

Differential Response of a Virginia Common Lambsquarters (*Chenopodium album*)
Collection to Glyphosate

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ABSTRACT

The purpose of this research was to evaluate a common lambsquarters (*Chenopodium album*) collection from Westmoreland County, Virginia, which exhibited a differential response to glyphosate treatments as compared to most other common lambsquarters. Plants from this site that survived glyphosate applications were collected in both 2002 and 2004. Greenhouse studies were conducted on F1, F2, and F3 progeny from this collection and compared to a wild type collection from Montgomery County, Virginia. Evaluations were conducted on these plants treated with a range of glyphosate rates. F1 progeny of the Westmoreland plants from both 2002 and 2004 collections showed reduced response to glyphosate relative to the Montgomery collection. Vigor reduction of F1 progeny from three 2004 Westmoreland source plants with 0.84 kg ae/ha of glyphosate ranged from 66 to 85% at 28 days after treatment (DAT), compared to 89% for the Montgomery collection. Evaluation of four Westmoreland F2 common lambsquarters lines derived from 2002 collections indicated significant differences in glyphosate sensitivity. Fifteen F2 lines were generated from 2004 collections from each of three Westmoreland source plants and from the Montgomery source. For the least sensitive Westmoreland source, vigor reduction ranged from only 24 to 36% across F2 lines in response to 1.68 kg/ha of glyphosate at 28 DAT, relative to 55 to 100% for the Montgomery source. I_{50} estimates for fresh weight reduction were 0.91 and 0.32 kg/ha, for these sources, respectively. Sequential treatments of 0.42, 1.26, and 1.68 kg/ha

applied at three-week intervals to the least susceptible 2004 Westmoreland F2 line resulted in only 37% vigor reduction and no mortality among 360 treated plants. Growth chamber studies were also conducted on the F2 progeny of these sources to determine if differential growth responses occur in noncompetitive environments and in the absence of glyphosate treatment. Generally, few differences were observed among the Westmoreland and Montgomery collections in growth parameters including height, leaf number, leaf area, leaf size, shoot weight, and reproductive output. However, significant differences were observed with regard to root weight, root length, and root density. In germination studies, it was determined that the Montgomery source had significantly faster and greater seed germination than the Westmoreland source. The susceptibility of F3 seedlings to glyphosate varied significantly with respect to F2 parent line and glyphosate rate. Mortality of 100% was observed in F3 seedlings from the Montgomery source in response to the 3.36 kg ae/ha glyphosate rate, while no mortality was observed in Westmoreland F3 seedlings in response to this glyphosate rate.

Nomenclature: Glyphosate; common lambsquarters, *Chenopodium album* L. CHEAL; soybean, *Glycine max* L. Merr.

Key words: Herbicide resistance, herbicide tolerance.

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Chapter 1

1.1 Introduction

1.1.1 Common Lambsquarters. Common lambsquarters is a summer annual broadleaf weed, considered to be one of the world's worst weeds (Holm et al. 1977). This weed is recognized as one of Virginia's ten most common and troublesome weeds in soybean and corn crops (Webster 2000; Webster 2001), due to its competitiveness with crops and reproductive fecundity. Reproduction occurs via self- or cross- pollination (Zomlefer 1994), resulting in 72,000 to 500,000 seed per plant (Crook and Renner 1990). Common lambsquarters can produce 300 to 600 million seeds/ha in field areas that lack competition with crop plants, such as field borders, areas with reduced crop stand, and non-crop land (Colquhoun et al. 2001).

1.1.2 Seed Characteristics. Great polymorphism exists within *Chenopodium* species in terms of seed properties and characteristics. An individual common lambsquarters plant can produce both brown and black seed varying in size, seed wall thickness, and surface appearance. The majority of seed are black and shiny, but larger light-brown seeds are also present in lower comparative proportions (Williams 1963). A plant may produce large thin-walled brown and small thick-walled black seed which have smooth or reticulated seed coats (Harper et. al 1970).

Although the seed coat does not serve as a dormancy mechanism, research has shown certain seed coat characteristics may affect germination timing (Williams 1963; Harper et al. 1970). A high proportion of common lambsquarters seed are innately dormant and can remain viable in soil for 30-40 years, contributing to future populations (Conn and Deck

1995; Holm et al. 1977; Williams 1963). A number of factors may influence germination of common lambsquarters seed. Seed polymorphism, soil temperature, nitrate content of soil, and light all seem effective in breaking dormancy of seed (Williams 1963; Holm et al. 1977). In freshly harvested seed, it has been reported that germination can be as great as 33% (Williams 1963). However, germination rates up to 64% can be obtained with temperature treatments of 0 to 5 C for 21-28 days exposure (Williams 1963). Williams (1963) also found that reticulate seeds, when exposed to acid treatment of ammonium salt, had a germination rate of 62% compared to a rate of 33% in smooth seed. It has also been reported that brown seeds germinate much more readily than black seeds (Harper et al. 1970).

Seed fitness and seedling vigor have been shown to be greatly influenced by maternal environments with regard to factors such as light penetration through the canopy, nutrient availability of parent plants, and interactions among these factors (Mahoney and Swanton 2006). It was found that seed collected from low light and high nitrogen areas had higher seedling vigor, regardless of the nitrogen availability of the progeny environment (Mahoney and Swanton 2006). Common lambsquarters seed meeting this description may have greater fitness and adaptability, thus increasing persistence and annoyance within agricultural cropping systems. This persistence is problematic when considering methods of herbicidal weed control. It has been found that low nitrogen environments may significantly decrease the effectiveness of glyphosate via decreased net export from mature leaves of velvetleaf and lambsquarters species (Mithila et al. 2006). Although this physiological relationship is not yet fully understood, decreased weed sensitivity is of concern when implementing postemergence weed control programs with glyphosate.

1.1.3 Competition. Common lambsquarters has a competitive advantage over some other crop and weed species because of its ability to germinate at lower temperatures, hence allowing seedling weed emergence before crop emergence (Chu et al. 1978; Wiese and Binning 1987). Although common lambsquarters is considered a summer annual, emergence begins in the early spring and continues throughout the summer, and late-season emergence is not unusual (Holm et al. 1977; Schuster et al. 2007). Thus, the ability of a few plants to escape control may lead to the production of thousands of seeds, continued infestations, and reduced crop yields in subsequent years.

Crop yield losses due to common lambsquarters competition can be dramatic. Soybean yield reductions of 15 and 20% occurred when 16 and 32 common lambsquarters plants per 10 m of row were present season-long in studies conducted in Michigan (Crook and Renner 1990) and North Carolina (Shurtleff and Coble 1985), respectively. The competitiveness of common lambsquarters in corn has been investigated by several researchers who have determined a range of crop losses due to varying environmental conditions and weed densities (Fischer et al. 2004). Researchers have reported corn yield losses due to common lambsquarters competition which include 12% in Illinois (Beckett et al. 1988), 38% in Quebec, Canada (Ngouajio et al. 1999), and 58% in Ontario, Canada (Sibuga and Bandeen 1980). Corn yield losses ranging from 0 to 100% were observed in response to common lambsquarters competition in research conducted in seven locations in the northcentral United States (Fischer et al. 2004). Common lambsquarters has also been shown to reduce yield in other crops including peanut (*Arachis hypogaea*) (Wilcut et al. 1991), tomato (*Solanum lycopersicum*) (Bhowmik and Reddy 1988), and sugarbeet (*Beta vulgaris*) (Schweizer 1983).

1.1.4 Herbicidal Control of Common Lambsquarters. Herbicides play a critical role in current agricultural production practices, and are especially important in no-till systems. However, variable herbicidal performance has been observed in common lambsquarters control in cropping systems (Fuerst et al. 1986; Glenn et al. 1997; Hagood 1989; Myers and Harvey 1993). Adverse environmental conditions during and after applications greatly influence the effectiveness of many herbicides, as was noted in these studies. Nevertheless, it was observed that the utilization of both preemergence and postemergence herbicides proved most effective in season long common lambsquarters weed control. Repeated use of chemical programs involving specific modes of action in consecutive growing seasons, however, increases selection pressures toward the development of resistance in weed populations.

1.1.5 Herbicide Resistance in Common Lambsquarters. Herbicide resistance in common lambsquarters has been well documented worldwide, with evidence of resistance to chemical groups which include ureas, amides, acetolactate synthase inhibitors, and extensive evidence of resistance to photosystem II inhibitors (Heap 2007). Donaldson et al. (2002) reported that the Environmental Protection Agency estimated that atrazine, which functions as an inhibitor of the photosynthetic electron transport pathway, was the most heavily used pesticide in the United States in 1999. Resistance to this mode of action has been documented in weed species which include *Amarathus* species (Conard and Radosevich 1979; Weaver and Warwick 1982), *Senecio vulgaris* (Conard and Radosevich 1979; Holt 1988), *Brassica campestris* (Mapplebeck et al. 1982) and *Chenopodium* species (Bandeem and McLaren 1976; Marriage and Warwick 1980; Warwick and Black 1981), among many others.

Many authors have conducted research not only with regard to differential growth and response of atrazine resistant and susceptible common lambsquarters populations, but also identifying relative fitness and competitive characteristics among such populations (Bandeem and McLaren 1976; Marriage and Warwick 1980; Warwick and Black 1981). Marriage and Warwick (1980) noted differential growth and response of atrazine resistant and susceptible common lambsquarters plants when grown in noncompetitive conditions. In germination studies comparing of two susceptible *Chenopodium album* populations (SI and SII) to a resistant biotype (R), it was found that germination of seed from SII was greater than SI and R, which did not differ significantly. Maturity of seedlings, as well as seedling weight, was least in R populations and greatest in SI. These results are in agreement with others in observing that resistant *Chenopodium album* populations emerged 2 to 3 days later than susceptible biotypes, and suggest a competitive disadvantage during early stages of plant development (Bandeem and McLaren 1976; Marriage and Warwick 1980). It was found, however, that once the seedling stage passed, the resistant population increased in height and leaf number and no longer expressed a competitive disadvantage in comparison with the susceptible biotypes (Marriage and Warwick 1980). Due to the early-flowering of some individuals in the SI population, the group was divided into early- and late-flowering and further identified as ESI and LSI, respectively. It was found that these populations of mature individuals differed in vegetative and floral weights with the ESI producing significantly less floral and vegetative dry weight than the other susceptible and the resistant population. However, the ESI had the greatest percentage of total dry weight represented in floral production in comparison to the others. In evaluation of all plant growth parameters, the

resistant biotype was always intermediate among the responses of susceptible biotypes. The total and vegetative dry weights of LSI were significantly larger than the resistant biotype, but both were statistically equivalent to the SII susceptible biotype. No significant differences were observed between R and SII populations in total dry weight, vegetative dry weight, or floral dry weight; however, they differed significantly in terms of time to maturation. It was concluded that an increase in fitness of the susceptible biotype was not reflected in plant growth after the seedling stage, total biomass of plants, or seed production, which were generally equal to or less than that of the resistant biotype. They observed that a competitive advantage of susceptible populations was most appropriately defined in early population establishment with more aggressive seed and seedling growth characteristics.

Warwick and Black (1981) have also documented differences in atrazine resistant and susceptible *Chenopodium album* and *Chenopodium strictum* populations. In mixed populations, the triazine susceptible biotype of *Chenopodium album* was found to be more competitive than either the triazine resistant *Chenopodium album* biotype or the triazine susceptible *Chenopodium strictum* biotype. Triazine susceptible and resistant biotypes of *Chenopodium strictum*, however, were found to be equally competitive. In noncompetitive situations, and in the absence of atrazine, triazine susceptible *Chenopodium album* populations produced greater aboveground total, vegetative, and reproductive dry weight than triazine resistant *Chenopodium album* populations. Their results are similar to the findings of Conard and Radosevich (1979) with regard to *Senecio vulgaris* and *Amaranthus retroflexus*, but contrary to the observations of

Marriage and Warwick (1980) discussed previously with regard to common lambsquarters.

This variability in growth between resistant and susceptible populations of common lambsquarters, and the differential response to herbicides, creates challenges in terms of the development and efficacious application of selective herbicides. Schuster et al. (2007) have observed that the development of herbicide resistant common lambsquarters limits weed management options, and alternatives are generally less effective or more expensive.

1.1.6 Glyphosate. Glyphosate [N-(phosphonomethyl)glycine] is a relatively inexpensive, broad spectrum, nonselective, postemergence (POST) herbicide with no soil residual activity. Sold primarily under the trade name Roundup[®], glyphosate is the most widely used pesticide in the United States and in the world (Ware and Whitacre 2004). This pesticide has proven very effective on annual and perennial grasses and broadleaf weeds in crop and noncrop areas (Ware and Whitacre 2004). It functions to inhibit the biosynthesis of aromatic amino acids, which leads to several metabolic disturbances causing disruption of the shikimate pathway and resulting in shikimate accumulation in plant tissue (Nandula et al. 2005).

With the introduction of glyphosate-resistant (GR) crops in the mid 1990s, many farmers began utilizing this technology in crops including corn, cotton, soybeans, and canola. Nandula et al. (2005) reported that farmers in the United States have rapidly adopted GR crops, planting 85% of soybean, 60% of cotton, and 18% of corn acres to GR varieties in 2004. In the following year, Sankula (2006) reported that farmers in the United States increased the acreage of GR crops even further with 88% of soybean, 78%

of cotton, and 31% of corn acres planted with these crops. Virginia's utilization was 84%, 97%, and 16%, respectively, in 2005 (Sankula 2006). This technology allows the grower to treat established GR crops with a POST glyphosate application, and through subsequent crop competition and/or additional applications, maintain a weed-free crop until harvest (VanGessel et al. 2001).

Since the introduction of glyphosate-tolerant crops, POST applications of glyphosate have generally provided effective annual grass and broadleaf weed control. Research studies conducted in GR soybean (Lee et al. 2002; Ateh and Harvey 1999), cotton (Askew et al. 2002; Askew and Wilcut 1999), and corn (Sparks et al. 1999; Crooks et al. 2000) indicate the effectiveness of this chemical weed control program. However, repeated use of glyphosate has led to increased infestation levels of weeds with inherent glyphosate tolerance, and in some instances to the development of glyphosate resistance in certain weed species (Heap 2007).

Beginning in the late 1990s, glyphosate resistance has become a growing topic of concern in not only the United States, but also in other countries which utilize glyphosate. Several rigid ryegrass biotypes from Australia have since been documented to exhibit as much as 11-fold resistance to glyphosate (Powles et al. 1998; Pratley et al. 1999). To date, glyphosate-resistance has been documented in other grass species such as goosegrass (*Eleusine indica*) in 1997 in Malaysia (Lee and Ngim 2000) and Italian ryegrass (*Lolium multiflorum*) in 2003 in Chile (Perez and Kogan 2003). The first report of glyphosate resistance occurring in a broadleaf weed was a horseweed (*Conyza canadensis*) biotype in Delaware (VanGessel 2001). Research studies confirmed that greenhouse grown seedlings collected from seed of the resistant population exhibited 8-

to 13-fold glyphosate resistance (VanGessel 2001). Since then, other confirmed cases of glyphosate resistance in broadleaf weeds have been observed in species such as buckhorn plantain (*Plantago lanceolata*) and hairy fleabane (*Conyza bonariensi*) in 2003 in South Africa, and Palmer amaranth (*Amaranthus palmeri*) in 2005 in the United States (Heap 2007). Greenhouse investigations of potential glyphosate resistance in a Missouri biotype of common waterhemp (*Amaranthus tuberculatus*) have also been conducted. Initial results indicated that this biotype, selected through repeated use of glyphosate in GR crops, exhibited several fold resistance relative to susceptible waterhemp biotypes (Bradley et al. 2006). It was later found in greenhouse experiments that selected waterhemp populations were as much as 19-times more resistant to glyphosate than the susceptible population (Legleiter and Bradley 2008). Greenhouse and field studies have also been conducted investigating differential responses in less sensitive giant ragweed (*Ambrosia trifida*) populations which were identified where giant ragweed was not adequately controlled within a four year exclusive glyphosate/GR soybean cropping system (Stachler and Loux 2006).

Another weed species which many farmers are having difficulty controlling in GR cropping systems is common lambsquarters. Greenhouse and field research has been conducted to determine glyphosate sensitivity within selected biotypes that express varying response to glyphosate application.

Greenhouse studies conducted in Ohio identified a total of 12 lambsquarters biotypes which appeared to have reduced sensitivity to glyphosate in one or more studies (Loux et al. 2005). Based on the variable glyphosate responses observed in greenhouse grown common lambsquarters, field studies were conducted in GR soybean in six locations

(Taylor et al. 2005). Labeled glyphosate rates provided common lambsquarters control in five locations, and control with sequential applications was observed in all locations. In only one location, individual plants were observed to have survived sequential applications. Reduced control of individual plants in the field was theorized to result from adverse environmental conditions.

In 2002, a grower in eastern Virginia applied glyphosate in combination with imazethapyr POST at 1.05 and 0.07 kg ai/ha, respectively, for weed control in GR soybean, however some common lambsquarters plants were not controlled. Dead common lambsquarters plants were observed throughout the field in close proximity to the surviving common lambsquarters plants, which were stunted but not controlled by the herbicide combination. The size of dead and live plants indicated that they were cohorts. The grower reported a history of successful control with glyphosate treatments. This grower has been using glyphosate for POST weed control in GR soybean since their commercial introduction and this field had received glyphosate treatments as a burndown or in-crop application in two of the last four years.

Greenhouse studies at Virginia Tech were conducted to investigate the differential response of this common lambsquarters biotype to glyphosate. It was found that at 28 days after treatment, 36 and 86% control was observed in a less sensitive biotype (Westmoreland) and a susceptible biotype (Montgomery), respectively, in response to glyphosate application of 0.56 kg ai/ha (King et al. 2004). At a treatment rate of 1.12 kg ai/ha, the Westmoreland and Montgomery biotypes were controlled 73 and 97%, respectively (King et al. 2004). Four F2 generations were generated from the F1 Westmoreland County lambsquarters. Two of the F2 lines exposed to glyphosate

application responded in a manner similar to the Montgomery biotype, while two exhibited reduced sensitivity. One Westmoreland F2 population was controlled only 68% with 2.24 kg ai/ha of glyphosate compared to 100% control of the Montgomery biotype (King et al. 2004).

The following studies were conducted to elucidate the nature of the common lambsquarters population from eastern Virginia. The specific objectives of my research include further evaluation of the sensitivity of this common lambsquarters collection to applications of glyphosate, elucidation of differential growth response among this collection in comparison to other common lambsquarters populations with no known history of glyphosate use, and examination of the inheritance of resistance or tolerance in progeny of these common lambsquarters collections.

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Chapter 2

Differential Response of a Virginia Common Lambsquarters

(*Chenopodium album*) Collection to Glyphosate

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2.1 Abstract. Control of common lambsquarters with POST applications of glyphosate in glyphosate-resistant crops generally has been effective. In 2002, common lambsquarters plants from Westmoreland County, Virginia were identified after not being controlled with a POST glyphosate application in glyphosate-resistant soybean. Plants from this site which survived glyphosate applications were collected in both 2002 and 2004. The objective of this research was to evaluate the susceptibility of F1 and F2 progeny from these common lambsquarters plants, relative to the susceptibility of common lambsquarters collected in Montgomery County, Virginia. F1 progeny of the Westmoreland plants from both 2002 and 2004 collections showed reduced response to glyphosate relative to the Montgomery collection. Vigor reduction of F1 progeny from three 2004 Westmoreland source plants with 0.84 kg ae/ha of glyphosate ranged from 66 to 85% at 28 days after treatment (DAT), compared to 89% for the Montgomery collection. Evaluation of four Westmoreland F2 common lambsquarters lines derived from 2002 collections indicated significant differences in glyphosate sensitivity. Fifteen F2 lines were generated from 2004 collections from each of three Westmoreland source plants and from the Montgomery source. For the least sensitive Westmoreland source, vigor reduction ranged from only 24 to 36% across F2 lines in response to 1.68 kg/ha of glyphosate at 28 DAT, relative to 55 to 100% for the Montgomery source. I_{50} estimates

for fresh weight reduction were 0.91 and 0.32 kg/ha, for these sources, respectively. Sequential treatments of 0.42, 1.26, and 1.68 kg/ha applied at three-week intervals to the least susceptible 2004 Westmoreland F2 line resulted in only 37% vigor reduction and no mortality among 360 treated plants.

Nomenclature: Glyphosate; common lambsquarters, *Chenopodium album* L. CHEAL; soybean, *Glycine max* L.

Key words: Herbicide resistance, herbicide tolerance.

2.2 Introduction. Common lambsquarters (*Chenopodium album*), an annual broadleaf weed, is recognized in Virginia as one of the ten most common and troublesome weeds in soybean (*Glycine max*) (Webster 2001) and corn (*Zea mays*) (Webster 2000). Common lambsquarters is also considered a principal weed pest in corn and soybean in the United States and is a competitive weed in 40 crops worldwide (Holm et al. 1977). One common lambsquarters plant can produce 72,000 seeds; however, a large plant is capable of producing as many as 500,000 seeds (Crook and Renner 1990). Common lambsquarters can produce 300 to 600 million seeds/ha in field areas that lack competition with crop plants, such as field borders, areas with reduced crop stand, and non-crop land (Colquhoun et al. 2001).

The ability of a few common lambsquarters escaping control may lead to the production of thousands of seed, continued infestations, and reduced crop yields in subsequent years. Common lambsquarters seed may remain viable in soil for as long as 30 to 40 years (Conn and Deck 1995; Holm et al. 1977). Common lambsquarters also has a competitive advantage over some other crop and weed species because of its ability to germinate at lower temperatures, hence allowing seedling weed emergence before crop emergence (Chu et al. 1978; Wiese and Binning 1987).

Crop yield losses due to common lambsquarters competition can be dramatic. Soybean yield reductions of 20 and 15% occurred when 32 and 16 common lambsquarters plants per 10 m of row were present season-long in studies conducted in Michigan (Crook and Renner 1990) and North Carolina (Shurtleff and Coble 1985), respectively. As few as 5 common lambsquarters plants per m of row reduced corn yield by 12% in research conducted in Illinois (Beckett et al. 1988). Corn yield reductions of 19 to 29% were

observed in response to common lambsquarters competition in corn in Quebec, Canada (Ngouajio et al. 1999). Corn yield losses ranging from 0 to 100% were observed in response to common lambsquarters competition in research conducted in seven locations in the northcentral United States (Fischer et al. 2004). Common lambsquarters also has been shown to reduce yield in other crops including peanut (*Arachis hypogaea*) (Wilcut et al. 1991), tomato (*Solanum lycopersicum*) (Bhowmik and Reddy 1988), and sugarbeet (*Beta vulgaris*) (Schweizer 1983).

Since the introduction of glyphosate-resistant (GR) crops in the mid 1990s, many farmers have utilized this technology in crops including soybean, cotton, corn, and canola. Sankula (2006) reported that farmers in the United States have rapidly adopted GR crops, planting 88% of soybean, 78% of cotton, and 31% of corn acres to GR varieties in 2005. Virginia's utilization was 84%, 97%, and 16%, respectively, in 2005. This technology allows the grower to treat established GR crops with a POST glyphosate application, and through subsequent crop competition and/or additional applications, maintain a weed-free crop until harvest (VanGessel et al. 2001). However, repeated use of glyphosate led to increased infestation levels of weeds with inherent glyphosate tolerance and, in some instances, development of glyphosate resistance in certain weed species (Heap 2007).

Beginning in the late 1990s, glyphosate resistance became a growing topic of concern not only in the United States but also in other countries which utilized glyphosate. Several rigid ryegrass (*Lolium rigidum*) biotypes from Australia have been documented to exhibit as much as 11-fold resistance to glyphosate (Powles et al. 1998; Pratley et al. 1999). To date, glyphosate resistance has been documented in other grass species such as

goosegrass (*Eleusine indica*) in 1997 in Malaysia (Lee and Ngim 2000) and Italian ryegrass (*Lolium multiflorum*) in 2003 in Chile (Perez and Kogan 2003). The first report of glyphosate resistance occurring in a broadleaf weed was a horseweed (*Conyza canadensis*) biotype in Delaware (VanGessel 2001). Studies confirmed that greenhouse grown seedlings collected from seed from the resistant population exhibited 8- to 13-fold glyphosate resistance. Since then, glyphosate resistance has been confirmed in broadleaf weeds including buckhorn plantain (*Plantago lanceolata*) and hairy fleabane (*Conyza bonariensis*) in 2003 in South Africa, and Palmer amaranth (*Amaranthus palmeri*) in 2005 in the United States. (Heap 2007). Greenhouse investigations of potential glyphosate resistance in a Missouri biotype of common waterhemp (*Amaranthus tuberculatus*) also have been conducted. The results indicate that this biotype, selected through repeated use of glyphosate in GR crops, exhibits several-fold resistance relative to susceptible waterhemp biotypes (Bradley et al. 2006). Greenhouse and field studies also have been conducted to investigate differential responses of giant ragweed (*Ambrosia trifida*) populations to glyphosate applications. These biotypes of giant ragweed that were not adequately controlled were identified in a GR soybean cropping system (Stachler and Loux 2006).

Greenhouse studies conducted in Ohio identified a total of 12 common lambsquarters biotypes which exhibited reduced sensitivity to glyphosate in one or more studies (Loux et al. 2005). Based on the variable glyphosate responses observed in greenhouse grown common lambsquarters, field studies were conducted in GR soybean in six locations (Taylor et al. 2005). Labeled glyphosate rates provided common lambsquarters control in five locations, and control with sequential applications was observed in all locations. In

only one location, individual plants were observed to have survived sequential applications. Reduced control of individual plants in the field was theorized to result from adverse environmental conditions.

In 2002, a grower in eastern Virginia applied glyphosate in combination with imazethapyr POST at 0.79 kg ae/ha and 0.07 kg ai/ha, respectively, for weed control in GR soybean. However, some common lambsquarters plants were not controlled. Dead common lambsquarters plants were observed throughout the field in close proximity to the surviving common lambsquarters plants, which were stunted but not controlled by the herbicides. The size of the dead and live plants indicated that they had both been approximately 15 to 20 cm in height at the time of herbicide application. The grower reported that in previous years all common lambsquarters plants had been controlled with this rate of glyphosate. This grower has been using glyphosate for POST weed control in GR soybean since 1996. This field had received glyphosate treatments as a burndown or in-crop application in two of the last four years.

Because common lambsquarters is considered to be one of the worst weeds in agronomic crops throughout the world, tolerance or the development of resistance to glyphosate could negatively affect current crop production practices. The present studies were conducted to evaluate glyphosate sensitivity in this common lambsquarters collection from eastern Virginia.

2.3 Materials and Methods

2.3.1 Plant Material. *2002 Collection.* Common lambsquarters seed was collected from plants surviving two applications of glyphosate in a GR soybean field in eastern Virginia in the fall of 2002. Specifically, an initial herbicide treatment of 0.63 kg ae/ha of glyphosate plus 0.07 kg ai/ha of imazethapyr¹ was applied and supplemented with an additional 0.16 kg ae/ha of glyphosate². Because this treatment did not control all common lambsquarters plants in the field, a second application of 1.68 kg ae/ha of glyphosate was applied. Although most common lambsquarters plants were controlled completely or stunted to the point where seed was not produced, some plants survived and produced seed. Seeds from two of these plants were collected prior to soybean harvest. This collection will be referred to as Westmoreland, which indicates the county of origin. Common lambsquarters seed also was collected from plants in a field in Montgomery County with no glyphosate use history, and plants derived from this location represent the susceptible collection.

2004 Collection. Because the grower's normal crop rotation would not have challenged the common lambsquarters population in Westmoreland County with glyphosate in 2003, no observations or collections were made in this growing season. In 2004, the site was monitored for common lambsquarters sensitivity to glyphosate. Wheat (*Triticum aestivum*) present at the site was controlled with 1.68 kg ae/ha of glyphosate² in February, 2004, after which the site was undisturbed until common lambsquarters emergence. A randomized complete block design was utilized containing six glyphosate treatments in four replications. Individual plots were 38.1 x 3.1 m. Glyphosate² treatments included 0,

0.42, 0.84, 1.68, 3.36, and 6.72 kg ae/ha and were applied on May 18, 2004, to common lambsquarters plants 6 to 7.5 cm in height. Applications were made with flat-fan nozzle tips³ delivering 210 L/ha of spray solution at 270 kPa. Individual common lambsquarters plants present at the time of application were flagged, and response to glyphosate was monitored. Most common lambsquarters plants were controlled with all application rates of glyphosate, and all plants were controlled by rates of 1.68 kg ae/ha or greater. However, six plants from either 0.42 or 0.84 kg ae/ha treatments survived and produced seed. These plants were collected, and progeny were evaluated for glyphosate susceptibility relative to the Montgomery County collection.

2.3.2 F1 Experiments. Greenhouse experiments were conducted at Virginia Polytechnic Institute and State University in Blacksburg, Virginia. Seed from the two Westmoreland common lambsquarters plants from the 2002 collections, and from the Montgomery County collection, were planted separately in 52- by 27- by 6-cm flats containing commercial potting soil⁴. One Westmoreland plant did not produce a sufficient number of seed for comprehensive evaluation of F1 response to glyphosate. The second Westmoreland plant produced abundant seed, and this plant was the source of all seedlings used from 2002 collections. Three of the six Westmoreland common lambsquarters plants collected in 2004 produced sufficient seed to allow evaluation, and were designated Westmoreland 1, 2, and 3. These seed and seed from the Montgomery seed collection were planted in the manner described above.

When seedlings were approximately 4 cm tall, 16 F1 plants from the 2002 Westmoreland source plant and 16 plants from the Montgomery collection were transplanted into 11-cm pots with one plant per pot. Seedlings were grown using

supplemental lighting⁵ of approximately 450 $\mu\text{mol}/\text{m}^2/\text{sec}$ with a 12 h photoperiod and were maintained at 22 to 27 C. Common lambsquarters plants were allowed to acclimate from transplanting, watered as needed, and fertilized⁶ once prior to herbicide application. Glyphosate treatments were applied when common lambsquarters plants were 7.5 to 9 cm tall. Sixteen F1 plants from each of the three source plants from 2004 Westmoreland collections, and 16 plants from the Montgomery source, were transplanted and maintained in the manner described above. For 2004 collections, however, common lambsquarters plants were approximately 5 cm tall at transplanting, and 6 to 9 cm tall at application.

Glyphosate² was applied at rates of 0, 0.21, 0.42, and 0.84 kg ae/ha using a stationary track sprayer⁷ containing a single even-edge nozzle tip⁸ that delivered 230 L/ha of spray solution at 270 kPa to plants derived from each collection. The experiments were arranged in a randomized complete block design with four replicates. Data were subjected to two-way factorial analysis of variance, with biotype and glyphosate rate as the factors analyzed. Common lambsquarters vigor reduction was visually rated on a scale of 0 to 100% at 7, 14, 21, and 28 days after treatment (DAT), and plant heights were determined at the same intervals. Plants were harvested 30 DAT and fresh weights were collected. The experiments were conducted twice and homogeneity of variance evaluation indicated no significant effect of repetition. Hence, data are presented as an average of the two repetitions. Means were separated using Fisher's protected LSD test at the 5% level of probability.

2.3.3 F2 Experiments. Fifteen additional F1 seedlings from the 2002 Westmoreland source plant, which had not been treated with glyphosate, were transplanted into

individual 11- cm pots, placed in a greenhouse isolated from other common lambsquarters plants, and allowed to mature and produce seed. Resulting progeny from these plants were designated F2.

The F2 seed were planted into 15 individual 52- by 27- by 6-cm flats and were designated as lines F2₁ through F2₁₅. Seed from the Montgomery source also were planted in a separate flat. Production of F2 seed was not uniform among Westmoreland lines, such that only four lines produced a sufficient number of seedlings for a dose-response study. These populations are referred to as F2₅, F2₁₁, F2₁₃, and F2₁₅. Twenty seedlings from each of these F2 lines and the Montgomery source were grown to approximately 5 cm in height and transplanted into individual pots. Growing conditions were the same as described from the F1 experiment above. Glyphosate treatments of 0, 0.21, 0.42, 0.84, and 1.68 kg ae/ha were applied to plants 7.5 to 9 cm tall.

Fifteen F1 seedlings from each of the three 2004 Westmoreland source plants were transplanted into individual pots and allowed to mature and produce seed. Each set of seedlings was maintained in a separate greenhouse. F2 seed from each of the 15 F1 plants were planted in individual flats, and transplanted as described above. Fifteen F2 lines from the Montgomery source also were generated. In each instance, the 15 F1 plants produced sufficient seed to allow dose-response evaluations. These 60 F2 lines were designated as Montgomery F2₁₋₁₅, Westmoreland 1 F2₁₋₁₅, Westmoreland 2 F2₁₋₁₅, and Westmoreland 3 F2₁₋₁₅. Common lambsquarters plants were approximately 5 cm in height when transplanted and 6 to 9 cm tall at application.

Glyphosate was applied at 0, 0.21, 0.42, 0.84, and 1.68 kg ae/ha. Herbicide treatments, data collection, and experimental design and analysis were conducted as described for the

F1 experiments. The F2 experiments were repeated, and homogeneity of variance evaluation indicated no significant effect of repetition for vigor reduction, height, and fresh weight. For this reason, data were combined over repetition before the final statistical analysis.

For the 2004 collections, the effects of F1 source plant and F2 line within each source on the relationship between glyphosate rate and common lambsquarters fresh weight were analyzed in three stages as follows. First, for each repetition, data were fit to a two-parameter log-logistic model of the form:

$$W_r = 100/[1 + (r/I_{50})^\beta] = 100/(1 + \exp\{\beta[\log(r) - \log(I_{50})]\}), r, \beta, I_{50} > 0 \quad [1]$$

where W_r is fresh weight as % of control at rate $r = 0.21, 0.42, 0.84,$ and 1.68 kg ae/ha; where β is the slope; and where I_{50} is the glyphosate application rate at which fresh weight is halved. This is a special case of the four-parameter log-logistic model recommended by Seefeldt et al. (1995) and it was fit using the SAS NLIN procedure⁹.

The log-logistic model described above provided an observation of the parameter I_{50} for each of the 2 repetitions of the experiment, and for each of the 15 lines within each of the 4 source plants, for a total of 120 observations. Using these, nested analysis of variance of the effects of F1 source plant, and F2 line within F1 source plant, as components of the variance of I_{50} was conducted with repetitions as blocks, using the REML method of SAS PROC MIXED⁹. The statistical model is:

$$\ln(I_{50})_{ijk} = \mu + D_i + S_j + L_{k(j)} + e_{k(ij)}, i = 1,2; j = 1, \dots, 4; k = 1, \dots, 15 \quad [2]$$

where D_i is the random block effect of repetition, S_j is the random effect of source j , $L_{k(j)}$ is the random effect of line k nested within source j , and where $e_{k(ij)}$ is the residual random effect of the interaction of repetition with the other factors in the model. The

effects of F1 source plants were considered as fixed, and estimates and comparisons of mean I_{50} were conducted using the Tukey-Kramer HSD multiple-comparison procedure (Kramer 1956).

Finally, for each of the four F1 source plants, I_{50} and slope parameters were estimated by nonlinear regression of fresh weight on glyphosate application rate, again using the two-parameter log-logistic model shown above. The parameter estimates, standard errors, and 95% confidence intervals are the result of nonlinear regression and SAS PROC NLIN⁹.

2.3.4 Sequential Experiments. For selected 2002 and 2004 F2 lines, the effects of sequential glyphosate applications were evaluated. Four hundred seedlings from the 2002 F2₅ Westmoreland line, and an equal number of Montgomery seedlings, were transplanted into individual pots as described above. All plants were treated with 0.42 followed by (fb) 1.26 fb 1.68 kg ae/ha of glyphosate at three-week intervals. Identical treatments were made to 360 seedlings from several 2004 Westmoreland F2 lines and the Montgomery F2 line. Common lambsquarters vigor reduction, height, and mortality were recorded beginning at 7 days after the initial application and were recorded at weekly intervals until 3 weeks after the final application. Plant vigor reduction values from the final evaluation were grouped in 11 response categories, ranging from 0 (no response) to 100 (death of plant). A one-way analysis of variance and t-Test were used to compare overall vigor responses between Montgomery and Westmoreland F2 lines to sequential applications, and 95% confidence intervals for overall population mean vigor reduction values were determined⁹.

2.4 Results and Discussion

2.4.1 F1 Experiments. Progeny of the Westmoreland common lambsquarters plants from both 2002 and 2004 collections showed reduced response to glyphosate rates of 0.21, 0.42, and 0.84 kg ae/ha relative to the response in the Montgomery collection. For 2002, the differential response between collections was observed as early as 7 DAT when glyphosate was applied at 0.84 kg ae/ha, when Westmoreland and Montgomery collections exhibited vigor reductions of 28 and 74%, respectively (Table 2-1). Higher levels of common lambsquarters vigor reduction were observed in the Montgomery collection compared to the Westmoreland collection throughout the duration of the experiment. At 28 DAT, the Westmoreland and Montgomery collections expressed vigor reductions of 73 and 97%, respectively.

For 2004 collections, similar responses to 2002 collections were observed (Table 2-1). A differential response to glyphosate application between Montgomery and Westmoreland collections was observed as early as 14 DAT, where Westmoreland sources 1 and 3 exhibited 59% vigor reduction relative to 75% in the Montgomery source. By 28 DAT, Westmoreland sources 2 and 3 expressed 76 and 66% vigor reduction, respectively, relative to 89% for the Montgomery source.

2.4.2 F2 Experiments. Evaluation of the response of F2 plants to glyphosate was conducted to provide information regarding heritability of the trait causing reduced sensitivity to glyphosate. Comparison of the four Westmoreland F2 common lambsquarters lines derived from 2002 collections to the Montgomery collection indicated differing levels of glyphosate sensitivity at 30 DAT (Table 2-2). Vigor

reduction in the Montgomery collection was greater than in any of the Westmoreland F2 lines when glyphosate was applied at 0.21 kg ae/ha. However, levels of vigor reduction similar to the Montgomery collection occurred in lines F2₁₃ and F2₁₅ when glyphosate was applied at 0.84 kg ae/ha, and in the F2₁₃ line at the 1.68 kg ae/ha glyphosate rate. The Westmoreland F2₅ line demonstrated consistently lower sensitivity to glyphosate across all rates in comparison to the Montgomery collection. In response to the 0.84 kg ae/ha rate of glyphosate, common lambsquarters fresh weight at 30 DAT was reduced significantly in the Montgomery collection relative to the F2₅ Westmoreland line, where values of 1.1 and 6.6 g/plant, respectively, were observed (LSD, $\alpha = 0.05$, data not presented). Significant reductions in fresh weight between the F2₅ Westmoreland line and the Montgomery collection also were observed in response to the 1.68 kg ae/ha glyphosate rate, where values of 0 and 2.2 g/plant, respectively, were observed (LSD, $\alpha = 0.05$, data not presented). Fresh weights for the other three Westmoreland F2 lines were intermediate. Fresh weights for the F2₁₁ line were 4.6 and 2.3 g/plant in response to glyphosate rates of 0.84 and 1.68 kg ae/ha, respectively, and were statistically equivalent to fresh weights for the Westmoreland F2₅ line. Fresh weight reductions for the Westmoreland F2₁₃ and F2₁₅ lines in response to these glyphosate rates were equivalent to those observed in the Montgomery collection.

For each of the 15 F2 lines generated from the three Westmoreland source plants from 2004 collections, and for the Montgomery source plant, factorial analysis of variance indicated significant effects of F1 source, F2 line, and the interaction between F1 source and F2 line for common lambsquarters vigor reduction ($p < 0.01$). Figure 2-1 contains ranked vigor reduction data for these Westmoreland sources and for the Montgomery

source in response to 1.68 kg ae/ha of glyphosate at 28 DAT. Line number values are assigned arbitrarily solely on the basis of vigor reduction rank. For the Montgomery source, vigor reduction values ranged from 55 to 100% and 4 of the 15 lines exhibited vigor reduction less than 80%. In response to this application rate of glyphosate, Westmoreland F2 sources 1 and 2 demonstrated vigor reductions ranging from 49 to 93% and 42 to 84% across lines, respectively. For these sources, 12 and 13 lines exhibited vigor reduction less than 80%, respectively. Glyphosate sensitivity was much lower for Westmoreland source 3, however, where vigor reductions ranged from only 24 to 36% across the 15 lines. The data contained in Figure 2-1 demonstrate the effect of F1 source on sensitivity of F2 progeny to glyphosate, particularly with respect to vigor reduction across F2 lines for Westmoreland source 3.

Also demonstrated by these data is the inherent variability of common lambsquarters in terms of susceptibility to this herbicide, in that 4 of the 15 F2 lines derived from the Montgomery source exhibited plant vigor reductions of only 55 to 71%. This variability in response to glyphosate in glyphosate-susceptible common lambsquarters has been previously reported (Taylor et al. 2005; Mithila et al. 2006; Schuster et al. 2007), and may account for reduced control of this species in GR crops, particularly with larger weeds and adverse environmental conditions.

Significant effects of F1 source plant ($p < 0.001$) and F2 line ($p = 0.0021$) also were observed for common lambsquarters fresh weight in response to varying rates of glyphosate from the 2004 collection. Further, no significant departure from a normal distribution was observed for the random effects of F2 lines ($p = 0.81$, $n = 60$) based on a Shapiro-Wilk test (Shapiro and Wilk 1965). On this basis, I_{50} glyphosate rates were

calculated for each of the three Westmoreland F1 source plants and for the Montgomery F1 source plant across F2 lines (Table 2-3). The I_{50} estimate for the Montgomery source was 0.32 kg ae/ha of glyphosate, and I_{50} estimates for Westmoreland F1 source plants 1 and 2 were 0.26 and 0.34 kg ae/ha, respectively. For the Westmoreland F1 source 3 plant, however, the I_{50} estimate was 0.91 kg ae/ha, which represents a 2.8-fold increase in glyphosate rate relative to the Montgomery F1 source. The overall relationship between glyphosate rate and common lambsquarters fresh weight for Montgomery F1 and Westmoreland F1 source plant 3, as determined by the log-logistic regression model, is illustrated in Figure 2-2.

2.4.3 Sequential Experiments. Sequential glyphosate applications made to 400 Montgomery F1 common lambsquarters seedlings caused significantly greater plant vigor reduction than did sequential applications made to the same number of Westmoreland F2₅ seedlings from 2002 collections (Table 2-4). At 2 weeks after the final application, 390 of 400 Montgomery seedlings exhibited 100% vigor reduction, and the remainder 90% vigor reduction. For the Westmoreland F2₅ line, however, only 49 of 400 seedlings exhibited 100% vigor reduction, and 150 seedlings exhibited vigor reductions of 50% or less. Average vigor reduction for the 400 seedlings was 100 and 61% for Montgomery and Westmoreland sources, respectively.

Because 15 F2 lines were created for each of the three 2004 Westmoreland source plants, and from the Montgomery source plant, the total number of lines prohibited evaluation of the effects of sequential treatments on all lines. For this reason, sequential experiments were conducted using the Montgomery F2 lines which had exhibited the lowest and highest levels of plant vigor reduction in response to single applications of

1.68 kg ae/ha of glyphosate, and using the Westmoreland source 3 lines which exhibited lowest and highest levels of plant vigor reduction in response to this application. These lines are designated F2₁ and F2₁₅, respectively, for the Montgomery source plants and the Westmoreland source 3 plant, and correspond to those lines as designated in Figure 2-1.

For the 2004 sequential experiments, factorial analysis of variance was conducted for the 2 x 2, source x F2 line factorial, using 360 replications. This analysis demonstrated highly significant ($p < 0.001$) main effects of source and line, and a nonsignificant ($p = 0.7968$) interaction. For this reason, one-way analysis of variance was conducted as described above for vigor reduction for Montgomery and Westmoreland sources within lines (Table 2-4). Relatively small, but significant, differences in common lambsquarters vigor were observed between Montgomery and Westmoreland sources, where 44 and 37% reduction was observed in the F2₁ lines, and 95 and 88% reduction for the F2₁₅ lines, respectively. While no mortality was observed in the least sensitive F2₁ lines, common lambsquarters mortality of 71 and 39% was observed for the Montgomery and Westmoreland F2₁₅ lines, respectively.

The results of sequential applications also demonstrate the inherent variability of common lambsquarters in terms of susceptibility to glyphosate. The use of only the least and most sensitive F2 lines in evaluation of sequential treatments does not provide information regarding the overall response of all of the F2 lines from either the Montgomery or Westmoreland 2004 sources. However, the fact that lines from both sources were identified that exhibited no mortality in response to these sequential applications is indicative of this variability. The selection of less sensitive individuals to glyphosate could account for the development of common lambsquarters populations that

are more difficult to control, even in the absence of the development of herbicide resistance.

The overall results of these experiments confirm reduced sensitivity to glyphosate in common lambsquarters progeny from Westmoreland sources relative to progeny from Montgomery sources. Further, these results could be interpreted to confirm low level glyphosate resistance in common lambsquarters as defined by the Weed Science Society of America and the International Survey of Herbicide Resistant Weeds (Heap 2007). For 2002 collections, evaluations of glyphosate susceptibility were made by comparing the response of F2 Westmoreland seedlings to the response of F1 Montgomery or wild type seedlings. Had the response of F2 seedlings derived from 2004 Westmoreland collections been compared to the response of Montgomery F1 seedlings, the case for confirmation of resistance would have appeared much stronger. Glyphosate resistance in Palmer amaranth (*Amaranthus palmeri*) was recently confirmed in comprehensive research which compared the response of greenhouse generated F2 plants of the resistant biotype to the response of F1 plants derived from field collections of seed from the susceptible biotype (Culpepper et al. 2006). It is preferred, however, that second generation seed from greenhouse grown plants of resistant and susceptible populations are collected and tested for resistance (Heap 2007). In our experiments, this approach documented a 2.8-fold differential glyphosate sensitivity between the 2004 Westmoreland source 3 and Montgomery populations, when responses were compared over the 15 F2 lines within each source. While the response to glyphosate was confirmed to be normally distributed for each of these sources, the differential response among lines within each source is indicative of the natural variability within common lambsquarters

biotypes with respect to glyphosate susceptibility. Further research is needed to elucidate the physiological bases for the differential responses observed, and thereby determine whether these responses derive from an introduced mechanism for resistance, or rather reflect the selection of naturally occurring more tolerant individuals from a species with substantial inherent variability with regard to this trait.

2.5 Sources of Materials

¹Extreme herbicide, BASF Corporation, Research Triangle Park, NC 27709

²Roundup Ultramax, Monsanto Co., St. Louis, MO 63167.

³Teejet 8003VS, Spraying Systems Co., Wheaton, IL 60166.

⁴Premier Promix, Premier Horticulture Inc., Quakerstown, PA 18951.

⁵Sunlight Supply, Inc., Vancouver, WA 98665.

⁶Peters 20-20-20, Scotts-Sierra Horticultural Products, Marysville, OH 43041.

⁷Allen Machine Works, Midland, MI 48640.

⁸Teejet 8001EVS, Spraying Systems Co., Wheaton, IL 60166.

⁹SAS Institute Inc., Cary, NC 27513.

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Table 2-1. Time course for plant vigor response of Westmoreland and Montgomery F1 common lambsquarters treated with 0.84 kg ae/ha of glyphosate^a.

Year of Collection	Line	Common lambsquarters vigor reduction ^b			
		Days after treatment			
		7	14	22	28
		----- % -----			
2002	Montgomery	74 a	89 a	95 a	97 a
	Westmoreland	28 b	41 b	60 b	73 b
2004	Montgomery	50 a	75 a	86 a	89 a
	Westmoreland #1	41 a	59 b	76 ab	85 ab
	Westmoreland #2	41 a	66 ab	64 bc	76 bc
	Westmoreland #3	45 a	59 b	55 c	66 c

^a Values represent mean vigor response of eight plants. Values within a column and within a year followed by the same letter do not differ significantly, LSD ($\alpha = 0.05$).

^b Common lambsquarters vigor reduction (%), where 0 = no effect and 100 = death of plant.

Table 2-2. Vigor response of the Montgomery common lambsquarters biotype and of 2002 Westmoreland F2 lines at 30 DAT with a range of glyphosate rates^a.

Collection	Common lambsquarters vigor reduction ^b			
	Glyphosate rate (kg ae/ha)			
	0.21	0.42	0.84	1.68
	————— % —————			
Montgomery	27 a	43 a	89 a	100 a
Westmoreland F2 ₅	05 b	23 b	40 c	68 b
Westmoreland F2 ₁₁	10 b	30 ab	53 bc	77 b
Westmoreland F2 ₁₃	10 b	19 b	79 ab	99 a
Westmoreland F2 ₁₅	10 b	33 ab	74 ab	83 b

^a Values represent mean vigor response of eight plants. Values within a column followed by the same letter do not differ significantly, LSD ($\alpha = 0.05$).

^b Common lambsquarters vigor reduction (%), where 0 = no effect and 100 = death of plant.

Table 2-3. I_{50} estimates derived from nonlinear regression for common lambsquarters fresh weight reduction in response to glyphosate rate of application.

Source plant	I_{50} Estimate ^a	95% Confidence interval	
		Lower	Upper
		kg ae/ha	
Montgomery	0.32 b	0.29	0.34
Westmoreland #1	0.26 c	0.23	0.27
Westmoreland #2	0.34 b	0.31	0.36
Westmoreland #3	0.91 a	0.83	0.98

^a Values followed by the same letter do not differ significantly, Tukey-Kramer HSD, ($\alpha = 0.05$).

Table 2-4. Frequency of plant vigor reduction responses among 2002 Montgomery F1 and Westmoreland F2₅ or 2004 Montgomery and Westmoreland F2₁ or F2₁₅ common lambsquarters seedlings to sequential glyphosate treatments^a at 2 weeks after the final application.

Source	Line	Plant vigor ^b reduction category (%)											Mean ^c
		0	10	20	30	40	50	60	70	80	90	100	
		number of plants											
Montgomery	2002 F1	0	0	0	0	0	0	0	0	0	10	390	100
Westmoreland	2002 F2 ₅	6	5	17	32	37	53	61	93	32	15	49	61*
Montgomery	2004 F2 ₁	0	0	1	44	156	135	19	2	3	0	0	44
Westmoreland #3	2004 F2 ₁	0	0	5	150	157	45	3	0	0	0	0	37*
Montgomery	2004 F2 ₁₅	0	0	0	0	0	0	5	10	40	51	254	95
Westmoreland #3	2004 F2 ₁₅	0	0	0	0	0	0	5	47	98	69	141	88*

^a Sequential treatments of 0.42 followed by 1.26 followed by 1.68 kg ae/ha at three-week intervals.

^b Common lambsquarters vigor, 0-100, where 0 = no effect and 100 = death of plant.

^c Mean plant vigor reduction (0-100) averaged over 400 plants for 2002 and 360 plants for 2004 sources. * indicates significant reduction in response between means within 2002 and within 2004 common sources, LSD ($\alpha = 0.05$). Upper and lower 95% confidence intervals for means presented = 99.5 – 99.9, 58.8 – 63.5, 43.1 – 44.9, 36.2 – 37.7, 94.0 – 95.9, and 86.6 – 89.3, respectively.

Figure 2-1. Ordered plant vigor responses of 15 F2 common lambsquarters lines from Westmoreland (WEST) and Montgomery (MONT) sources to 1.68 kg ae/ha of glyphosate at 28 DAT. Factorial analysis of variance indicated significant effects of source, line number, and source by line number interaction ($p = 0.01$).

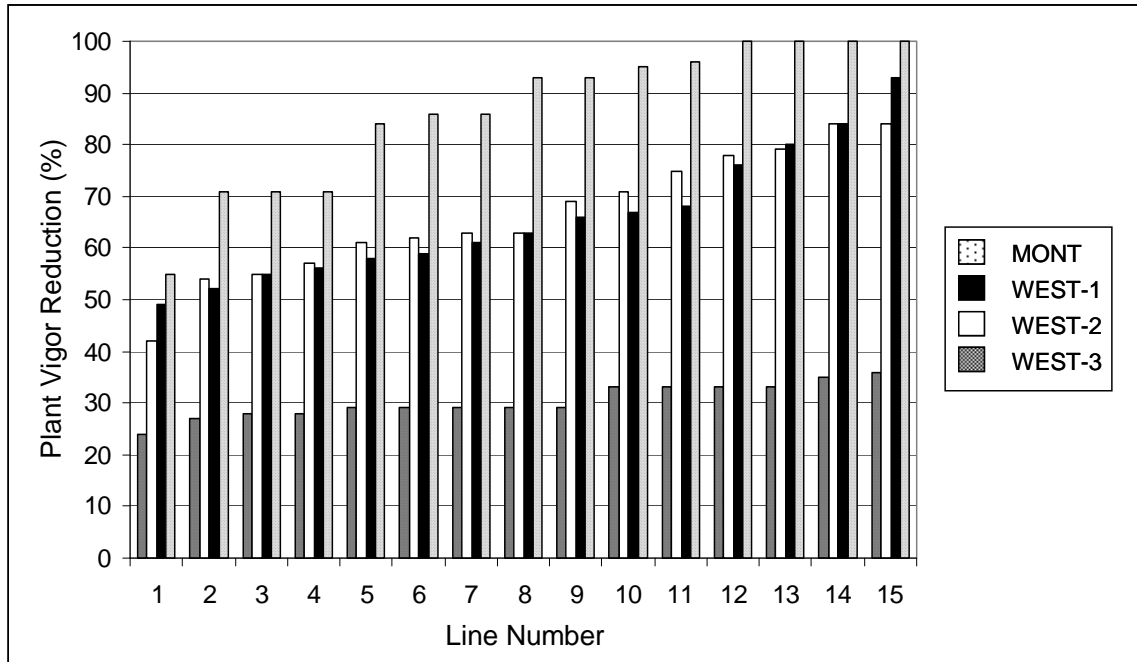
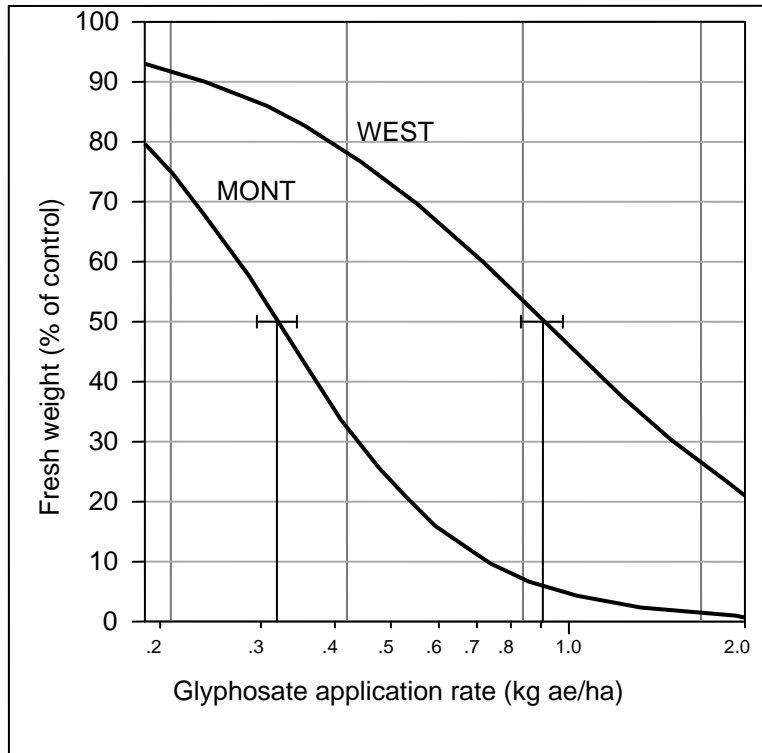


Figure 2-2. Log-logistic regression relationships for common lambsquarters fresh weight as a function of glyphosate application rate over all 15 F2 lines derived from Montgomery (MONT) or Westmoreland # 3 (WEST) F1 source plants. Error bars represent 95% confidence intervals for I_{50} values.



Chapter 3

Comparison of the Growth and Development of Common Lambsquarters (*Chenopodium album*) Progeny from Collections which Exhibit Differential Susceptibility to Glyphosate

3.1 Abstract. Previous work by the authors showed that differential responses were exhibited among collections of common lambsquarters plants in response to applications of glyphosate. F1 and F2 progeny from a suspected resistant population from Westmoreland County, Virginia showed reduced sensitivity to glyphosate relative to the Montgomery County, Virginia wild type population evaluated. However, variation in response did occur within F2 lines from each source. The objective of this research was to evaluate the growth, development, seed production, and seed viability of the least susceptible F2 line from the Westmoreland collection, an F2 line from the Westmoreland collection which was considered intermediate in response, and the most susceptible F2 line from the Montgomery collection. Common lambsquarters growth characteristics including height, leaf number, leaf area, leaf size, and shoot weight generally did not vary among Westmoreland and Montgomery F2 lines. Significant differences were observed, however, for root weight, root length, and root/shoot ratio variables. Common lambsquarters roots from the Montgomery source were shorter than those from the least susceptible Westmoreland source. However, root weights for plants from the Montgomery source were greater than those from the least susceptible Westmoreland source, as were root/shoot ratios. Seed production was similar among sources, although individual seed weight was reduced in the Montgomery source relative to the least

susceptible Westmoreland source. The susceptibility of F3 seedlings to glyphosate varied significantly with respect to F2 parent line and glyphosate rate. Mortality of 100% was observed in F3 seedlings from the Montgomery source in response to the 3.36 kg ae/ha glyphosate rate, while no mortality was observed in Westmoreland F3 seedlings in response to this glyphosate rate.

Nomenclature: Glyphosate; common lambsquarters, *Chenopodium album* L. CHEAL; soybean, *Glycine max* L. Merr.

Key words: Herbicide resistance, herbicide tolerance.

3.2 Introduction. The occurrence of glyphosate resistance has been well documented in a number of grass and broadleaf weed species worldwide (Heap 2007). Although there is limited research on the relative growth and development of glyphosate resistant and susceptible weed populations, more extensive documentation has been reported with respect to triazine resistant and susceptible biotypes.

In research conducted on the germination and seedling growth of atrazine resistant and susceptible biotypes of *Brassica campestris*, no significant differences were determined in overall percent germination between the biotypes; however, it was noted that after 24 hours, seed from susceptible biotypes germinated four times more than those from resistant biotypes (Mapplebeck et al. 1982). It was also found that susceptible seeds emerged from greater soil depths, displayed faster seedling growth, and exhibited more photosynthetic efficiency than did resistant seed and seedlings (Mapplebeck et al. 1982). Conard and Radosevich (1979) have reported that atrazine susceptible populations in both *Senecio vulgaris* and *Amaranthus retroflexus* species generated significantly greater dry matter and total seed amounts, and displayed increased overall competitive ability in comparison to resistant populations. Similar results were also found with respect to biomass and seed production among atrazine resistant and susceptible populations of *Amaranthus retroflexus* and *Amaranthus powellii* (Weaver & Warwick 1982). When triazine resistant and susceptible *Senecio vulgaris* were compared by Holt (1988), it was determined that, while the susceptible population was significantly greater in height and total dry weight at each harvest, no significant differences were detected in leaf number, root/shoot ratio, or total leaf area. It was also found that the susceptible plants concentrated more energy on stem tissue development for greater height, while the

resistant populations allocated resources toward more leaf tissue, and demonstrated greater leaf area ratios. The susceptible plants also generated greater reproductive output. These reports generally seem to support the theories advanced by Gressel and Segel (1978) that, in the absence of herbicides, resistant biotypes are less fit than susceptible biotypes.

Similar research has been conducted in atrazine resistant and susceptible *Chenopodium* species. Marriage and Warwick (1980) noted differential growth and response of common lambsquarters (*Chenopodium album*) plants when grown in noncompetitive conditions. In germination studies comparing two susceptible *Chenopodium album* populations (SI and SII) to a resistant biotype (R), germination of seed from SII was greater than SI and R, which were not significantly different. Maturity of seedlings, as well as seedling weight, was least in the R population and greatest in SI. These results are in agreement with others in observing that resistant *Chenopodium album* populations emerged 2 to 3 days later than susceptible biotypes, and suggest a competitive disadvantage during early stages of plant population development (Bandeem and McLaren 1976; Marriage and Warwick 1980). It was found, however, that beyond the seedling stage, the resistant population increased in height and leaf number and no longer expressed a competitive disadvantage in comparison with the susceptible biotypes. Due to the early-flowering of certain individuals in the SI population, the group was divided into early- and late-flowering, and further identified as ESI and LSI, respectively. It was found that these populations of mature individuals differed in their vegetative and floral weights and reproductive efforts with the ESI producing significantly less floral and vegetative dry weight than the other susceptible and the resistant populations. However,

the ESI had the greatest amount of total dry weight directed towards floral production. In evaluation of all plant growth parameters, the resistant biotype was always intermediate among the responses of the susceptible biotypes. The total and vegetative dry weights of LSI were significantly larger than the resistant biotype, but both were statistically equivalent to the SII susceptible biotype. No significant differences were observed between R and SII populations in total dry weight, vegetative dry weight, floral dry weight, or reproductive effort; however, they differed significantly in time to maturation. It was concluded that an increase in fitness of the susceptible biotype was not reflected in plant growth after the seedling stage, total biomass of plants, or seed production. It was observed that a competitive advantage of susceptible populations was most appropriately defined in early population establishment with more aggressive seed and seedling growth characteristics.

Warwick and Black (1981) have also documented differences in atrazine resistant and susceptible *Chenopodium album* and *Chenopodium strictum* populations. In mixed populations, the triazine susceptible biotype of *Chenopodium album* was found to be more competitive than either the triazine resistant *Chenopodium album* biotype or the triazine susceptible *Chenopodium strictum* biotype. Triazine susceptible and resistant biotypes of *Chenopodium strictum*, however, were found to be equally competitive. In noncompetitive situations, and in the absence of atrazine, triazine susceptible *Chenopodium album* populations produced greater aboveground total, vegetative, and reproductive dry weight than triazine resistant *Chenopodium album* populations. These results are similar to the findings of Conard and Radosevich (1979) with regard to *Senecio vulgaris* and *Amaranthus retroflexus*, but contrary to the observations of

Marriage and Warwick (1980) discussed previously with regard to common lambsquarters.

Previous work by the authors showed that differential responses were exhibited among collections of common lambsquarters plants in response to applications of glyphosate (Hite et al. 2008). F1 and F2 progeny from a suspected resistant population from Westmoreland County, Virginia showed reduced sensitivity to glyphosate relative to the Montgomery wild type population evaluated. However, variation in response did occur within F2 lines for each source. The objective of this research was to evaluate the growth, development, seed production, and seed viability of the least susceptible F2 line from the Westmoreland collection, an F2 line from the Westmoreland collection which was considered intermediate in response, and the most susceptible F2 line from the Montgomery collection.

3.3 Materials and Methods

Previous work conducted by the author involved glyphosate sensitivity of F1 and F2 common lambsquarters progeny from 2002 and 2004 collections made in Westmoreland County, Virginia (Hite et al. 2008). One aspect of the investigation involved the response of 15 F2 lines derived from three F1 Westmoreland sources and a wild type source from Montgomery County, Virginia. Substantial differences in vigor reduction were observed both among sources and among lines within each source (Hite et al. 2008). The objective of this research is to compare growth parameters within selected F2 lines from Westmoreland and Montgomery sources which had been shown to exhibit significant differences in susceptibility to glyphosate.

Seed from two 2004 F2 Westmoreland lines were used in this study. These F2 lines were created from F1 progeny from separate plants collected in 2004. These two lines, designated WR and WI, were selected because they exhibited differential susceptibility to a glyphosate rate of 1.68 kg ae/ha at 28 days after treatment (DAT). At this evaluation, WR and WI lines exhibited mean vigor response of 27 and 42%, respectively (LSD, $\alpha = 0.05$). A 2004 F2 Montgomery line, designated MS, was included in this research because this population demonstrated a vigor reduction of 100% in response to this rate of glyphosate at 28 DAT, significantly greater than the Westmoreland lines described above (LSD, $\alpha = 0.05$). Seed from these lines were selected for use in the growth chamber study because they represented F2 lines which exhibited least, intermediate, and greatest sensitivity to glyphosate.

3.3.1 Growth Chamber Experiments. Seed were planted separately in 52- by 27- by 6-cm flats containing commercial potting soil¹. Thirty six seedlings from each source flat were selected and transplanted into 11-cm pots with one plant per pot. The plants were allowed to acclimate in the pots and fertilized² once prior to placement in the growth chamber³. When plants were approximately 11.5 cm in height, they were placed in a growth chamber with a 12 h photoperiod of 450 $\mu\text{mol}/\text{m}^2/\text{s}$, day/night temperatures of 30/19 C, and 40-60 % relative humidity. Plants were watered daily.

Plants were harvested every 10 days for a total of 6 harvest timings. Plant height, number of axils, leaf number, leaf area⁴, and leaf size were recorded at each harvest. During reproductive growth stages, inflorescence length was also recorded. Roots and shoots were separated at the soil surface, potting soil was carefully removed from the roots, and root length was measured. The root and shoot material was placed in separate paper bags and dried in a constant temperature cabinet⁵ for 48 hours at approximately 74 C, and dry weights were recorded. For each harvest date, total plant dry weights were calculated and root/shoot ratios were determined. Plants in the final harvest were allowed to fully mature and senesce, and seed were collected and counted. These seed were used in the germination study, and served as the source of seedlings for F3 experiments. The experiment had three replications and was conducted twice. Factorial analysis of variance evaluation indicated no significant effect of repetition. Hence, data presented are combined over the two repetitions. Means were separated using Fisher's protected LSD test at 5 % level of probability.

3.3.2 Seed Germination. Twenty seed from each plant from the final harvest in the growth chamber experiments were randomly selected, and the perianth and pericarp from

each seed removed by hand. The seed were then placed between two pieces of filter paper in a 9-cm diameter plastic Petri dish, watered with 3 mL of distilled water on day one, and watered with 1 ml every other day throughout the remainder of the study. Petri dishes were evaluated weekly over a period of 28 days. Percent germination was defined as the occurrence in which the radicle protruded through the seed coat of each seed.

3.3.3 F3 experiments. Greenhouse experiments were also conducted on F3 seedlings from each replication and both repetitions from the WR and MS sources used in the growth chamber experiments. The seed were planted in separate 52- by 27- by 6-cm flats containing commercial potting soil¹. When the seedlings were approximately 2 cm in height, plants were transplanted into 11-cm pots with one plant per pot. Seedlings were grown using supplemental lighting⁶ of approximately 450 $\mu\text{mol}/\text{m}^2/\text{sec}$ with a 12 h photoperiod and were maintained at 22 to 27 C. Common lambsquarters plants were allowed to acclimate from transplanting and watered as needed. Glyphosate treatments were applied when plants were 7 to 11 cm in height. Glyphosate was applied at rates of 0.42, 0.84, 1.68, and 3.36 kg ae/ha using a stationary track sprayer⁷ containing a single even-edge nozzle tip⁸ that delivered 230 L/ha of spray solution. Four individual plant replications derived from plants from each repetition and replication of the growth chamber experiment for the WR source were treated with 0, 0.42, 0.84, 1.68, 3.36 kg ae/ha of glyphosate. Four individual plant replications for each repetition and replication of the growth chamber experiment for the MS source were treated with 0 and 0.84 kg ae/ha of glyphosate. Due to a lack of seed, four individual plants from repetition 2 and replication 1 from the MS source were treated with the full range of glyphosate rates. Factorial analysis of variance indicated a nonsignificant effect of repetition for plant

vigor and height variables on the F3 experiments, and data are therefore combined over repetitions. Factorial analysis of variance for the F2 source and collection site/glyphosate rate variables was conducted, and appropriate mean separation applied using Fisher's protected LSD at the 5% probability level.

3.4 Results and Discussion

3.4.1 Growth Chamber Experiments. Growth characteristics were compared among WR, WI, and MS common lambsquarters sources grown under uniform conditions within a growth chamber environment. Although many parameters observed were equivalent among the sources, some differences were identified. No significant differences were observed in common lambsquarters height among the sources across all harvest periods with the exception of harvest four, where plants from the WR and the MS sources were significantly shorter than those from the WI source (Table 3-1). No significant differences were detected among plants from the three sources in terms of the number of axils (data not presented). Significant effects of common lambsquarters source plant were observed for leaf number (Table 3-2). Generally, leaf number was greatest in MS plants relative to the Westmoreland sources. Significantly greater leaf number was observed for the MS source relative to the WR source at 30 and 40 days after introduction into the growth chamber. In research conducted by Marriage and Warwick (1980), somewhat similar results were reported, where one susceptible common lambsquarters biotype had significantly greater height and leaf number than the resistant biotype.

No significant differences in leaf area were exhibited among the three sources at any harvest periods except harvest four, where WR and MS plants were equivalent, but plants from the WI source had significantly greater leaf area (Table 3-3). Because leaf area was maximized for all sources by harvest two, the increased leaf area in WI at harvest four indicates delayed senescence and leaf drop for this source relative to WR and MS sources. Individual leaf size among the sources decreased through the course of the study

(Table 3-4). Generally, the MS source had smaller leaf sizes while the WR source had the largest. However, no consistent statically significant differences were observed with regard to this parameter.

Shoot dry weights in all sources were maximized in harvest four (Table 3-5). No statistical differences were observed until harvests four and five, where WI source shoots were significantly heavier than the shoots from WR and MS sources, which were equivalent. These results concur with those reported by Marriage and Warwick (1980), in that the vegetative dry weight of the resistant biotype was not significantly different from one of the susceptible sources, and intermediate among the other two susceptible sources. However, these results are contrary to those reported by Warwick and Black (1981), where it was observed that the atrazine susceptible common lambsquarters biotype had significantly greater vegetative dry weight than did the resistant biotype evaluated.

Significant differences in root dry weight among sources were observed in all harvests after 10 days in the growth chamber (Table 3-6). Roots from the MS plants were consistently heavier than those from WR and WI sources. It was also observed that the MS plants had significantly shorter root lengths than the WR plants in harvests three and four (Table 3-7). Although not directly quantified, it was observed upon washing that plants from the MS source had a noticeably denser tertiary root mass nearer the soil surface than plants from the WR source. For harvest three, this difference is exemplified by root densities of 0.017 and 0.034 g dry weight/cm for WR and MS sources, respectively. Essentially no information is available in the literature regarding root development in herbicide resistant and susceptible *Chenopodium* species. Holt et al.

(1988) observed greater root mass and root/shoot ratio in triazine resistant *Senecio vulgaris* relative to the susceptible biotype. Conard and Radosevich (1979), however, did not identify significant differences in root dry weight in comparing triazine resistant and susceptible biotypes of both *Senecio vulgaris* and *Amaranthus retroflexus*. Differential root development is also reflected in root/shoot ratios (Table 3-8). The MS source had significantly greater root/shoot ratios than the WR and WI sources at all harvests in the experiment.

Significant differences were observed in total weight among plants from the three sources at harvests two, four, and five (Table 3-9). Plants from the WR source were consistently the lightest at all harvest periods, while plants from the WI source, in most cases, were heaviest. Total weights of the MS plants were generally intermediate among the other two sources. Marriage and Warwick (1980) found similar data in that the atrazine resistant common lambsquarters biotype was intermediate in total dry weight among the susceptible biotypes studied.

Inflorescence lengths were measured in the four later harvests. Although some differences occurred in harvests two and four, there were no consistent statistically significant differences observed with regard to this parameter (data not presented). At the final harvest, seed was collected and seed characteristics were determined. No significant differences were observed for seed weight per plant or seed number per plant; however, the WR source had slightly, but significantly heavier individual seed weight than the MS source (Table 3-10).

3.4.2 Seed Germination. The percent germination of seed collected from the three sources differentiated dramatically after the first rating (Table 3-11). Beginning at 14

days after initiation of the experiments, the MS source was observed to germinate most rapidly, and to the greatest extent, followed by the WR and WI sources, respectively. Marriage and Warwick (1980) found that percent germination of *Chenopodium album* seed from an atrazine resistant biotype was significantly less than one of the susceptible biotypes, but statistically equivalent to the other susceptible biotype evaluated in the study.

Overall, the results of the growth chamber studies showed similar growth and development characteristics among the WR, WI, and MS sources. Differences were observed, however, with regard to root length, root weight, and therefore, root density. It could be speculated that reduced root density could represent a fitness penalty for the WR source relative to the MS source. Similarly, significantly reduced seed germination in the WR and WI sources relative to the MS source could represent a competitive disadvantage.

3.4.3 F3 Experiments. While it was not the intent of these experiments to comprehensively evaluate the susceptibility of F3 progeny to glyphosate, the collection of seed from plants grown in the growth chamber for viability evaluations also allowed the establishment of a limited number of F3 seedlings for evaluation of susceptibility to glyphosate.

In response to the 0.84 kg ae/ha application rate of glyphosate, no significant effect of F2 parent was observed within either the MS or WR sources (Table 3-12). The MS and WR sources did, however, differ significantly overall in response to glyphosate application at this rate. A differential response was observed between the MS and WR F3 lines as early as 14 DAT ($\alpha = 0.05$, data not presented). It was found that the MS

source was significantly more susceptible to glyphosate, with a mean vigor response of 55% at 35 DAT, relative to 21% vigor reduction in the WR source (Table 3-13).

Expanded evaluation of the WR F3 lines' susceptibility to a range of glyphosate rates demonstrated significant main effects of F2 parent and site of collection/glyphosate rate variables, and of the interaction between these variables for common lambsquarters vigor at 35 DAT (Table 3-13). At 35 DAT, the vigor reductions for the F3 MS lines at the 0.84 kg ae/ha glyphosate rate were found to be statistically equivalent to the responses of the WR F3 lines at the 1.68 kg ae/ha rate. Further, two of the three MS F3 lines at the 0.84 kg ae/ha glyphosate rate were equivalent to the WR F3 lines treated with 3.36 kg ae/ha of glyphosate. No mortality was observed in any of the WR F3 plants at the 3.36 kg ae/ha glyphosate rate. Vigor response to this rate ranged from 63 to 84% across lines, compared to a range of 41 to 76% for the MS source across lines in response to the 0.84 kg ae/ha rate.

Significant effects of F2 source and collection site/glyphosate rate variables were also observed for common lambsquarters height at 35 DAT. No significant interaction between these variables was observed, and therefore means for both variables from the factorial analysis of variance are presented (Table 3-14). Common lambsquarters height was similar at the 0.84 kg ae/ha rates for both WR and MS sources across F2 parent lines. Common lambsquarters height was significantly reduced with the 1.68 and 3.36 kg ae/ha glyphosate rates relative to heights observed with the 0.84 kg ae/ha rate of application. These results differ from those discussed above with regard to vigor reduction, where vigor was reduced significantly in response to the 0.84 kg ae/ha glyphosate rate in lines

from the MS source relative to vigor reductions in response to this rate in lines from the WR source.

From repetition 2 of the seed viability experiment, sufficient seedlings were obtained to allow evaluation of the response of MS common lambsquarters seedlings to 0.42, 0.84, 1.68, and 3.36 kg ae/ha rates of glyphosate (Table 3-15). A significant interaction between glyphosate rate and collection site/F2 source variables was observed, and therefore means separation is presented for one variable within individual levels of the second variable. Vigor reduction ranged from 85 to 100% for the MS source in response to glyphosate rates from 0.84 to 3.36 kg ae/ha. All WR F2 parent lines showed significantly reduced responses to the 0.84 kg ae/ha glyphosate application rate relative to the MS source, as did two of the WR F2 sources in response to the 1.68 kg ae/ha glyphosate rates. Response to the 3.36 kg ae/ha glyphosate rate among the WR sources was significantly lower than that observed in the MS source. Mortality of 100% was observed in the MS source in response to the 3.36 kg ae/ha, however, no mortality occurred in any WR sources in response to this rate of application (data not presented).

In conclusion, growth characteristics among the WR, WI, and MS sources grown in uniform conditions generally did not vary. It was observed that significant differences were evident in root growth and development, as well as in seed weight and germination rate. In comparing the response of F3 progeny of each source to a range of glyphosate rates, it was determined that the Westmoreland sources were significantly less sensitive than the Montgomery source.

3.5 Sources of Materials

¹ Sun Gro Metro-Mix 360 Growing Medium, Sun Gro Horticulture Distribution Inc., Bellevue, WA 98008.

² Peters 20-20-20, Scotts-Sierra Horticultural Products, Marysville, OH 43041.

³ M-12 Chamber, Environmental Growth Chambers, Chagrin Falls, OH 44022.

⁴ LI-COR Model LI-3100 Area Meter, LI-COR Inc., Lincoln, NE 68504.

⁵ STABIL-THERM constant temperature cabinet, Blue-M Electric Company, Blue Island, IL 60406.

⁶ Supplemental lighting, Sunlight Supply Inc., Vancouver, WA 98665.

⁷ Spray table, Allen Machine Works, Midland, MI 48640.

⁸ Teejet 8001EVS, Spraying Systems Co., Wheaton, IL 60166.

3.6 Literature Cited

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Warwick, S.I. and L. Black. 1981. The relative competitiveness of atrazine susceptible and resistant populations of *Chenopodium album* and *C. strictum*. *Can. J. Bot.* 59:689-693.

Weaver, S.E. and S.I. Warwick. 1982. Competitive relationships between atrazine resistant and susceptible populations of *Amaranthus retroflexus* and *A. powellii* from southern Ontario. *New Phytol.* 92:131-139.

Table 3-1. Common lambsquarters height (cm) at each harvest timing for WR, WI, and MS sources grown in the growth chamber^a.

Source	Harvest Timing				
	One	Two	Three	Four	Five
WR	11.5 a	29.0 a	55.6 a	59.6 b	58.5 a
WI	12.0 a	27.0 a	56.8 a	65.8 a	58.8 a
MS	10.7 a	28.5 a	54.4 a	56.8 b	55.0 a

^a Values within a column followed by the same letter do not differ significantly, LSD (0.05).

Table 3-2. Common lambsquarters total leaf number at each harvest timing for WR, WI, and MS sources grown in the growth chamber^a.

Source	Harvest Timing				
	One	Two	Three	Four	Five
WR	24.5 b	70.5 a	85.7 b	65.8 b	16.3 a
WI	32.2 a	78.8 a	93.7 b	87.7 a	11.5 a
MS	28.2 ab	89.3 a	110.8 a	88.8 a	24.5 a

^a Values within a column followed by the same letter do not differ significantly, LSD (0.05).

Table 3-3. Common lambsquarters leaf area (cm²) at each harvest timing for WR, WI, and MS sources grown in the growth chamber^a.

Source	Harvest Timing				
	One	Two	Three	Four	Five
WR	71.7 a	179.3 a	165.4 a	81.8 b	12.2 a
WI	80.1 a	187.0 a	186.4 a	124.6 a	5.6 a
MS	76.6 a	198.3 a	189.0 a	83.2 b	15.8 a

^a Values within a column followed by the same letter do not differ significantly, LSD (0.05).

Table 3-4. Common lambsquarters average leaf size (cm²) at each harvest timing for WR, WI, and MS sources grown in the growth chamber^a.

Source	Harvest Timing				
	One	Two	Three	Four	Five
WR	2.96 a	2.68 a	1.95 a	1.25 ab	0.69 a
WI	2.50 b	2.42 a	1.99 a	1.42 a	0.37 a
MS	2.76 ab	2.22 a	1.70 a	0.92 b	0.59 a

^a Values within a column followed by the same letter do not differ significantly, LSD (0.05).

Table 3-5. Dry weight of shoots (g) at each harvest timing for WR, WI, and MS sources grown in the growth chamber^a.

Source	Harvest Timing				
	One	Two	Three	Four	Five
WR	0.35 a	1.35 a	2.28 a	2.69 b	2.29 b
WI	0.44 a	1.49 a	2.83 a	3.64 a	3.12 a
MS	0.40 a	1.39 a	2.55 a	2.88 b	2.37 b

^a Values within a column followed by the same letter do not differ significantly, LSD (0.05).

Table 3-6. Common lambsquarters dry weight (g) of roots at each harvest timing for WR, WI, and MS sources grown in the growth chamber^a.

Source	Harvest Timing				
	One	Two	Three	Four	Five
WR	0.15 a	0.43 b	0.38 b	0.40 b	0.31 b
WI	0.20 a	0.37 b	0.49 a	0.55 ab	0.38 b
MS	0.27 a	0.72 a	0.56 a	0.63 a	0.49 a

^a Values within a column followed by the same letter do not differ significantly, LSD (0.05).

Table 3-7. Common lambsquarters root length (cm) at each harvest timing for WR, WI, and MS sources grown in the growth chamber.

Source	Harvest Timing				
	One	Two	Three	Four	Five
WR	17.3 a	19.0 a	22.9 a	18.7 a	16.2 a
WI	17.0 a	17.2 a	19.6 ab	17.3 a	18.3 a
MS	15.8 a	16.7 a	16.2 b	15.0 b	17.0 a

^a Values within a column followed by the same letter do not differ significantly, LSD (0.05).

Table 3-8. Common lambsquarters root/shoot ratios at each harvest timing for WR, WI, and MS sources grown in the growth chamber^a.

Source	Harvest Timing				
	One	Two	Three	Four	Five
WR	0.402 b	0.321 b	0.168 b	0.146 b	0.133 b
WI	0.446 b	0.256 b	0.172 b	0.153 b	0.120 b
MS	0.671 a	0.524 a	0.230 a	0.222 a	0.214 a

^a Values within a column followed by the same letter do not differ significantly, LSD (0.05).

Table 3-9. Common lambsquarters total weight (g) at each harvest timing for WR, WI, and MS sources grown in the growth chamber^a.

Source	Harvest Timing				
	One	Two	Three	Four	Five
WR	0.49 a	1.78 b	2.66 a	3.08 b	2.60 b
WI	0.63 a	1.86 ab	3.32 a	4.19 a	3.50 a
MS	0.67 a	2.11 a	3.11 a	3.51 b	2.86 b

^a Values within a column followed by the same letter do not differ significantly, LSD (0.05).

Table 3-10. Seed characteristics of mature individuals from WR, WI, and MS sources grown in the growth chamber^a.

Source	Seed weight (g per plant)	Seed weight (g per 100 seeds)	Seed per plant
WR	1.33 a	0.054 a	2483 a
WI	1.42 a	0.051 ab	2767 a
MS	1.19 a	0.048 b	2418 a

^a Values within a column followed by the same letter do not differ significantly, LSD (0.05).

Table 3-11. Germination rate among seed from WR, WI, and MS common lambsquarters sources grown in the growth chamber.

Seed germination				
Source	7 d	14 d	21 d	28 d
	%			
WR	9.2 a	40.0 b	47.5 b	55.0 b
WI	4.2 a	15.0 c	29.2 b	31.7 c
MS	4.2 a	65.8 a	80.0 a	80.0 a

^a Values within a column followed by the same letter do not differ significantly, LSD (0.05).

Table 3-12. Vigor response of F3 common lambsquarters lines from MS and WR collections to 0.84 kg ae/ha of glyphosate at 35 DAT^a.

Common lambsquarters vigor reduction ^b		
F2 parent number	Site of collection	
	MS	WR
	%	
1	76	20
2	41	24
3	48	20
Mean	55 a	21 b

^a Values for means followed by the same letter do not differ significantly, LSD (0.05).

^b Common lambsquarters vigor reduction (%), where 0 = no effect and 100 = death of plant.

Table 3-13. Effect of site of collection, glyphosate rate, and F2 parent on susceptibility of F3 common lambsquarters lines to glyphosate at 35 DAT^a.

Common lambsquarters vigor reduction ^b						
		Site of collection				
F2 parent number	MS	WR				
		Glyphosate rate (kg ae/ha)				
		0.84	0.42	0.84	1.68	3.36
		%				
1	76	6	20	51	84	
2	41	0	24	48	68	
3	48	1	20	34	63	

^a LSD (0.05) for rate means within a source = 27, LSD (0.05) for source means within a rate = 21.

^b Common lambsquarters vigor reduction (%), where 0 = no effect and 100 = death of plant.

Table 3-14. Effect of site of collection on F3 common lambsquarters height (cm) in response to varying rates of glyphosate at 35 DAT^a.

F2 parent number	Site of collection					Mean
	MS	WR				
	Glyphosate rate (kg ae/ha)					
	0.84	0.42	0.84	1.68	3.36	
1	12.3	22.9	18.3	11.8	7.4	14.5 c
2	25.8	32.1	22.3	17.1	16.1	22.7 b
3	28.8	43.9	34.6	28.9	17.4	30.7 a
Mean	22.3 bc	33.0 a	25.0 b	19.2 c	13.6 d	

^a Values for means followed by the same letter do not differ significantly, LSD (0.05).

Table 3-15. Vigor reduction of selected MS and WR F3 lines in response to glyphosate treatments at 35 DAT^a.

Common lambsquarters vigor reduction ^b				
Glyphosate rate	Site of collection			
	MS	WR		
	F2 one	F2 one	F2 two	F2 three
kg ae/ha	%			
0.42	8	13	0	3
0.84	85	13	30	30
1.68	95	43	65	40
3.36	100	75	78	68

^a LSD (0.05) for rate means within a source = 16, LSD (0.05) for source means within a rate = 16.

^b Common lambsquarters vigor reduction (%), where 0 = no effect and 100 = death of plant.