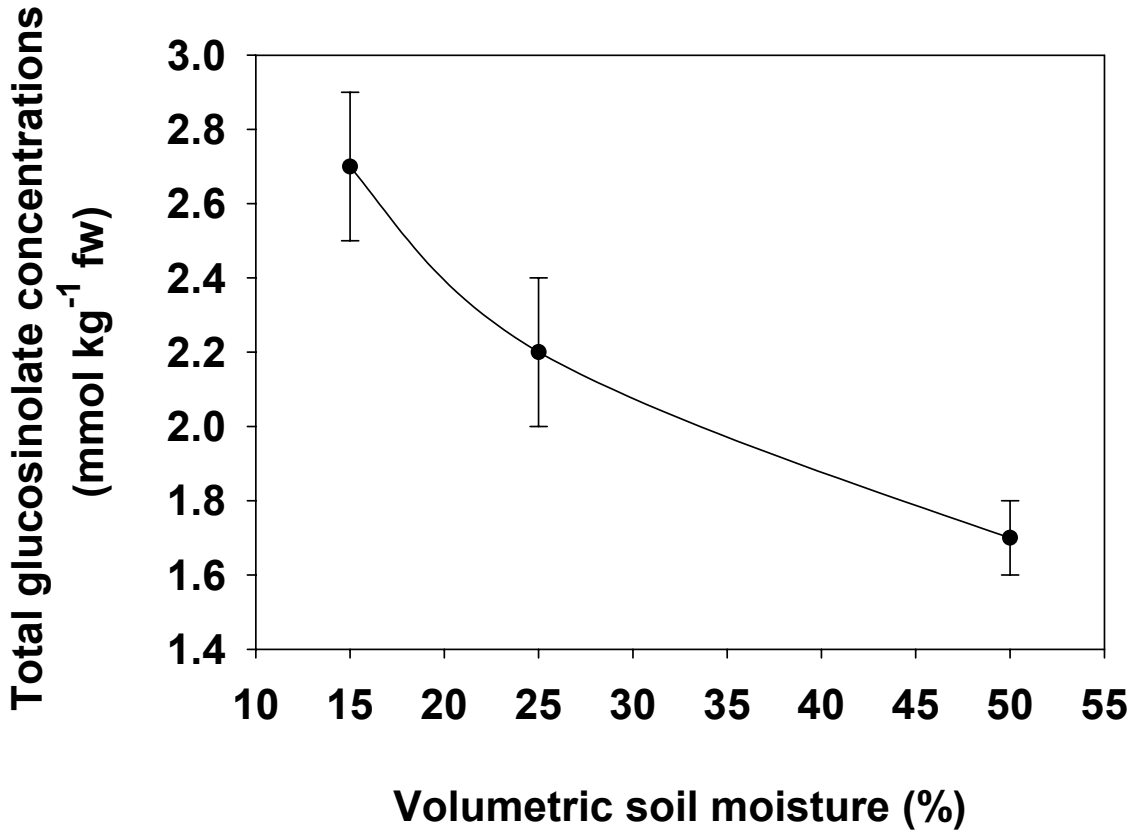

Figure B.6. Relationship between average volumetric soil moisture of treatment range and total glucosinolate concentrations in leaves of radish grown in controlled environments at 40% RH in 2003 and 2004 at the Ohio Agricultural Research and Development Center in Wooster, Ohio. Values are treatment means across two runs of the experiment. Error bars are standard errors.

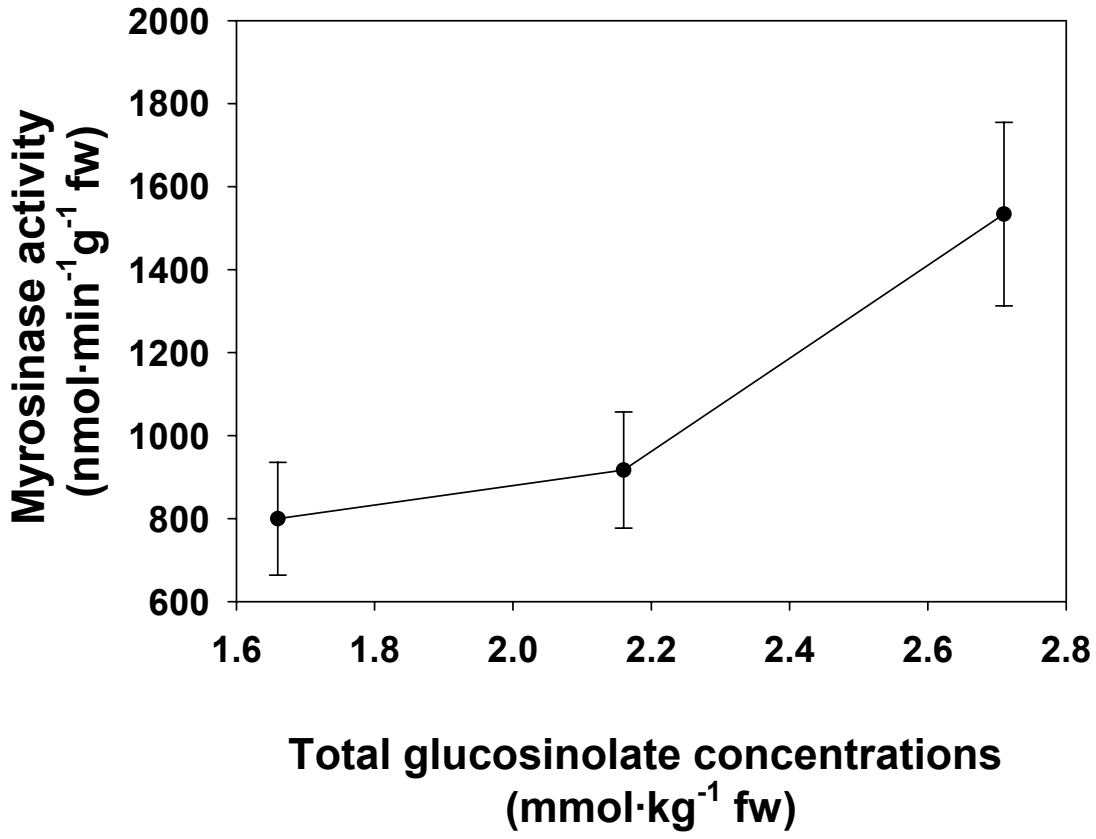

Figure B.7. Relationship between total glucosinolate concentrations and myrosinase activity on a fresh weight basis in leaves of radish grown in controlled environments in 2003 and 2004 at the Ohio Agricultural Research and Development Center in Wooster, Ohio at 40% RH. Values are treatment means across two runs of the experiment. Error bars are standard errors.

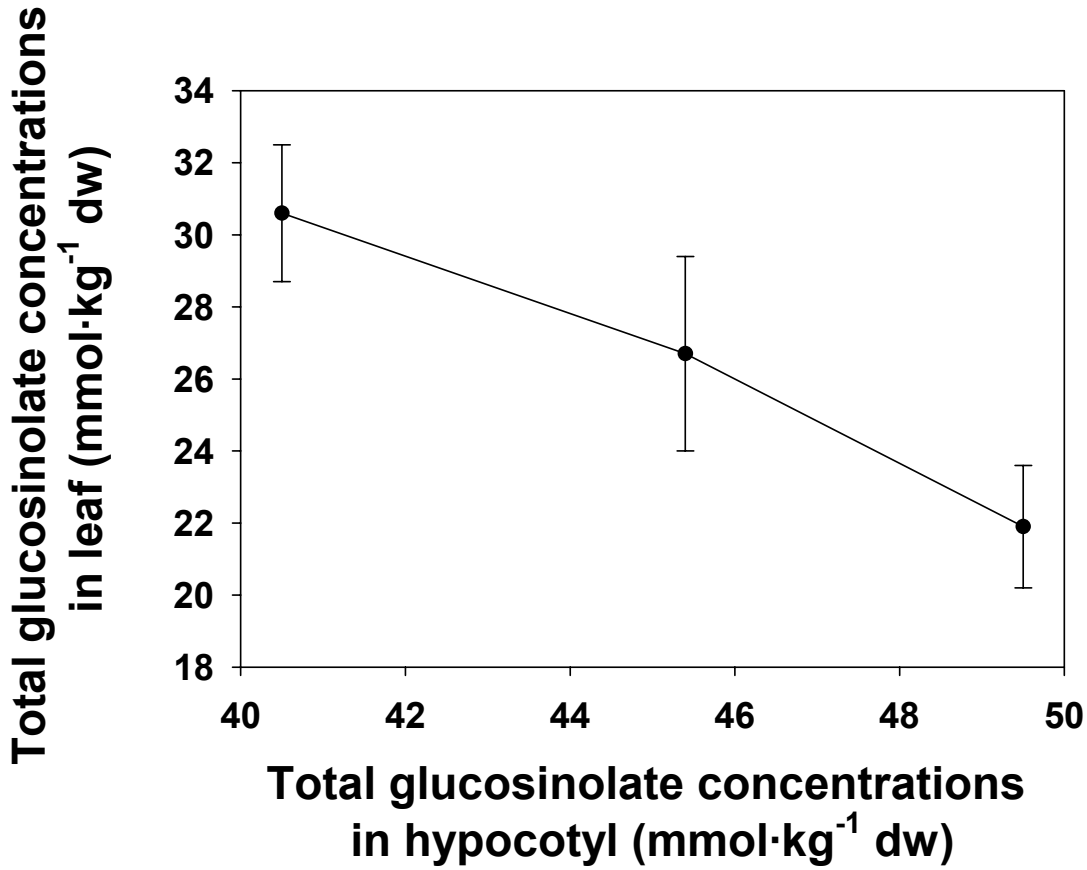

Figure B.8. Relationship between leaf and hypocotyl total glucosinolate concentrations on a fresh weight basis in radish grown in controlled environments in 2003 and 2004 at the Ohio Agricultural Research and Development Center in Wooster, Ohio. Values are treatment means across two runs of the experiment at 40 % RH. Error bars are standard errors.

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