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ALLELOPATHIC EFFECT OF HAIRY VETCH (*Vicia villosa*) AND COWPEA (*Vigna unguiculata*) ON WEED AND VEGETABLE CROPS

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ALLELOPATHIC EFFECTS OF HAIRY VETCH (*Vicia villosa*) AND COWPEA (*Vigna unguiculata*) ON WEED AND VEGETABLE CROPS

By

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ABSTRACT

ALLELOPATHIC EFFECTS OF HAIRY VETCH (*Vicia villosa*) AND COWPEA (*Vigna unguiculata*) ON WEEDS AND VEGETABLE CROPS

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Hairy vetch (*Vicia villosa* Roth) and cowpea (*Vigna unguiculata* (L.) Walp), two leguminous cover crops, have been suspected of containing allelochemicals that allow them to suppress weeds and in some cases to affect the growth of vegetables. These studies were conducted to analyze species specific responses to the presence of residues of these two cover crops. Due to its suitability to Michigan's climate, hairy vetch served as the focus for our field study investigating the impact of hairy vetch residues on the weed community in pickling cucumber. The duration of hairy vetch phytotoxicity was examined by delaying cucumber planting dates after its incorporation. In the laboratory, the effect of hairy vetch and cowpea water, methanol, and ethyl acetate extracts on germination and radicle elongation of several vegetables and weeds was examined. Our results indicate that hairy vetch significantly reduces quackgrass populations in the field and consistently increases cucumber yield compared to no cover crop. Planting cucumbers 3 to 4 weeks after hairy vetch incorporation best maximizes yields. In the laboratory, vegetable and weed germination percentages varied in response to the extracts; however, the radicle lengths of most species tested were reduced. Occasional stimulation occurred at low extract concentrations in certain vegetable crops. Based on these findings it appears that both hairy vetch and cowpea possess allelopathic compounds which are able to help suppress weed growth when grown as a cover crop or applied as an extract.

Dedicated to my grandparents, parents, and brother

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CHAPTER 1: Introduction

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After decades of successfully increasing crop yields through intensive tillage and synthetic inputs (i.e. fertilizers and pesticides), the negative effects of conventional agriculture practices on the environment are becoming evident (Waller 2004). Field soils are eroding and ground waters are being polluted with nitrates from fertilizers and contaminants from pesticides. Resistance to pesticides by weeds, insects, and plant pathogens is also a result of constant selection pressure. Sustainable agricultural practices are born out of the realization that the environment needs to be preserved.

Sustainable agriculture promotes reduced reliance on synthetic inputs and the integration of cultural practices such as the use of green manures, conservation tillage, biological pest management, cover crops, and crop rotations to alleviate pest and nutrient problems (Waller 2004; Weston 1996).

Taking this concept a step further, organic agriculture bans the use of synthetic inputs and encourages the use of on farm resources. Certified organic cropland acreage in the U.S. nearly quadrupled between 1992 and 2003 (USDA-ERS 2003). In 2003, certified organic cropland in active production totaled over 1.4 million acres. The growth in organic agriculture can be partially attributed to an increasing demand by consumers to know when, where, and how their food is produced (Sooby 2004). It has also become easier to start growing organic crops due to clarified certification standards and in some cases state-funded subsidies for conversion from conventional to organic production.

In Michigan, vegetable production is one area concerned with sustainability. Many Michigan interest groups have specifically stated the need to look at improving cultural practices in vegetable production in their priority statements.

- Michigan Vegetable Council (MVC): *"research cultural practices to improve weed control in vegetable crops"* and *"Study cropping systems for vegetable crops, including the use of cover crops."*
- Michigan Integrated Food and Farming Systems (MIFFS) recommends research on sustainable production practices: *"Identify niche produce crops and determine sustainable production practices."*
- Michigan Organic Food and Farm Alliance (MOFFA): *"Research is needed to evaluate and assess the use of cover crops for nutrient management and for weed and pest control in organic production."*

From the listed priorities it is evident that weed management is a top concern. This is no surprise as herbicides account for 70% of the total volume of pesticides used in the U.S. agriculture (Duke 1996). In order to facilitate continued development in sustainable practices, new weed management strategies are being explored. Before the advent of synthetic herbicides, several systems were used to build weed suppressive cropping systems. One of these practices was the exploitation of allelopathy.

This research looks at the allelopathic potential of two leguminous cover crops, hairy vetch (*Vicia villosa*) and cowpea (*Vigna unguiculata*), as it may pertain to weed suppression and crop vigor.

CHAPTER 2: Literature Review

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ALLELOPATHY

Interactions occurring between plants that are biochemically facilitated by secondary compounds were first referred to as allelopathic by Hans Molisch in 1937. Though the study of allelopathy can encompass both positive and negative interactions among plants; negative interactions are generally the focus of most research studies. More recently, allelopathy has come to also include the microbial breakdown of secondary plant compounds that result in chemicals with suppressive properties (Weston and Duke 2003). These chemicals are referred to as allelochemicals and can be found in the tissues of nearly all plants. However, not all of these compounds with allelopathic potential will be released into the environment. The quantity of allelochemicals released or those created by microbes varies by species, chemical composition, and environmental conditions. The latter two factors also affect the persistence of the compounds (Putnam 1988).

In agriculture, allelopathy may serve as an alternative or a supplement to the use of synthetic herbicides for weed control. Alfalfa (*Medicago sativa*) and rice (*Oryza sativa*) are two examples of crops that have been shown to produce weed suppressing allelochemicals (Chon et al. 2002; Singh et al. 2003; Xuan et al. 2005). If isolated and identified, allelochemicals have the potential to be used to generate new herbicides (Cheema and Khaliq 2000; Putnam 1986). Allelochemical-based herbicides are natural products and thus could be broken down easily by microorganisms, making them less persistent in the environment (Rice 1984; Rice 1995). The allelochemicals of higher plants discovered as of 1988 [e.g. 2,4-dihydroxy-1,4(2H)-benzoxazin-3-one (DIBOA)

found in rye] lacked the potency to be widely used as herbicides creating the demand to examine compounds that are more effective (Putnam 1988). Another option is the transformation of plant allelochemicals to increase activity or suitability as herbicides. One example is the herbicide cinmethylin which is derived from 1,4-cineole, a terpene found in desert plants (Duke et al. 2000). The substitution made to create cinmethylin makes it more stable than 1,4-cineole. Research has also been conducted to exploit microbially derived allelochemicals as herbicides (Rice 1995; Duke 2000). The herbicide glufosinate is a synthetic version of phosphinothricin which is produced by *Streptomyces viridochromogenes* and *S. hygroscopicus* during the break down of bialaphos (Duke et al. 2000). Though currently natural products only make up a small portion of the herbicide industry, they remain important as they have the potential to exploit novel sites of action. Dakshini et al. (1999) stated the need for research on the "use of allelochemicals in biocontrol of specific weedy taxa, especially in cultivated areas" as a part of an integrated tactic to the understanding of allelochemical interactions.

Another option being explored is the utilization of cover crops with allelopathic properties for weed management. Cover crops are generally used as a management strategy to add organic matter to the soil, control erosion, prevent the leaching of nitrates between growing seasons, and physically suppress weeds. If cover crops possess allelochemicals that can inhibit the germination and growth of some weed species, then the reliance on synthetic herbicides could be reduced, saving money and preserving the environment. Examples of cover crops that have been found to produce allelochemicals include cereal rye (Weston 1996, Barnes et al. 1987), sorghum (Weston 1996; Cheema and Kaliq 2000), certain Brassicaceae species(Weston 1996; Weston and Duke 2003;

Netzley and Butler 1986; Bell and Muller 1973; Strivers-Young 1998) and legumes (Caamal-Maldonado 2001; Ohno et al. 2000; White et al. 1988).

ALLELOPATHIC COVER CROPS

Cereal rye, sorghum, Brassicaceae species, and legumes are currently some of the most commonly utilized cover crops in vegetable production. Each is under examination for the presence of allelochemicals and/or their potential to improve weed management. The activity of allelochemicals however, is not always restricted to weed species. There are several reports of cash crop injury (Burgos and Talbert 2000; Caamal-Maldonado et al. 2001; Chon et al. 2000; Putnam and DeFrank 1983). A broad list of potentially allelopathic cover crops is presented in Table 1, along with the corresponding weed and crop species the cover crops have been observed to suppress.

Cereal Rye

Cereal rye (*Secale cereale*) is a cool season annual grass. It is native to the mountains of Southwestern Asia. Cereal rye can grow to between 0.91-1.83 m tall and accumulate 4.47-11.22 tons·ha^{-1} of biomass. On average the above ground biomass contains 1% nitrogen. Cereal rye is winter hardy and drought tolerant (UC SAREP 2002).

Cereal rye can be used as a winter cover (Weston 1996) that once killed in the spring will control weeds for 30-75 days (Weston and Duke 2003). The compounds DIBOA (Dihydroxy-1,4(2H)-benzoxazin-3-one) and its breakdown product BOA (2(3H)-benzoxazolinone) have been found to be primarily responsible for rye's negative effects on crops and weeds (Barnes and Putnam, 1987) (Fig. 1a). Furthermore, BOA is

transformed by a soil dwelling bacterium, *Acinetobacter caloaceticus* into AZOB (2,2'-oxo-1,1'-azobenzene), another allelochemical with suppressive activity (Chase et al. 1991ab). Aqueous extracts of rye were shown to suppress the radicle growth of cucumber (*Cucumis sativus*), cantaloupe (*Cucumis melo*), summer squash (*Cucumis pepo*), lettuce (*Lactuca sativa*), sweet corn (*Zea mays*), and tomato (*Lycopersicon esculentum*), as well as weeds such as barnyardgrass (*Echinochloa crus-galli*), goosegrass (*Eleusine indica*), large crabgrass (*Digitaria sanguinalis*), Palmer amaranth (*Amaranthus palmeri*), and velvetleaf (*Abutilon theophrasti*) (Burgos and Talbert 2000). Stimulation of radicle growth in summer squash and sweet corn has been shown at low concentrations of the extract. It has become apparent that the susceptibility of weeds and crops to cover crop residues is species specific (Teasdale 1996), with smaller seeded species appearing to be the most sensitive (Burgos and Talbert 2000).

In a two year field study, rye was found to decrease weed biomass in pickling cucumber by 27% the first year and 77% the second compared to a no cover crop control (Ngouajio and Mennan 2005). Total marketable cucumber yield was increased 154 and 41% in those years, respectively.

Sorghum, Sudangrass, and Sorghum-Sudangrass

Sorghum, sudangrass (both referred to as *Sorghum bicolor*), and their hybrid, sorghum-sudangrass, (*S. bicolor* x *S. sudanese*) are all annual grasses. Sorghum is native to Africa. It can grow to between 0.46-5.00 m tall and accrue 16.80-22.41 tons of biomass per hectare with cutting. The average nitrogen content of the aboveground biomass is 1.5% of the total biomass. Sorghum is frost sensitive (UC SAREP 2002).

Sorghum residues kill existing weeds and prevent the germination of some weed seeds, thereby increasing the cash crop yield (Weston 1996). The compound responsible for these effects has been identified as a quinone named sorgoleone (Netzley and Butler 1986; Weston and Duke 2003) (Fig. 1b). The isolation and use of this chemical is more cost effective than synthetic herbicides (Cheema and Kaliq 2000).

Brassicas

Several members of the Brassicaceae family are used as cover crops. They are all broadleaf, winter annuals that quickly provide ground cover to reduce erosion (Stivers-Young 1998). Brassica cover crops observed to be allelopathic include: wild radish (*Raphanus raphanistrum*), black mustard (*Brassica nigra*), white mustard (*Brassica hirta*), rapeseed (*Brassica napus*), brown mustard (*Brassica juncea*), and turnip rape (*Brassica rapa*) (Al-Khatib et al. 1997; Bell and Muller 1973; Boydston and Hang 1995; Krishnan et al. 1998; Muller 1969; Norsworthy 2003; Ohno et al. 1999; Peterson et al. 2001; Singh et al. 2003; Weston and Duke 2003). The glucosinolates present in the tissue of Brassica species are converted into isothiocyanates, thiocyanates, and nitriles upon release during tissues damage (Bell and Muller 1973; Boydston and Hang 1995; Haramoto and Gallandt 2004; Kirkegaard and Sarwar 1998). The forms of glucosinolates and their hydrolyzed products vary by species, some of which suppress weeds, nematodes, and/or diseases (Figure 1c).

As an example, the presence of rapeseed and white mustard residues inhibit the emergence and growth of common lambsquarters (*Chenopodium album*), redroot pigweed (*Amaranthus retroflexus*), hairy nightshade (*Solanum sarrachoides*), and

longspine sandbur (*Cenchrus longispinus*) (Boydston and Hang 1995). Additionally, wild radish (*Raphanus raphanistrum*) restrains the germination and growth of corn, cotton, and wheat, showing that Brassica cover crops can pose a risk to both weeds and cash crops (Norsworthy 2003).

Legumes

Legume cover crops are popular in monoculture and in mixtures because of their association with rhizobia. In the presence of rhizobium, legumes form nodules in their root systems that house the nitrogen fixing bacteria. In addition to providing structure, the plants also provide the bacteria with nutrients and carbohydrates in exchange for nitrogen (Taiz and Zeiger 2002). The cover crop's symbiosis adds available nitrogen to the system that could be used by the following cash crop. Therefore, in addition to the previously discussed benefits of cover crops, legumes can also reduce the amount of external fertilizer needed to maintain a healthy cash crop. A survey conducted by the University of California Sustainable Agriculture Research and Education Program found that 52% of growers currently using cover crops stated that nitrogen fixation was a characteristic they desired when choosing cover crops (Ridgley and Van Horn 1994).

Allelopathy adds further appeal to the use of legume cover crops. Several legume species have been and are currently being examined for allelopathic properties. Included in this list are: alfalfa (*Medicago sativa*), clovers (*Trifolium* spp.), peas and beans, and vetches (*Vicia* spp.) (Abdul-Rahman and Habib 1989; Akemo et al. 2000; Caamal-Maldonado et al. 2001; Chikoye et al. 2002; Chon et al. 2000; Chon et al. 2002; Chung and Miller 1995; Harrison et al. 2004; Jones et al. 1999; Mohler and Teasdale 1993;

Moyer and Huang 1997; Ohno et al. 1999; Singh et al. 2003; Teasdale 1996; Teasdale and Daughtry 1993; White et al. 1989; Worsham and Blum 1992; Xuan et al. 2001; Xuan and Tsuzuki 2001). Hairy vetch and cowpea are examined more closely in the present study.

Hairy Vetch

Hairy vetch (*Vicia villosa* Roth) is a winter annual native to Europe and Asia. Other common names include: sand vetch, winter vetch, woolypod vetch, Russian vetch, and Siberian vetch. It has a climbing/trailing habitat and can grow to heights between 0.30-0.51 m in monoculture. The compound leaves of hairy vetch are made up of several pairs of leaflets. Hairy vetch is winter hardy and grows best on well drained soils. It can accrue between 4.82-7.68 tons/ha in biomass (UC SAREP 2002), and can contribute approximately 44.8 kg/ha of nitrogen (Ngouajio, personal communication) to the soil due to fixation. These characteristics along with its allelopathic potential against weeds make hairy vetch an ideal cover crop for Michigan.

In 1989, White et al. found that aqueous extracts of hairy vetch reduced corn and cotton (*Gossypium hirsutum*) germinations by up to 44 and 42%, respectively, depending on concentration. Corresponding radicle length reductions were 39 and 62%. In the same study, they found that the germination and radicle growth of pitted morningglory (*Ipoema lacunosa*), wild mustard (*Sinapis arvensis*), and Italian ryegrass (*Lolium multifolorum*) were all inhibited to some degree in the presence of the hairy vetch extract. Screening tests conducted by Fujii (2001) showed that water extracts of hairy vetch (67 g of material was extracted with 1 L of water 150 times) reduced the radicle elongation of

lettuce by 88% and hypocotyl growth by 11% compared with a non-treated control. However, germination was not affected. A methanol extract of hairy vetch (250 g of material was extracted with 1 L of methanol 40 times) reduced the radicle length was reduced by 82% and hypocotyl growth by 48%. In this case, germination was also reduced by 10%.

In 1993, Hoffman et al. conducted a two year field study comparing various killing methods for hairy vetch prior to crop planting. They found that both a chopped and a living hairy vetch cover crop reduced weed emergence and density, whereas hairy vetch that was rolled or killed using glyphosate did not. All treatments however, were found to reduce corn yield compared with the no cover crop, weed-free control. Hairy vetch that is left living has been shown to suppress weeds longer than desiccated hairy vetch; however, weed densities in both systems were less than density in the no cover crop control (Teasdale and Daughtry 1993). This finding suggests that something other than light transmission or temperature buffering is contributing to the reduction in weed density. More recently, in a two year study Ngouajio and Mennan (2005) reported reduced marketable cucumber yields in the presence of hairy vetch residues compared with a no cover crop control during the second year. The yields in the hairy vetch plots during both years were significantly lower than those in the rye plots and sorghum-sudangrass plots. The same study has shown that hairy vetch reduced weed density and biomass by 99 and 91%, respectively, when compared with the no cover crop control.

In cover crops that accrue a large amount of biomass, competition appears to play a large role in reducing weed populations. However, the amount of biomass accumulated by hairy vetch does not account for the degree of weed reduction observed, providing

further evidence of allelopathy (Fujii 2001). Drought conditions have been shown to exacerbate the growth inhibition caused by hairy vetch (Hoffman et al. 1993; Ngouajio and Mennan 2005). Therefore, it follows that the responsible allelochemicals are likely water soluble, resulting in higher concentrations under conditions, such as drought, that reduce leaching.

Cowpea

Cowpea (*Vigna unguiculata*) is a warm season annual. It is native to Africa and is also known by the names blackeye(d) pea, blackeye bean, crowder pea, and southern pea. Cowpea can reach heights between 0.48-0.61 m and accumulate biomasses of 4.25-5.39 tons/ha (in California). Cowpea stands erect with glabourous foliage. It has a taproot with large nodules. Cowpea needs warm temperatures and is sensitive to frost and flooding (UC SAREP 2002). For these reasons, cowpea is an ideal cover crop for the dry southern portions of the United States.

Increased death has occurred in transplanted broccoli (*Brassica oleracea*) following a cowpea 'Pinkeye Purplehull BVR' cover crop (Schroeder et al. 1998). Additionally, cowpea has displayed phytotoxic effects on 'Purple top' turnip (*Brassica rapa*); injury which was attributed to allelopathy (Wang et al. 2003). More recently, cowpea grown as a summer cover crop in pepper and lettuce production was shown to reduce weed density (Hutchinson and McGiffen 2000; Ngouajio et al. 2003).

To the best of our knowledge the above studies are the only published works looking at cowpea allelopathy on specific species. For this reason, any new knowledge gained on this cover crop is of value.

The allelopathic effects of the cover crops previously discussed, and those mentioned in Table 1 are all at varying stages of research and development. Cover crops such as rye and sorghum have already had their allelochemicals isolated and identified and researchers are currently looking at ways to put this knowledge to use. Others cover crops such as hairy vetch and cowpea are at the beginning stages of research to collect evidence to support claims of allelopathic effects. More information is necessary prior to the identification and understanding of the allelochemicals and their interactions in these two cover crops (Dakshini et al. 1999).

As a part of this effort, this thesis research aims to examine the response of vegetable crops and weeds to hairy vetch and cowpea cover crops in a series of field and laboratory experiments. It also will look to estimate the duration of any suppressive activities of hairy vetch under field conditions.

OBJECTIVES OF STUDY

Understanding the potential allelopathic effects of hairy vetch and cowpea on vegetables and weeds could help to select an appropriate cover crop and crop rotation. The subsequent research, described in this thesis, examines the use of hairy vetch and cowpea to improve weed management and the sustainability of vegetable production systems. Specific objectives of this research are to:

1) study the effect of a hairy vetch cover crop on weed populations and cucumber density and yield under field conditions (Chapter 3),

2) compare the yield and quality of cucumbers planted at delayed intervals following hairy vetch kill (Chapter 3), and

3) test the activity of hairy vetch and cowpea water, methanol, and ethyl acetate extracts on weeds and vegetable crops using laboratory bioassays (Chapters 4 and 5).

Table 1. Cover crop species known to negatively impact the growth of various weeds and crops (pg. 16-22).

Cover Crop Latin Name	Common Name	Crop and Weed Species Affected	Citation
Avena sativa	Oat	*Cassia obtusifolia*	Singh et al. 2003
		Chenopodium album	Singh et al. 2003
		Descurainia sophia	Moyer and Huang 1997, Singh et al. 2003
		Digitaria sanguinalis	Putnam and DeFrank 1983
		Echinochloa crus-galli	Singh et al. 2003
		Lactuca sativa	Putnam and DeFrank 1983
		Lolium spp.	Singh et al. 2003
		Portulaca oleracea	Putnam and DeFrank 1983
		Rhaphanus sativus	Putnam and DeFrank 1983
		Thlaspi arvense	Moyer and Huang 1997, Singh et al. 2003
Brassica hirta	White Mustard	*Amaranthus retroflexus*	Krishnan et al. 1998, Singh et al. 2003
		Capsella bursa-pastoris	Al-Khatib et al. 1997, Krishnan et al. 1998, Singh et al. 2003
		Kochia scoparia	Al-Khatib et al. 1997, Krishnan et al. 1998, Singh et al. 2003
		Setaria viridis	Al-Khatib et al. 1997, Krishnan et al. 1998, Singh et al. 2003
Brassica juncea	Brown Mustard	*Amaranthus retroflexus*	Krishnan et al. 1998, Singh et al. 2003
		Capsella bursa-pastoris	Krishnan et al. 1998, Singh et al. 2003
		Kochia scoparia	Krishnan et al. 1998, Singh et al. 2003
		Setaria viridis	Krishnan et al. 1998, Singh et al. 2003
Brassica napus	Canola/Rapeseed	*Amaranthus retroflexus*	Krishnan et al. 1998, Boydston and Hang 1995, Singh et al. 2003
		Avena ludoviciana	Jones et al. 1999, Singh et al. 2003
		Capsella bursa-pastoris	Al-Khatib et al. 1997, Krishnan et al. 1998, Singh et al. 2003
		Cenchrus longspinus	Boydston and Hang 1995, Singh et al. 2003
		Chenopodium album	Boydston and Hang 1995, Singh et al. 2003
		Descurainia sophia	Moyer and Huang 1997, Singh et al. 2003
		Echinochloa crus-galli	Boydston and Hang 1995, Singh et al. 2003
		Kochia scoparia	Al-Khatib et al. 1997, Krishnan et al. 1998, Singh et al. 2003
Brassica napus	Canola/Rapeseed	*Phalaris paradoxa*	Jones et al. 1999, Singh et al. 2003
		Rapistrum rugosum	Jones et al. 1999, Singh et al. 2003

Cover Crop Latin Name	Common Name	Crop and Weed Species Affected	Citation
Brassica napus	Canola/Rapeseed	*Setaria viridis*	Al-Khatib et al. 1997, Krishnan et al. 1998, Singh et al. 2003
		Sonchus oleraceus	Jones et al. 1999, Singh et al. 2003
		Thlaspi arvense	Moyer and Huang 1997, Singh et al. 2003
Brassica nigra	Black mustard	*Avena fatua*	Bell and Muller 1973
		Bromus mollis	Bell and Muller 1973
		Bromus rigidus	Bell and Muller 1973
		Grasses	Muller 1969, Weston and Duke 2003
Brassica rapa	Turnip rape	*Matricaria inodora*	Pertersen et al. 2001, Singh et al. 2003
		small seeded weed species	Peterson et al. 2001, Weston and Duke 2003
		Sonchus asper inodora	Pertersen et al. 2001, Singh et al. 2003
Canavalia ensiformis	Jackbean	*Amaranthus hypochondriacus*	Caamal-Maldonado et al. 2001
		Echinochloa crus-galli	Caamal-Maldonado et al. 2001
		Lycopersicon esculentum	Caamal-Maldonado et al. 2001
Cicer arietinum	Chickpea	*Avena ludoviciana*	Jones et al. 1999, Singh et al. 2003
		Phalaris paradoxa	Jones et al. 1999, Singh et al. 2003
		Rapistrum rugosum	Jones et al. 1999, Singh et al. 2003
		Sonchus oleraceus	Jones et al. 1999, Singh et al. 2003
Fagopyrum esculentum	Buckwheat	*Chenopodium album*	Rice 1984, Weston 1996
		Lactuca sativa	Iqbal et al. 2003
Hordeum vulagare	Barley	*Amaranthus retroflexus*	Putnam et al. 1983, Singh et al. 2003
		Ambrosia artemisiifolia	Putnam et al. 1983, Singh et al. 2003
		Avena ludoviciana	Jones et al. 1999, Singh et al. 2003
		Cassia obtusifolia	Singh et al. 2003
		Chenopodium album	Singh et al. 2003
		Descurainia sophia	Moyer and Huang 1997, Singh et al. 2003
		Echinochloa crus-galli	Singh et al. 2003
		Lactuca sativa	Putnam and DeFrank 1983
		Lolium perenne	Bertholdsson 2004
		Lolium spp.	Singh et al. 2003
		Lycopersicon esculentum	Putnam and DeFrank 1983
		Phalaris paradoxa	Jones et al. 1999, Singh et al. 2003

Cover Crop Latin Name	Common Name	Crop and Weed Species Affected	Citation
Hordeum vulagare	Barley	*Portulaca oleracea*	Putnam et al. 1983, Singh et al. 2003
		Rapistrum rugosum	Jones et al. 1999, Singh et al. 2003
		Rhaphanus sativus	Putnam and DeFrank 1983
		Sonchus oleraceus	Jones et al. 1999, Singh et al. 2003
		Thlaspi arvense	Moyer and Huang 1997, Singh et al. 2003
		Triticum aestivum	Ben-Hammouda et al. 2001
Lens culinaris	Lentil	*Descurainia sophia*	Moyer and Huang 1997, Singh et al. 2003
		Thlaspi arvense	Moyer and Huang 1997, Singh et al. 2003
Leucaena leucocephala	Jumbiebean	*Amaranthus hypochondriacus*	Caamal-Maldonado et al. 2001
		Echinochloa crus-galli	Caamal-Maldonado et al. 2001
		Lycopersicon esculentum	Caamal-Maldonado et al. 2001
Lysiloma latisliquum	Wild tamarind	*Amaranthus hypochondriacus*	Caamal-Maldonado et al. 2001
		Echinochloa crus-galli	Caamal-Maldonado et al. 2001
		Lycopersicon esculentum	Caamal-Maldonado et al. 2001
Medicago sativa	Alfalfa	*Abutilon theophrasti*	Chung and Miller 1995. Singh et al. 2003
		Amaranthus spp.	Chung and Miller 1995. Singh et al. 2003
		Avena factua	Ominski et al. 1999, Singh et al. 2003
		Brassica kaber	Ominski et al. 1999, Singh et al. 2003
		Bromus secalinum	Chung and Miller 1995, Singh et al. 2003
		Chenopodium album	Chung and Miller 1995, Singh et al. 2003
		Cirsium arvense	Ominski et al. 1999, Singh et al. 2003
		Cyperus difformis	Xuan and Tsuzuki 2001, Singh et al. 2003
		Digitaria ciliaris	Xuan and Tsuzuki 2001, Singh et al. 2003
		Digitaria spp.	Chung and Miller 1995, Singh et al. 2003
		Doparium junceum	Xuan et al. 2001, Singh et al. 2003
		Echinochloa crus-galli	Xuan and Tsuzuki 2001, Xuan et al. 2001, Chon et al. 2002, Singh et al. 2003
		Elatine tianda var. pedicellata	Xuan et al. 2001, Singh et al. 2003
		Eleocharis acicularis var. longiseta	Xuan et al. 2001, Singh et al. 2003
		Galium aparine	Ominski et al. 1999 Singh et al. 2003
		Imperata cylindrica	Abdul-Rahman and Habib 1989, Singh et al. 2003
		Lindernia pyxidaria	Xuan et al. 2001, Singh et al. 2003
		Medicago sativa	Chon et al. 2000, Chon et al. 2002

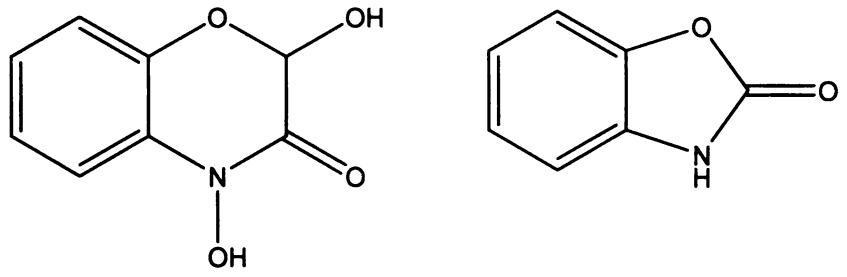
Cover Crop Latin Name	Common Name	Crop and Weed Species Affected	Citation
Medicago sativa	Alfalfa	*Monochoria vaginalis*	Xuan and Tsuzuki 2001, Singh et al. 2003
		Rotala indica var. uliginosa	Xuan et al. 2001, Singh et al. 2003
		Setaria faberi	Chung and Miller 1995, Singh et al. 2003
Muncuna deeringiana	Velvetbean	*Amaranthus hybridus*	Caamal-Maldonado et al. 2001
		Amaranthus hypochondriacus	Caamal-Maldonado et al. 2001
		Amaranthus spinosus	Caamal-Maldonado et al. 2001
		Brassica oleracea	Harrison et al. 2004
		Cenchrus insertus	Caamal-Maldonado et al. 2001
		Echinochloa crus-galli	Caamal-Maldonado et al. 2001
		Imperata cylindrica	Chikoye et al. 2002, Singh et al. 2003
		Lycopersicon esculentum	Caamal-Maldonado et al. 2001
		Parthenium hysterophorus	Caamal-Maldonado et al. 2001
Raphanus raphanistrum	Wild Radish	*Cyprerus esculentus*	Norsworthy 2003
		Gossypium hirsutum	Norsworthy 2003
		Ipomoea lacunosa	Norsworthy 2003
		Senna obtusifolia	Norsworthy 2003
		Sida spinosa	Norsworthy 2003
		Triticum aestivum	Norsworthy 2003
		Zea mays	Norsworthy 2003
Secale cereale	Cereal Rye	*Abutilon theophrasti*	Burgos and Talbert 2000
		Amaranthus palmeri	Burgos and Talbert 2000
		Amaranthus retroflexus	Putnam et al. 1983, Worsham and Blum 1992, Mohler and Teasdale 1993, Bhowmik and Inderjit 2003
		Amaranthus spinosus	Worsham and Blum 1992, Singh et al. 2003
		Ambrosia artemisiifolia	Barnes and Putnam 1983, 1986, Putnam et al. 1983, Singh et al. 2003
		Annual broadleaves	Barnes and Putnam 1983, Weston and Duke 2003
		Cassia obtusifolia	Singh et al. 2003
		Chenopodium album	Barnes and Putnam 1983, 1986, Singh et al. 2003, Mohler and Teasdale 1993
		Conzya canadensis	Przepiorkowski and Gorski 1994, Bhowmik and Inderjit 2003
		Cucumis melo	Burgos and Talbert 2000

Cover Crop Latin Name	Common Name	Crop and Weed Species Affected	Citation
Secale cereale	Cereal Rye	*Cucumis sativus*	Burgos and Talbert 2000
		Cucurbita pepo	Burgos and Talbert 2000
		Digitaria ischaemum	Putnam and DeFrank 1983
		Digitaria sanguinalis	Barnes and Putnam 1983, 1986, Burgos and Talbert 2000, Singh et al. 2003
		Echinochloa crus-galli	Burgos and Talbert 2000, Singh et al. 2003
		Eleusine indica	Burgos and Talbert 2000
		Epilobium cilatum	Przepiorkowski and Gorski 1994, Bhowmik and Inderjit 2003
		Ipoema lacunosa	Burgos and Talbert 2000
		Lactuca sativa	Barnes and Putnam 1983, Putnam and DeFrank 1983, Burgos and Talbert 2000
		Lolium spp.	Singh et al. 2003
		Lycopersicon esculentum	Burgos and Talbert 2000
		Panicum capillare	Mohler and Teasdale 1993
		Portulaca oleracea	Putnam and DeFrank 1983, Putnam et al. 1983, Singh et al. 2003
		Rhaphanus sativus	Putnam and DeFrank 1983
		Senna obtusifolia	Burgos and Talbert 2000
		Sesbania exaltata	Burgos and Talbert 2000
		Small seeded grasses	Burgos et al. 1999, Weston and Duke 2003
		Stellaria media	Mohler and Teasdale 1993
Sorghum bicolor 'Sorghum'	Sorghum	*Abutilon theophrasti*	Hoffman et al.1996, Weston 1996, Singh et al. 2003
		Amaranthus hybridus	Hoffman et al.1996, Weston 1996, Singh et al. 2003
		Amaranthus retroflexus	Putnam et al. 1983, Panasiuk 1986, Singh et al. 2003
		Ambrosia artemisiifolia	Putnam et al. 1983, Singh et al. 2003
		Annual broadleaves	Einhellig and Rasmussen 1989, Weston and Duke 2003
		Arachis hypogea	Sene et al. 2000, Weston and Duke 2003
		Digitaria ischaemum	Putnam and DeFrank 1983, Singh et al. 2003
		Echinochloa crus-galli	Panasiuk et al. 1986, Singh et al. 2003
		Lactuca sativa	Putnam and DeFrank 1983, Netzly and Butler 1986
		Lycopersicon esculentum	Putnam and DeFrank 1983
		Portulaca oleracea	Putnam et al. 1983, Putnam and DeFrank 1983, Singh et al. 2003

Cover Crop Latin Name	Common Name	Crop and Weed Species Affected	Citation
Sorghum bicolor 'Sorghum'	Sorghum	*Rhaphanus sativus*	Putnam and DeFrank 1983
		Rumex acetosella	Panasiuk et al. 1986, Singh et al. 2003
		Setaria virdis	Hoffman et al.1996, Weston 1996, Singh et al. 2003
Sorghum bicolor 'Sudangrass'	Sudangrass	*Digitaria ischaemum*	Putnam and DeFrank 1983
		Portulaca oleracea	Putnam and DeFrank 1983
Sorghum bicolor x S. Sudanese	Sorghum-sudangrass	*Lactuca sativa*	Putnam and DeFrank 1983
		Lycopersicon esculentum	Putnam and DeFrank 1983
		Rhaphanus sativus	Putnam and DeFrank 1983
Trifolium pratense	Red Clover	*Sinapis arvensis*	Ohno et al. 1999
Trifolium subterraneum	Subterranean Clover	*Amaranthus hybridus*	Worsham and Blum 1992, Singh et al. 2003
		Amaranthus retroflexus	Worsham and Blum 1992, Singh et al. 2003
		Amaranthus spinosus	Worsham and Blum 1992, Singh et al. 2003
		Cassia obtusifolia	Singh et al. 2003
		Chenopodium album	Singh et al. 2003
		Echinochloa crus-galli	Singh et al. 2003
		Lolium spp.	Singh et al. 2003
Triticum aestivum	Wheat	*Abutilon theophrasti*	Singh et al. 2003
		Amaranthus retroflexus	Putnam and DeFrank 1983, Blume et al. 2002, Singh et al. 2003
		Amaranthus spinosus	Singh et al. 2003
		Ambrosia artemisiifolia	Putnam et al. 1983, Singh et al. 2003
		Avena ludoviciana	Jones et al. 1999, Singh et al. 2003
		Cassia obtusifolia	Singh et al. 2003
		Digitaria ischaemum	Putnam and DeFrank 1983
		Echinochloa crus-galli	Singh et al. 2003
		Ipomoea hederacea	Singh et al. 2003, Blume et al. 2002
		Ipomoea lacunosa	Singh et al. 2003
		Ipomoea purpurea	Singh et al. 2003
		Lactuca sativa	Putnam and DeFrank 1983
		Lolium perenne	Al Hamdi et al. 2001
		Lycopersicon esculentum	Putnam and DeFrank 1983
		Phalaris paradoxa	Jones et al. 1999, Singh et al. 2003

Cover Crop Latin Name	Common Name	Crop and Weed Species Affected	Citation
Triticum aestivum	Wheat	*Portulaca oleracea*	Putnam and DeFrank 1983, Singh et al. 2003
		Rapistrum rugosum	Jones et al. 1999, Singh et al. 2003
		Rhaphanus sativus	Putnam and DeFrank 1983
		Sida spinosa	Blume et al. 2002, Singh et al. 2003
		Sonchus oleraceus	Jones et al. 1999, Singh et al. 2003
Triticum durum x *Secale cereale*	Triticale	*Cassia obtusifolia*	Singh et al. 2003
		Chenopodium album	Singh et al. 2003
		Echinochloa crus-galli	Singh et al. 2003
		Lolium spp.	Singh et al. 2003
Vicia villosa	Hairy vetch	*Abutilon theophrasti*	Mohler and Teasdale 1993, Teasdale 1996
		Amaranthus hybridus	Teasdale et al. 2005
		Amaranthus retroflexus	Mohler and Teasdale 1993, Teasdale 1996
		Chenopodium album	White et al. 1989, Mohler and Teasdale 1993, Teasdale 1996, Singh et al. 2003
		Cyprerus esculentus	Teasdale and Daughtry 1993
		Echinochloa crus-galli	Mohler and Teasdale 1993, Teasdale 1996
		Panicum capillare	Mohler and Teasdale 1993, Teasdale 1996
		Setaria glauca	Teasdale and Daughtry 1993
		Setaria virdis var. major	Mohler and Teasdale 1993, Teasdale 1996
		Solanum spp.	White et al. 1989, Singh et al. 2003
		Stellaria media	Mohler and Teasdale 1993, Teasdale 1996
		Taraxacum officinale	Mohler and Teasdale 1993
Vigna unguiculata	Cowpea	*Brassica oleracea*	Schroeder et al. 1998
		Brassica rapa	Wang et al. 2003

a.



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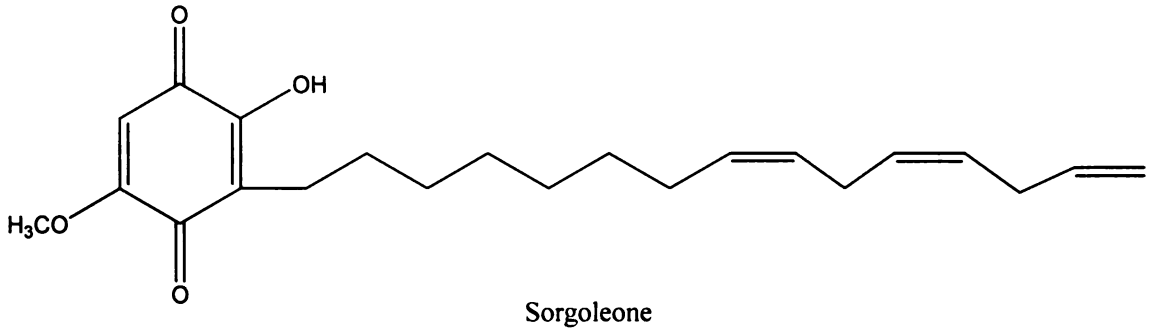
b.



Sorgoleone

c.

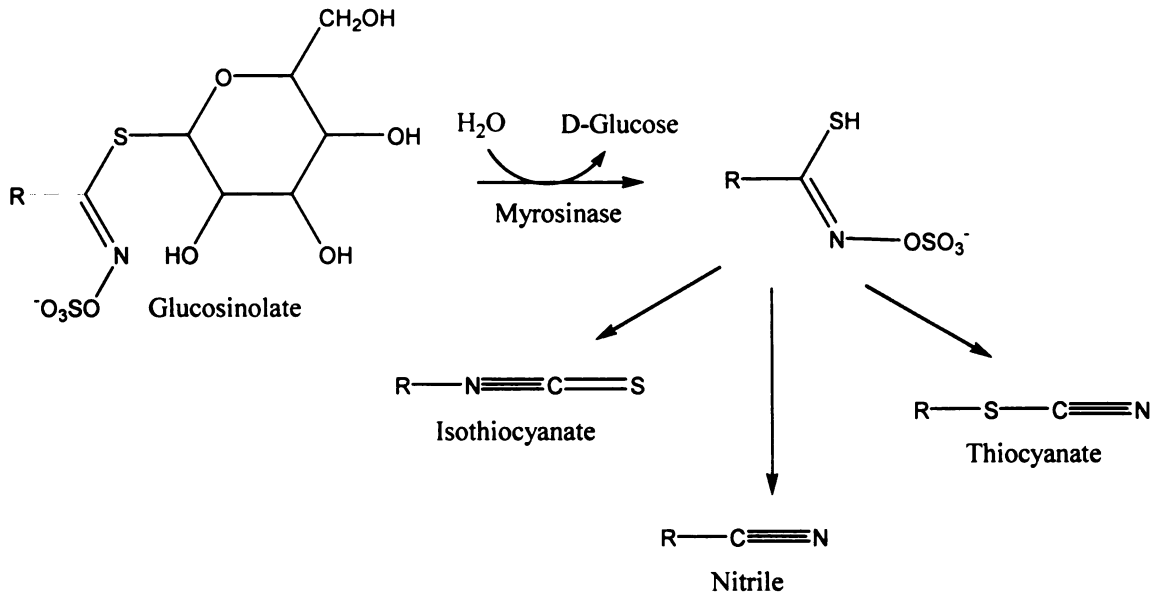


Figure 1. Allelochemical structures identified in cereal rye (a), sorghum (b), and brassica (c) cover crops.

CHAPTER 3: Response of Weed Species and Pickling Cucumber (*Cucumis sativus*) to a Hairy Vetch (*Vicia villosa*) Cover Crop

CHAPTER 3: Response of Weed Species and Pickling Cucumber (*Cucumis sativus*) to a Hairy Vetch (*Vicia villosa*) Cover Crop

ABSTRACT

Hairy vetch (*Vicia villosa* Roth) has been shown to affect the growth of some weed and crop species under field conditions. However, the response of individual weed species to hairy vetch residues and the duration of the effects following hairy vetch kill have not been investigated. A two year field study was conducted in East Lansing, Mich. to examine the effect of hairy vetch on weed populations and cucumber yield. The experiment was a split-plot design with four replications. The main factor was cover crop with two levels: hairy vetch and no cover (i.e. fallow). The subplot factor was staggered cucumber planting date with six levels: 0, 1, 2, 3, 4, and 5 weeks after hairy vetch kill (WAK). Nitrogen fertilizer was adjusted in the hairy vetch plots to account for symbiotic fixation. Weed density by species and total biomass were assessed at three and six weeks after cucumber plantings (WAP). Cucumber stand, vine biomass, yield, and fruit quality in each treatment were converted to percent of their corresponding fallow counterpart to remove confounding environmental factors during the staggered season and to allow for comparisons among planting dates. Overall, total weed biomass was not significantly different between the hairy vetch and fallow treatments; however there were significant changes to individual weed species densities (i.e. common purslane and quackgrass). Cucumber vine biomass per plant was increased for the latest planting dates. Fruit number was not significantly affected among planting dates. Yield reached two peaks during the season at the 0 and 4 WAK planting dates. The trend observed indicates that

any allelochemicals released are present between 1 and 3 WAK. All yields were higher in the hairy vetch treatments than the fallow. Finally, fruit firmness, specific gravity, and total soluble solid content were not different among planting dates. This study suggests that hairy vetch alone is not sufficient to achieve desired weed suppression. Other strategies should be combined with a hairy vetch cover crop to improve weed management. To achieve the highest yields and avoid difficulty planting in heavy residues, waiting 3-4 WAK would be the best planting time for cucumber.

INTRODUCTION

Michigan is the nation's largest producer of pickling cucumber (*Cucumis sativus*) with 14,000 ha harvested in 2004 valued at $35 million (MDA 2004). Pickling cucumbers have a short growing season allowing some growers to produce two crops per year. As a result of the intense production in pickling cucumber, and other vegetables in Michigan, growers and commodity groups (e.g. Michigan Organic Food and Farming Alliance and the Michigan Vegetable Council) are promoting the incorporation of cover crops into production systems to reduce nutrient losses and pesticide reliance (GREEEN 2005). Cover crops are noted for their abilities to add organic matter to the soil, improve soil structure, reduce erosion, prevent nutrient leaching, and suppress weeds. Legume cover crops also fix nitrogen and thus reduce the need for synthetic fertilizers for subsequent cash crops. In addition to these favorable qualities, increasing cases of weed suppression by cover crops via allelopathy have been reported (Caamal-Maldonado et al. 2001; Mohler and Teasdale 1993; Singh et al. 2003; Weston 1996; Weston and Duke 2003). Allelopathy is a mechanism by which some plants can positively or negatively

affect surrounding plants through the release of secondary plant compounds (Molisch 1937, Rice 1984). Some allelochemicals are produced directly by the plant, while others are the results of microbial breakdown of secondary plant compounds (Putnam 1986). Allelochemicals released from live cover crops or decaying residues have been deemed responsible for varying degrees of weed suppression and crop injury in field settings (Burgos and Talbert 2000; Singh et al. 2003; Weston and Duke 2003).

An ideal cover crop to incorporate into a pickling cucumber rotation in Michigan would be one that encompasses all of the beneficial traits mentioned above along with the ability to grow late in the season and to tolerate the cold winters. Hairy vetch (*Vicia villosa* Roth) is one cover crop that fits this profile. Hairy vetch is a cold hardy winter annual native to Europe and Asia which can contribute approximately 44.8 $kg \cdot ha^{-1}$ of nitrogen (Ngouajio, personal communication) to the soil due to fixation (Teasdale 1996; UC SAREP 2002).

Living hairy vetch has been shown to suppress weeds longer than desiccated hairy vetch; however, the weed densities of both were less than that of the fallow control (Teasdale and Daughtry 1993), suggesting that something other than light transmission, such as allelopathy, microenvironment changes, or enhanced predator or microbe populations, is contributing to the weed suppression. This idea is supported by Fujii (2001) who found that the biomass accumulated by hairy vetch does not account for the high degree of weed reduction observed compared with other cover crops such as rye (*Secale cereale*) and oat (*Avena sativa*). These cover crops have exhibited allelopathic potential (Singh et al. 2003; Putnam and DeFrank 1983; Burgos and Talbert 2000); therefore, it is possible that hairy vetch is even more allelopathic per unit of biomass.

Two studies have found that hairy vetch, living or killed, reduced corn (*Zea mays*) yields when compared with a no cover, weed-free control (Hoffman et al. 1993; Yenish et al. 1996). Likewise, Ngouajio and Mennan (2005) observed reduced marketable cucumber yields in the presence of hairy vetch residues during the second year of their study. In the same study, weed density and biomass in early spring were reduced by 99 and 91%, respectively, compared with a fallow control. These studies reported that drought conditions could have enhanced the inhibitory effects of hairy vetch (Hoffman et al. 1993; Ngouajio and Mennan 2005).

The above studies have found weed and/or crop suppression from hairy vetch. Few studies, however, have looked specifically at the response or individual weed species or the duration of the allelopathic effects of hairy vetch. Consequently, the objectives of this study were to: i) determine the effect of hairy vetch residue on weed populations and species composition, ii) assess the duration of any cucumber suppressive effects, and iii) establish if the timing of hairy vetch kill in relation to cucumber planting date has an impact on cucumber growth, yield, and fruit quality.

MATERIALS AND METHODS

Experimental Site and Procedures

Hairy vetch was planted at the Michigan State Horticultural Research Farm in East Lansing, Michigan on September 2, 2003 and September 13, 2004. The plot was previously fallow in the spring and summer of 2003. The soil was a Thetford loamy sand (pH 7.4 and 173 $kg \cdot ha^{-1}$ phosphorus, 269 $kg \cdot ha^{-1}$ potassium, 2466 $kg \cdot ha^{-1}$ calcium, and 377 $kg \cdot ha^{-1}$ magnesium, sandy, mixed, mesic, Psammaquentic Hapludalfs). The

experimental plot was divided into eight equal sized regions (each 215 m^2), four of which were seeded at a rate of 39.2 $kg \cdot ha^{-1}$ of hairy vetch and four of which were left fallow. The following springs, on May 28, 2004 and June 1, 2005, the whole field was disked and the seedbed smoothed using a cultipacker. Caution was taken to avoid transferring hairy vetch residues to fallow areas.

The field was organized as a split-plot with four replicates. Levels of the main plot factors were hairy vetch and no cover (i.e. fallow). Each main plot was subdivided into six subplots, each randomly assigned to the six cucumber planting dates. Therefore, each cucumber planting in the hairy vetch treatments had a corresponding planting in the fallow treatments to factor out environmental differences occurring over the six week planting period. Pickling cucumber 'Vlaspik' was planted at weekly intervals, 0, 1, 2, 3, 4, and 5 weeks after hairy vetch kill (WAK). Individual subplots consisted of four 9.14 m rows in 2004 and six 6.10 m rows in 2005. In both years, rows were spaced 46 cm apart, with an in row spacing of 13 cm. Prior to planting each week, the seed bed in each plot to be planted was prepared using hoes and rakes in 2004 and a rototiller in 2005. Two seeds per hole were planted by hand, as planting equipment was too large to fit in the individual plots. Also, hand planting prevented residues from the hairy vetch regions from contaminating the control plots and provided better control of the seeding rates in the small plots. Three weeks after planting, cucumbers were thinned to one plant per 13 cm within the rows.

Three weeks after planting, in 2004, the control and hairy vetch plots received 448 and 336 $kg \cdot ha^{-1}$ of a 34-0-0 (N_2-P_2O_5-K_2O) fertilizer, respectively. On account of symbiotic nitrogen fixation, the hairy vetch plots required less fertilizer. Previous studies

showed that hairy vetch can add approximately 44.8 $kg \cdot ha^{-1}$ of nitrogen in monoculture (Ngouajio, personal communication). In 2005, a 19-19-19 (N_2-P_2O_5-K_2O) fertilizer was applied to the entire field at 448 $kg \cdot ha^{-1}$ on April 29 (i.e. 34 days prior to hairy vetch kill). At the time of cucumber planting, 198 $kg \cdot ha^{-1}$ for the control and 119 $kg \cdot ha^{-1}$ for the hairy vetch plots of 34-0-0 (N_2-P_2O_5-K_2O) fertilizer was added to total the same amount of nitrogen that was applied in 2004.

In the summer 2004, rainfall was sufficient to forgo irrigation. However, in 2005, sprinklers were used to supplement rainfall. Irrigation was turned on for 1-2.5 hours (depending on soil moisture) following each planting to set in the fertilizer and improve seed germination. Beyond that, the irrigation was turned on as needed when plants were visibly water stressed.

Plots were weeded by hand as necessary leaving one 50-by-50 cm microplot per plot undisturbed for weed sampling.

Data Collection

Prior to field preparation, 50-by-50 cm plant samples were taken from the hairy vetch treatments to measure fresh and dry biomass. Samples were dried at 70 °C for 7 days.

During the cucumber season, all weeds in the 50-by-50 cm microplots were removed at three and six weeks after planting (WAP). These weeds were separated by species and counted. Due to the small quantity of most weed species, all weeds were recombined and dried at 70 °C for 7 days to obtain a dry biomass. To compare the weed biomass in the hairy vetch and fallow plots through out the season the 3 and 6 WAP

samples were combined for each cucumber planting date. At the end of each season, weed species were placed in one of three categories: 1) prominent weeds, those appearing at high density with uniform distribution (24 or more of the 48 microplots) across treatments, 2) important weeds, those appearing at high density with uneven distribution (10-23 microplots) or those appearing at low density with uniform distribution, and 3) other weeds, those appearing at low density with patchy distribution (less than 10 microplots).

Three weeks after planting cucumber stand was recorded. At harvest, a total of 60 feet of cucumbers were harvested (i.e. 2 rows in 2004 and 3 rows in 2005) from the center rows. Stand was recorded in the field along with total vine fresh biomass after the fruits were removed. Vine subsamples were collected and dried at 70 °C for 7 days to estimate total dry biomass. Fruits were sorted into grades 1, 2, 3 (USDA 1997), and over-sized, then counted and weighed by grade.

Ten grade 2 sized cucumbers were selected for laboratory analysis of specific gravity, firmness, and total soluble solids content the day of harvest. The water displacement method was used to measure volume for specific gravity (Ngouajio et al. 2003). In this method, the volume of the fruit is measured by the volume of water displaced from a column after submerging the fruit. Firmness was determined as the maximum force needed to puncture the mesocarp of a 2.5 cm thick cucumber slice from the midpoint of the fruit by a digital penetrometer (DPS-11, Imada, Inc. Northbrook, IL 60062) equipped with a 49 N load cell and a cylindrical plunger with a diameter of 5 mm and a flat head was recorded. After removing the seeds and peel of a second slice, closer to the base of the fruit, it was crushed using a hand garlic press and the exuded liquid was

placed on a digital refractometer with temperature compensation (Palette PR-32, National Microscopic Exchange, Carnation, WA 98014) to discern the percentage of soluble solids. Firmness data for 2005 were lost, therefore only the 2004 data will be presented.

Statistical Analysis

Because the important species of weeds changed from 3 WAP to 6 WAP and from year to year, data were analyzed separately. Cucumber data in each treatment were converted to percent of their corresponding fallow counterpart to remove confounding environmental factors during the staggered season and to allow for comparisons among planting dates. The converted cucumber data were then analyzed as a completely randomized design to test the effect of planting date among hairy vetch treatments. All data were subjected to analysis of variance to test the differences between hairy vetch and fallow treatments and to test for any treatment-by-year interactions for cucumber data only. Since there were no treatment-by-year interactions for any of the parameters, the cucumber data from both years was combined. Normality of the residuals was checked and outliers were removed. In the event of a significant treatment effect ($p<0.05$), means were separated in SAS (Version 8, SAS 2001) using Fisher's protected LSD. Regression analyses were then performed for vine biomass measurements using TableCurve™ 2D (Version 4, AISN Software, Inc., 1996). The following second degree polynomial equation was used:

$$y = \quad a + bx^c \qquad [1]$$

where y is the vine biomass per plant, x is the planting date in WAK, and a, b, and c are regression coefficients.

RESULTS

Hairy Vetch

Prior to disking, the hairy vetch fresh biomass was 34,700 $kg \cdot ha^{-1}$ in 2004 and 32,700 $kg \cdot ha^{-1}$ in 2005. The corresponding dry biomasses totaled 3,720 $kg \cdot ha^{-1}$ and 6,240 $kg \cdot ha^{-1}$, respectively.

Weed Density

In both years of study, quackgrass (*Elytrigia repens*, AGRRE) and common purslane (*Portulaca oleracea*, POROL) were prominent weeds at all sampling dates (Figure 1). In 2004, the important weed species collected 3 WAP included redroot pigweed (*Amaranthus retroflexus*, AMARE), yellow rocket (*Barbarea vulgaris*, BARVU), shepherd's-purse (*Capsella bursa-pastoris*, CAPBD), common lambsquarters (*Chenopodium album*, CHEAL), henbit (*Lamium amplexicaule*, LAMAM), eastern black nightshade (*Solanum ptycanthum*, SOLPT), and common chickweed (*Stellaria media*, STEME). At 6 WAP all of the above listed weeds were still important weeds except eastern black nightshade (Figure 2). In 2005, the important weed species observed 3 WAP were redroot pigweed, common lambsquarters, large crabgrass (*Digitaria sanguinalis*, DIGSA), witchgrass (*Panicum capillare*, PANCA), wild-proso millet (*Panicum miliaceum*, PANMI), eastern black nightshade, and common chickweed. At 6 WAP, redroot pigweed, quackgrass, shepherd's-purse, common lambsquarters, witchgrass, wild-proso millet, common purslane, and common chickweed were the major weed species (Figure 3). Of the above species listed, common purslane density was

significantly higher in the hairy vetch plots than in the fallow 3 WAP in 2005. Shepherd's-purse density was higher in the fallow plots 6 WAP in 2005. Finally, 3 WAP in 2004 and 6 WAP in 2004 and 2005, quackgrass density was significantly reduced in the hairy vetch plots.

Weed Biomass

When averaged over all planting dates, the hairy vetch and fallow treatments produced similar amounts of weed biomass in both 2004 and 2005 (Figure 4). Overall, the 2005 season had nearly double the weed biomass compared with the 2004 season. During individual growing seasons, weed biomass varied with cucumber planting date (Figure 5). In 2004 for example, the hairy vetch treatment had a greater weed biomass than the control when cucumber was planted immediately after hairy vetch kill. As the delay in cucumber planting increased, weed biomass in the hairy vetch plots tended to decrease compared with the fallow treatment, until 4 WAK. Beyond this date, weed biomass increased again in the hairy vetch treatments. In 2005 weed biomass was similar in the two treatments, except at 3 and 4 WAK when the hairy vetch treatments had a greater weed biomass than the fallow.

Cucumber Stand

Cucumber stand 3 WAP was significantly higher for the 2 WAK planting date compared with all other planting dates (Table 1). At 120% of the control, it was the only planting date that had more plants than the corresponding fallow control.

At harvest, cucumber stands were different among planting dates within the hairy vetch treatments. The 0 and 4 WAK planting dates had the lowest stands at 90 and 84% of their controls, while the 1 and 2 WAK dates were the highest at 113 and 103%, respectively.

Cucumber Vine Biomass

Changes in vine fresh and dry biomasses in response to planting date were examined on a per plant basis and were adequately described using Equation 1 (Figure 6). Fresh biomass per plant in the hairy vetch plots averaged around 160% of the no cover control for planting dates 0 through 3 WAK and then rose to a maximum of 242% for the 5 WAK planting date. Dry biomass per plant for the hairy vetch treatments was approximately 140% of the control for planting dates 0 through 4 WAK and then rose to 189% for the 5 WAK date.

Cucumber Yield

Total fruit number was not significantly different among planting dates for hairy vetch (Table 1). The largest number of fruits (150% of the control) was reached for the 1 WAK planting date, while the smallest number (120%) was observed for the 0 WAK date.

Many of the hairy vetch plots were ready for harvest up to a week prior to their corresponding fallow plots. Harvest was delayed to allow further maturation of the fruit in the control plots. Therefore, many of the hairy vetch plots had higher yields in the #3 and over-sized grades than would normally be acceptable by a grower. For this reason,

total yield was the focus of our analysis as opposed to marketable yield which would exclude the over-sized fruits (Figure 7).

Total cucumber yield in the hairy vetch plots was at a maximum of 236% of the control for the 0 WAK planting date. After this, yield declined, reaching a low of 113% for the 2 WAK date. Total yield then experienced another increase to 198% 4 WAK before settling to 141% for the 5 WAK date. The pattern of total yield change in response to planting date was consistent for both the 2004 and 2005 growing seasons, and was similar for marketable yield.

Cucumber Fruit Quality

The selected fruit quality measures (i.e. specific gravity, firmness, and the percentage of soluble solids) were not found to be affected by hairy vetch, nor by planting date within the hairy vetch plots (Table 1). Specific gravity for all dates was very close to 100% of the controls. Firmness was slightly higher for the first three planting dates than the last three, but the difference was not statistically significant. The percent of soluble solids for the hairy vetch plots were all slightly less than the controls. The closest to 100% of the control was the 2 WAK planting date at 99%.

DISCUSSION

Weeds

Quackgrass and common purslane were by far the most prominent weeds during the two years of study. Of the weeds sampled, quackgrass seems to be the most sensitive species to hairy vetch. Based on our observations it could be possible that the suppression

of quackgrass is due to allelopathy, competition during the off season, or a combination of the two factors (Wu et al. 2001). Future greenhouse and laboratory studies examining the relationship between hairy vetch and quackgrass will be useful in determining the processes involved in the suppression observed from the hairy vetch plots.

Common purslane occurred at greater densities in the hairy vetch plots than in the fallow plots, though only significantly so 3 WAP in 2004. Perhaps increased moisture retention caused by the hairy vetch residues resulted in favorable conditions for common purslane growth (Teasdale and Mohler 1993, Teasdale and Daughtry 1993). Mohler and Teasdale (1993) observed increased emergence in some weed species in the presence of low rates of hairy vetch residues. Another possibility is that a compound released from the hairy vetch is stimulating the germination of common purslane, similar to ethylene in the presence of witchweed (*Striga asiatica*) (Putnam 1988).

Weed biomass was almost doubled from 2004 to 2005. Overall, hairy vetch tended to have higher weed dry biomass than the controls at the sample dates. Due its high density, common purslane contributed significantly to the observed total weed biomass.

Cucumber

When comparing the stand counts taken at harvest to those 3 WAP, it was found that some planting dates resulted in reduced stands in the hairy vetch plots and others in the no cover plots. Based on this observation, hairy vetch does not seem to significantly impact stand between 3 WAP and harvest. This implies that any potential allelochemical from hairy vetch may be reducing cucumber growth as opposed to killing the seedling. It

is also interesting to note that some of the highest stands at harvest equated to some of the lowest yields (Table 1 and Figure 7). Therefore, yield per plant was low at that time; this could be related to allelopathic interference, or perhaps intraspecific competition.

Cucumber vine biomass per plant, both fresh and dry, increased with delayed planting date in the hairy vetch plots. Perhaps the increase in biomass toward the end of the season indicates a release from allelopathic pressure, a better synchrony between nutrient release from the hairy vetch residue and cucumber uptake, or a more favorable microenvironment.

When looking at cucumber yield, the hairy vetch plots planted at 0 WAK and 4 WAK produced the highest yields. The trend seen in Figure 7 seems to indicate that the potential allelochemicals from hairy vetch are not released immediately or not readily available after incorporation (Inderjit et al. 1995), but rather during the 1-3 WAK period. It also suggests that once plants are established their yield is not affected by these allelochemicals (e.g. the 0 WAK plants were able to grow sufficiently so that when the allelochemical concentration increased during hairy vetch decomposition they were no longer susceptible). Though the 0 and 4 WAK plantings performed the best among hairy vetch plantings, it is important to note that all planting dates within the hairy vetch plots yielded at or above their corresponding fallow plots. This cannot be attributed entirely to nitrogen fixation by hairy vetch since the fertilizer rates were reduced in the hairy vetch treatments unless hairy vetch was contributing more than our estimated 44.8 $kg \cdot ha^{-1}$ or it was enhancing the populations of nitrogen fixing mycorrhizal fungi. Teasdale and Shirley (1998) found hairy vetch residues occasionally could add over 112 $kg \cdot ha^{-1}$ of nitrogen during the growing season. Hairy vetch plots were ready to harvest up to a week ahead of

their fallow counter parts. This suggests that better growing conditions existed in the hairy vetch system. Several other benefits of cover crops including temperature buffering, moisture retention, and increased beneficial organism populations are documented in the literature (Teasdale and Daughtry 1993).

CONCLUSIONS

Results of this study suggest that incorporated hairy vetch does not sufficiently suppress weed biomass. An alternative to be considered could be the use of hairy vetch as a mulch, therefore also acting as a physical barrier to weeds. Planting cucumber two to three weeks after hairy vetch kill would best take advantage of hairy vetch's weed suppressing activity, reducing competition with cucumber.

Hairy vetch increases the populations of some species (i.e. common purslane), while decreasing those of others (i.e. quackgrass). Therefore, the composition of the pre-existing weed community will dictate the success of suppression by hairy vetch. Continuous use of hairy vetch could result in dramatic weed population shifts over short periods of time. For this reason, a rotation of cover crops may be needed to maximize the weed suppressing benefits.

The hairy vetch treatments in this study consistently out yielded the fallow treatments at all planting dates. Optimum yields were found for cucumbers planted immediately after hairy vetch kill (0 WAK) and 4 WAK. Planting immediately after hairy vetch kill is not practical due to the interference of the fresh residue with planting equipment (Teasdale and Shirley 1998). Therefore, we suggest that to optimize yields,

growers wait approximately three to four weeks after hairy vetch kill before planting cucumber.

Table 1. Effect of hairy vetch on cucumber stand and fruit quality at different planting dates following hairy vetch kill. Data from 2004 and 2005 were combined and are expressed as a percentage of the no cover (fallow) compliment. LSD values are presented for parameters that showed a significant difference ($p<0.05$) between planting dates. Data from 2004 and 2005 were combined.

Planting Date (WAK)	Stand (3WAP)	Stand (Harvest)	Total Fruit Number	Specific Gravity	Firmness (2004)	Soluble Solids
	Percent of No Cover Control					
0	99.07	90.24	120.05	100.25	102.77	98.98
1	95.51	113.42	151.78	100.20	105.85	96.67
2	120.07	103.81	124.86	100.63	108.24	99.17
3	96.85	98.22	126.09	100.24	96.93	94.64
4	83.81	84.28	122.83	100.13	98.16	96.58
5	93.97	97.97	128.30	99.85	90.06	96.70
LSD (0.05)	**19.61**	**13.75**	**NS**	**NS**	**NS**	**NS**

Planting dates are in weeks after hairy vetch kill (WAK).

NS=Not Significant

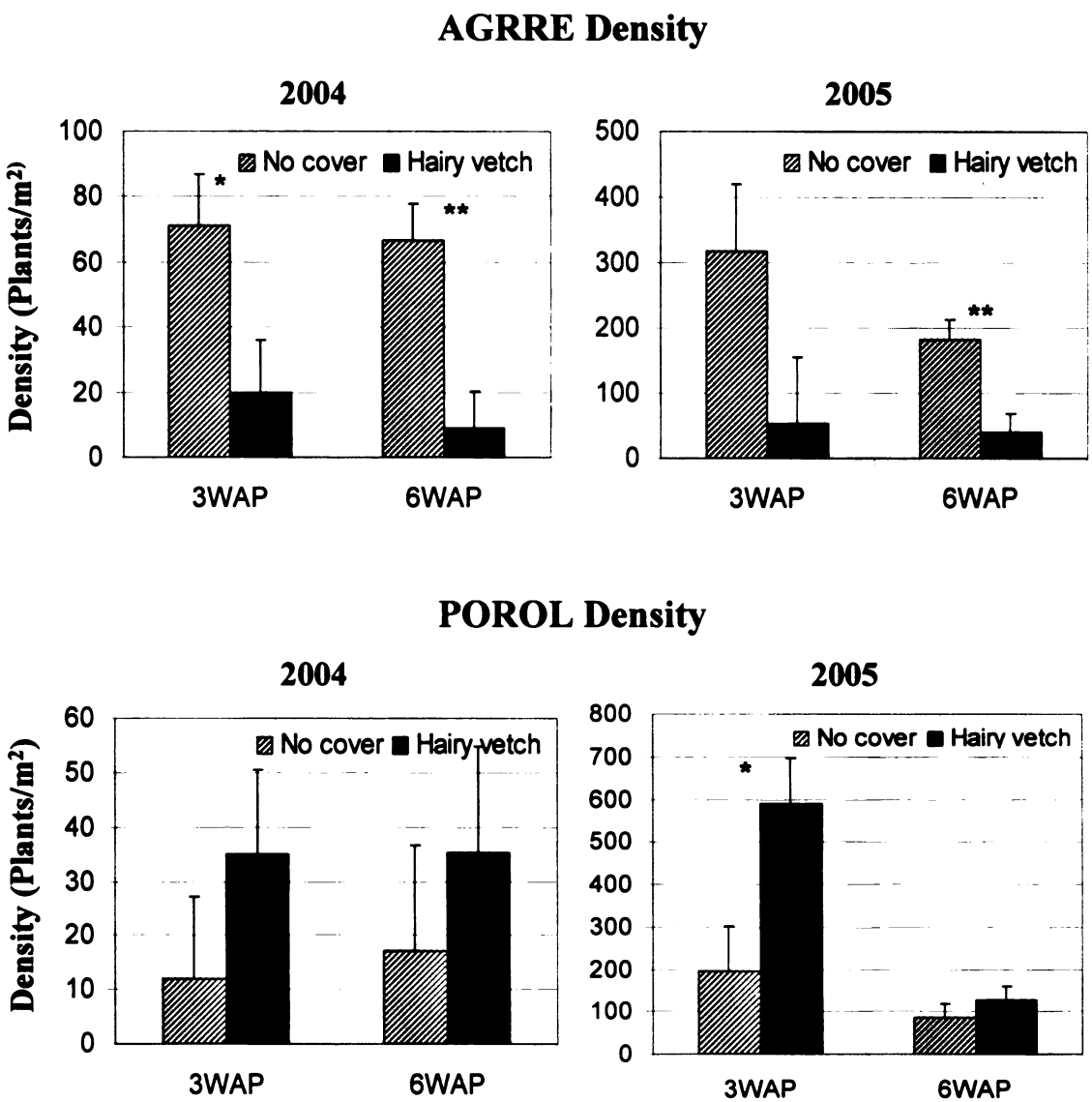


Figure 1. Densities of the two prominent weed species, quackgrass (AGRRE) (top) and common purslane (POROL) (bottom) for evaluations taken at 3 and 6 weeks after cucumber planting (WAP) in 2004 and 2005. Significance is indicated by * ($p<0.05$) or ** ($p<0.01$).

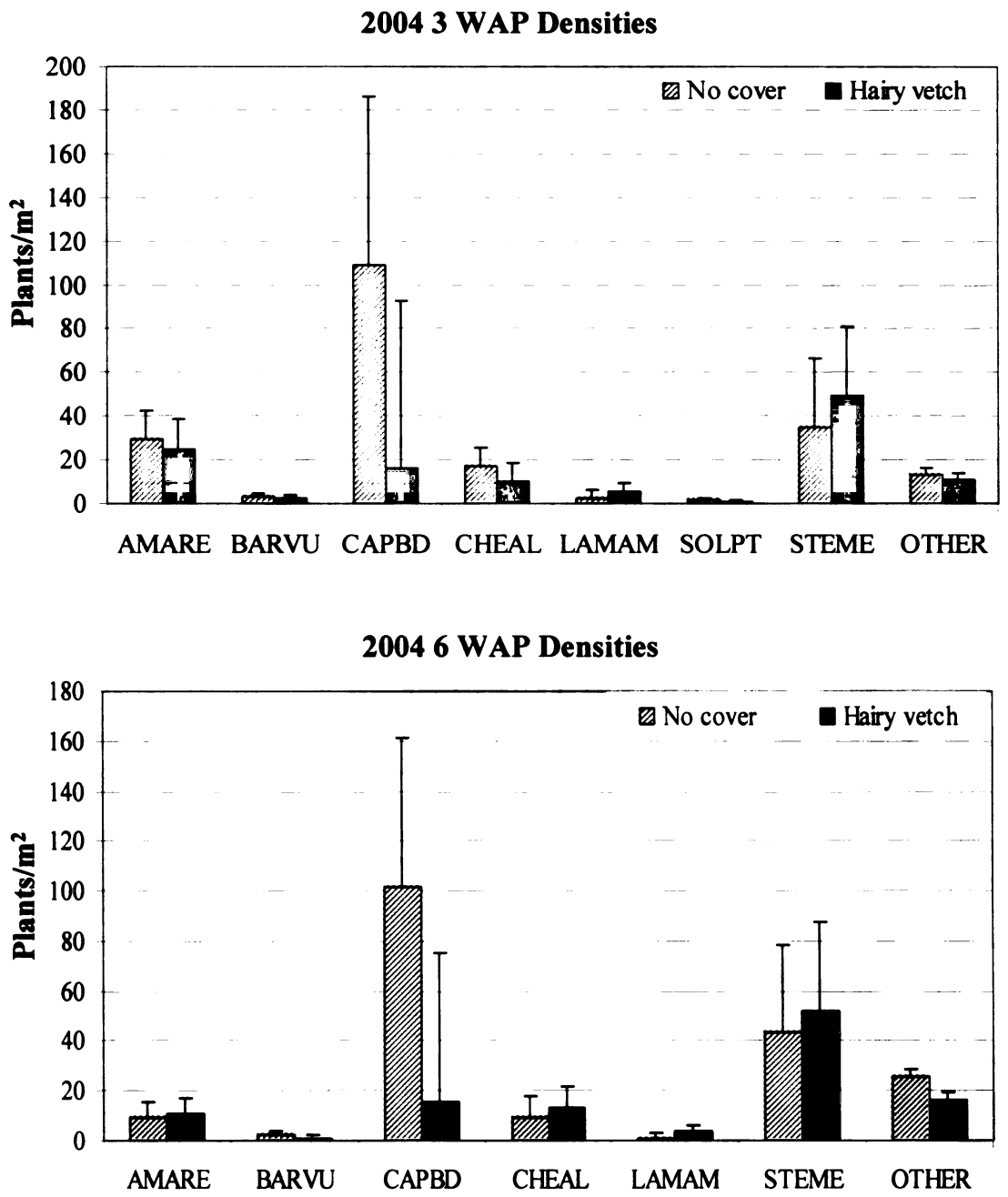


Figure 2. 2004 densities of important weed species measured at 3 (top) and 6 (bottom) weeks after planting (WAP) in 2004. OTHER includes all species appearing in less than ten of the 48 microplots.

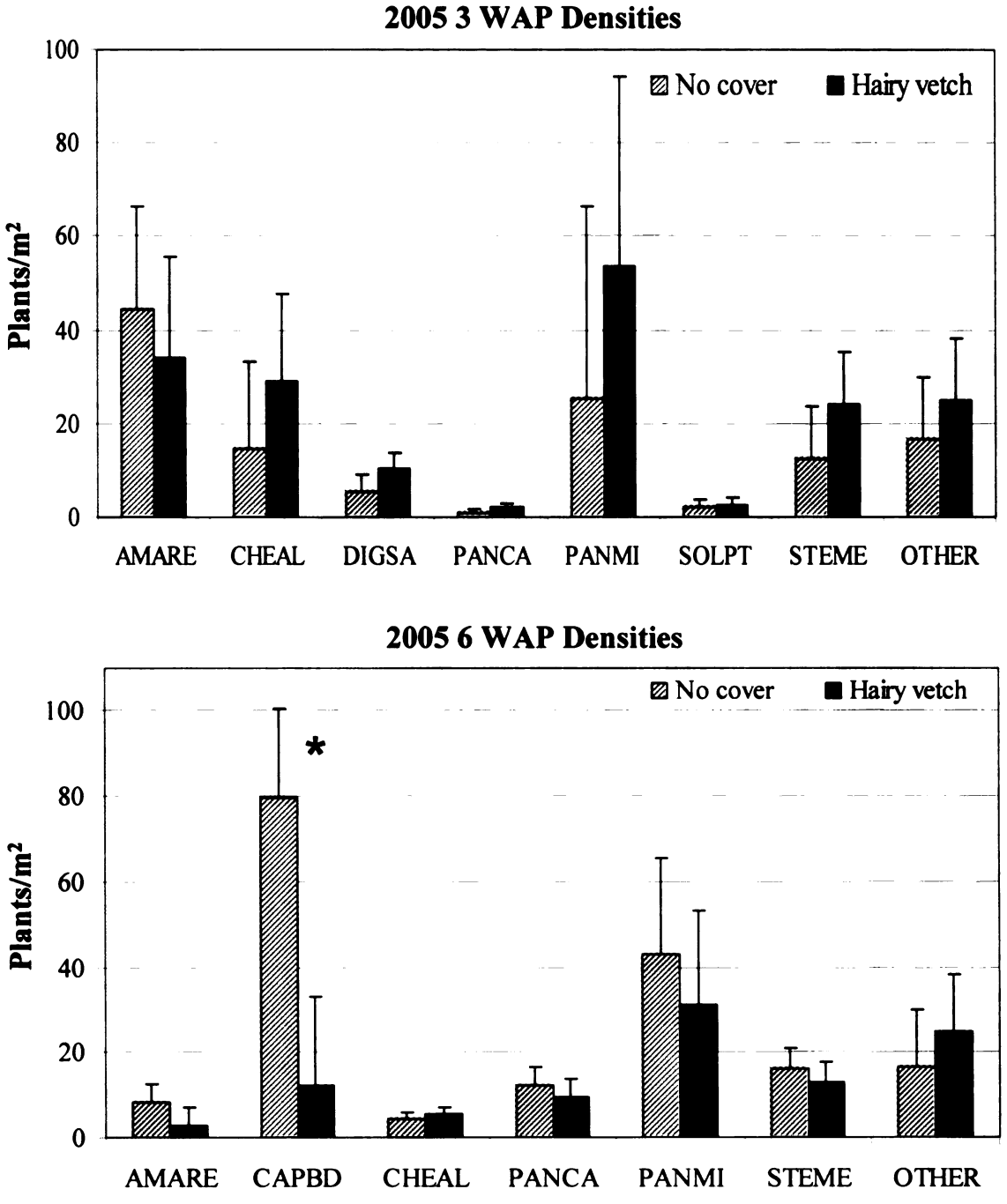


Figure 3. 2005 densities of important weed species measured at 3 (top) and 6 (bottom) weeks after planting (WAP) in 2005. OTHER includes all species appearing in less than ten of the 48 microplots. * Indicates significance ($p<0.05$).

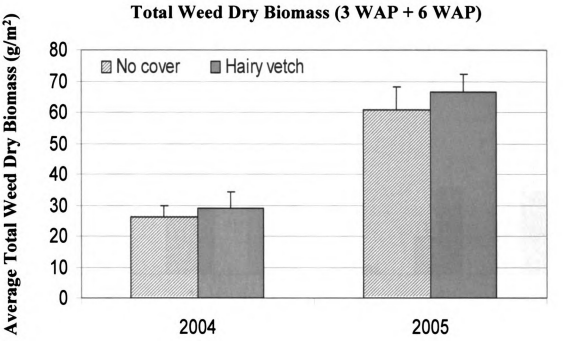
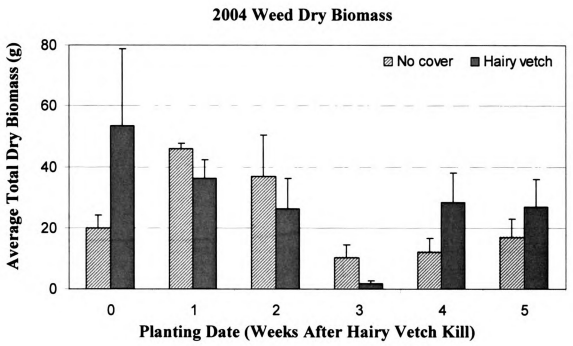


Figure 4. Average total weed dry biomass over all planting dates for the 2004 and 2005 seasons. Data are averages of the sum of weed biomass measured at three and six weeks after cucumber planting (WAP).



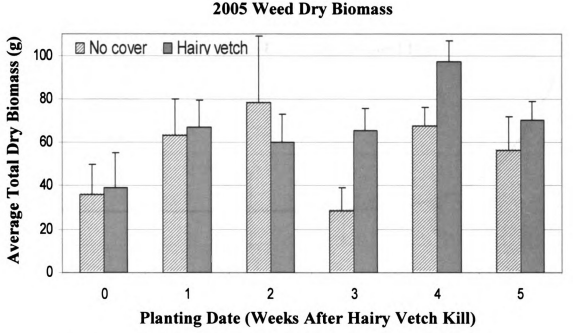


Figure 5. Total weed biomass in the fallow and hairy vetch treatments at various cucumber planting dates following hairy vetch kill for 2004 (top) and 2005 (bottom). Data are the sum of weed biomass measured at three and six weeks after cucumber planting (WAP).

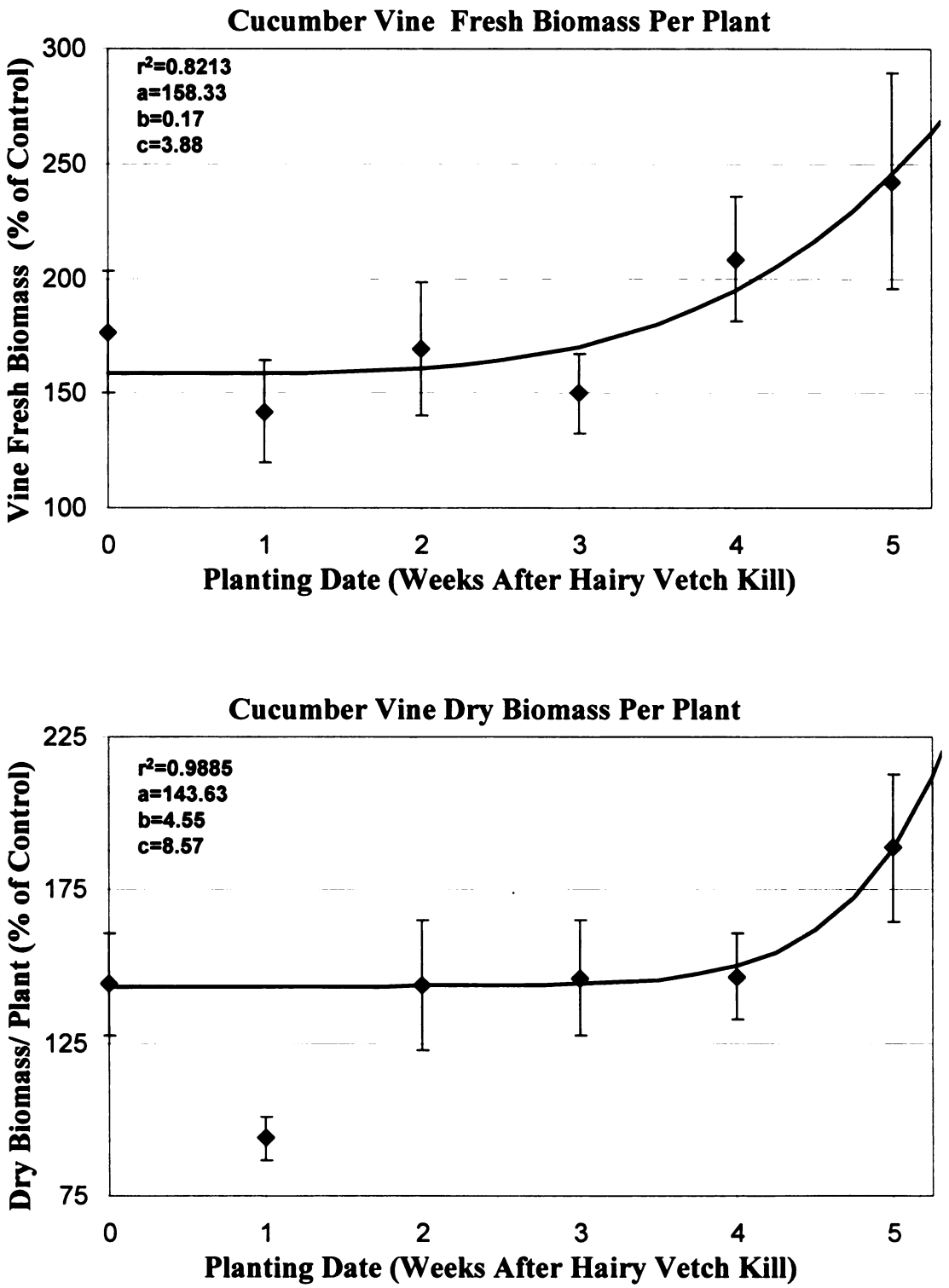


Figure 6. Combined 2004 and 2005 cucumber vine fresh (top) and dry (bottom) biomass per plant in response to changes in cucumber planting dates. Data were fitted to Eq. [1].

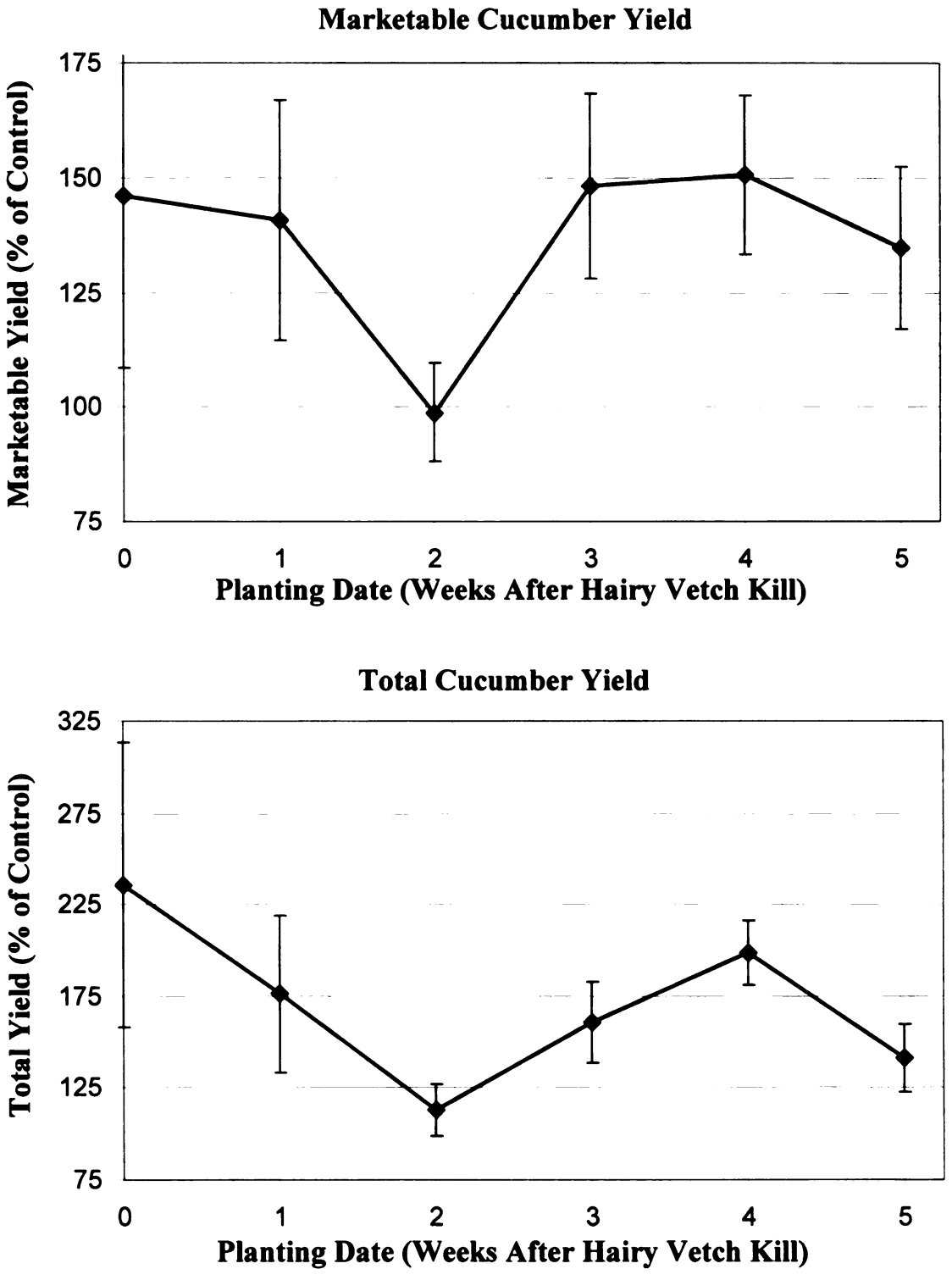


Figure 7. Marketable yield (top) and total yield (bottom) of cucumber for the hairy vetch plots combined for 2004 and 2005 at different planting dates following hairy vetch kill. Results are presented as a percentage of the fallow control.

CHAPTER 4: Allelochemical Effect of Hairy Vetch (*Vicia villosa*) and Cowpea (*Vigna unguiculata*) Water-soluble Extracts on Selected Vegetable Crops and Weeds

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ABSTRACT

The residues of two leguminous cover crops, hairy vetch (*Vicia villosa* Roth) and cowpea (*Vigna unguiculata* (L.) Walp), have been shown to injure vegetable crops and weeds both *in situ* and under laboratory conditions. Some of these observations have indicated that the responsible allelochemicals may be water-soluble in nature. Laboratory experiments were conducted by using a completely randomized design to study the effect of the water-soluble extracts of hairy vetch and cowpea on germination and subsequent radicle elongation in seven vegetable and six weed species. Lyophilized water extracts of hairy vetch and cowpea were dissolved in distilled water, yielding seven concentrations of 0, 0.25, 0.5, 1, 2, 4, and 8 $g \cdot L^{-1}$. Each treatment had 4 replicates and the full experiment was repeated. In general, seed germination was not affected by extracts of both cover crops studied. However, radicle growth of all species tested (except common milkweed exposed to cowpea extract) was affected by the cover crop residue extracts. Low concentrations of the hairy vetch extract stimulated the radicle growth of carrot, pepper, barnyardgrass, common milkweed, and velvetleaf. Likewise, low concentrations of the cowpea extract stimulated the growth of corn, barnyardgrass, and velvetleaf. The order of species sensitivity to the hairy vetch extract, as determined by the IC_{50} (concentration required to produce 50% radicle inhibition) values, was common chickweed > redroot pigweed> barnyardgrass 1 > carrot 1 > wild carrot > corn > carrot 2 > lettuce > common milkweed > tomato > onion > barnyardgrass 2 > velvetleaf > pepper

> cucumber (most sensitive to least sensitive). For the cowpea extract, the order was common chickweed > redroot pigweed > corn > tomato > lettuce > wild carrot > pepper > carrot > cucumber > onion > barnyardgrass and velvetleaf. This study shows that at low rates, water-soluble extracts of hairy vetch and cowpea are stimulatory to some vegetable and weed species. However, at higher concentrations all species were negatively affected, a situation that is beneficial for weed control, but negative for vegetable stand establishment. Future studies should seek to identify, isolate, and test the affects of the responsible allelochemicals in hairy vetch and cowpea water-soluble extracts.

INTRODUCTION

Cover crops are integrated into vegetable cropping systems for their many favorable traits including nutrient recycling and acquisition, erosion control, weed suppression. However, in some cases the cover crop residue has been detrimental to the cash crop, reducing establishment, growth, and yield (Putnam 1986; Teasdale 1996; Weston 1996). Allelopathy is one of the proposed causes of these reductions. Allelopathy, a term coined by Hans Molisch (1937), is defined as interactions between plants that are biochemically facilitated by secondary compounds above and below ground. Allelopathy includes both positive and negative interactions among plants. The microbial breakdown of secondary plant compounds can also result in chemicals with allelopathic properties (Molisch 1937; Rice 1984; Rice 1995; Weston and Duke 2003). All of these chemicals are referred to as allelochemicals. If released by the plant into the environment, allelochemicals have the potential to affect neighboring plant life to varying degrees based on the quantity and persistence of the compound (Putnam 1988). These

two factors vary by species, chemical composition, and environmental conditions. Allelochemicals, such as glufosinate, bialaphos, cinmethlyn, and leptospermone, have been studied for their potential to be used as natural herbicides, which could be safer for the environment than synthetics (Duke et al. 2000; Bhowmik and Inderjit 2003). Depending on their processing from the plant or microbes, some of these naturally produced chemicals may be allowable in organic production systems.

Extensive studies on the allelopathy of rye (*Secale cereale*) used as a cover crop have revealed that the compounds BOA and DIBOA are primarily responsible for its negative effects on crops and weeds (Barnes and Putnam 1987). Rye aqueous extracts have been shown to negatively influence the radicle growth of cucumber (*Cucumis sativus*), cantaloupe (*Cucumis melo*), summer squash (*Cucumis pepo*), lettuce (*Lactuca sativa*), sweet corn (*Zea mays*), and tomato (*Lycopersicon esculentum*), as well as barnyardgrass (*Echinochloa crus-galli*), goosegrass (*Eleusine indica*), large crabgrass (*Digitaria sanguinalis*), Palmer amaranth (*Amaranthus palmeri*), and velvetleaf (*Abutilon theophrasti*) (Burgos and Talbert 2000). At low concentrations a stimulation of radicle growth was observed in summer squash and sweet corn. Susceptibility to cover crop residues is species specific (Teasdale 1996), with smaller seeded species being the most sensitive (Burgos and Talbert 2000).

The use of legume cover crops is of particular interest because of their nitrogen fixing capabilities. Hairy vetch (*Vicia villosa*) and cowpea (*Vigna unguiculata*) are legume cover crops that are growing in popularity. Hairy vetch, a winter hardy species is primarily used in temperate regions, while cowpea, a warm season species, is used in

tropical regions. Both, however, have been seen to reduce weed populations and to negatively affect some vegetable crops.

In 1989, White et al. found that hairy vetch aqueous extracts of 16.7 and 33.3 $g \cdot L^{-1}$ reduced corn and cotton (*Gossypium hirsutum*) germinations by 18, 44 and 36, 42%, respectively. Corn and cotton seeds receiving the 33.3 $g \cdot L^{-1}$ aqueous hairy vetch extract showed a reduction in radicle lengths by 39 and 62%, respectively. In the same study, they found that the germination and radicle growth of pitted morningglory, wild mustard, and Italian ryegrass were inhibited in the presence of hairy vetch extract. In a two year field study, Hoffman et al. (1993) found that live and chopped hairy vetch cover crops reduced weed emergence and thus density. However, all hairy vetch treatments, live, rolled, chopped, or killed with glyphosate, reduced corn yield to varying degrees compared with the no cover, weed-free control. Live hairy vetch has been shown to suppress weeds longer than desiccated hairy vetch; however, weed densities in both were less than a no cover control (Teasdale and Daughtry 1993). This finding would suggest that something other than light transmission or temperature buffering is contributing to the reduction in weed density. Recently, Ngouajio and Mennan (2005) reported reduced marketable cucumber yields in the presence of hairy vetch residues compared with a no cover control during the second year of their two year study. During both years, yields in hairy vetch plots were significantly lower than those in the sorghum sudangrass plots (*Sorghum bicolor* x *S. sudanense*) and rye plots. In the same study hairy vetch was shown to reduce weed density and biomass by up to 99 and 91%, respectively, compared with the bare ground control.

Growth inhibition after a hairy vetch cover crop has been shown to be exacerbated under drought conditions (Hoffman et al. 1993; Ngouajio and Mennan 2005). It is therefore possible that the responsible allelochemicals are water soluble, resulting in higher concentrations under conditions, such as drought, that reduce leaching.

Cowpea 'Pinkeye Purplehull BVR' has been linked to increased transplant death of broccoli (*Brassica oleracea*) (Schroeder et al. 1998). Phytotoxicity to 'Purple top' turnip (*Brassica rapa*) observed following a cowpea cover crop was attributed to allelopathy (Wang et al. 2003). Also, cowpea grown as a summer cover crop in pepper and lettuce production has been shown to reduce the density of weeds (Hutchinson and McGiffen 2000; Ngouajio et al. 2003).

The studies mentioned above strongly suggest that hairy vetch and cowpea cover crops may be able to reduce weed density via allelochemicals, however, there is the risk of negative effects on subsequent vegetable crops. Understanding the allelopathic effects of hairy vetch and cowpea on vegetables and weeds could help select the appropriate cover crop and crop rotation. Therefore, this work intends to examine the germination and radicle response of a range of vegetables and weeds to hairy vetch and cowpea water-soluble extracts.

MATERIALS AND METHODS

Plant Material Extraction

Hairy vetch was planted in the field at the Horticulture Teaching and Research Center on the campus of Michigan State University in East Lansing, Mich. on September 3, 2003 and whole plants were harvested on May 12 , 2004. Cowpea was planted on June

17, 2004 and whole plants were harvested on August 27, September 10, and September 28, 2004. For each cover crop, the area harvested was recorded to allow for the calculation of the field rate of each extract. All plants were rinsed once with tap water and once with distilled water to remove soil. After rinsing, the plants were allowed to air dry before being weighed. The fresh plant material (i.e. 25.49 kg of hairy vetch and 31.15 kg of cowpea) was chopped by hand and blended with 2.3 and 1.8 $L \cdot kg^{-1}$ of distilled water for hairy vetch and cowpea, respectively, in an industrial blender (CB-10; Waring Commercial®, Torrington, Conn.) for 30-60 sec. The crude blend was filtered through cheese cloth resulting in 2.7 and 2.1 $L \cdot kg^{-1}$ of liquid for hairy vetch and cowpea, correspondingly. After centrifugation of the liquids (RC5C; Sorvall® Instruments, DuPont, Wilmington, Del.) at 10,000 rpm for 10 min, the resulting supernatants (i.e. extracts) were freeze dried using a tray-lyophilizer. The resulting extract powders were mixed to allow for uniformity within each species. The powders were then stored at -20°C until use. For the assays, the lyophilized extracts for each cover crop were dissolved in distilled water to afford concentrations of 0, 0.25, 0.5, 1, 2, 4, and 8 $g \cdot L^{-1}$ by serially diluting a stock solution containing 10 $g \cdot L^{-1}$.

Germination and Radicle Elongation Assays

The vegetable crops examined were carrot, cucumber, lettuce, onion, pepper, sweet corn, and tomato, along with barnyardgrass, common chickweed, common milkweed, redroot pigweed, velvetleaf, and wild carrot weed species (Table 1). The experiment was a randomized complete block design comprised of seven extract concentrations, thirteen species, and four replications with ten seeds each. The

experiment was repeated once in its entirety. For each species, the seeds were sterilized in a 1% sodium hypochlorite solution, rinsed three times with distilled water, dried, and placed on a 90 mm Whatman No. 1 filter paper in a 100 mm plastic Petri dish. The weed species required 24 h (48 h for velvetleaf) of soaking in distilled water, after sterilization, to increase germination rates. Once placed on the filter paper, each dish received 2.5 ml of extract (sweet corn received 3.0 ml, necessary for imbibition). After extract administration, the Petri dishes were sealed using Parafilm® and incubated in the dark for 4 to 11 days at the temperatures specified in Table 1, depending on species germination time. Preliminary tests were conducted to determine the appropriate incubation time and temperature for each species (Table 1). Temperature was monitored using data loggers (Watch Dog 100-Temp 2K, Spectrum Technologies, Plainfield, Ill.) set to record temperature every 15 min. After the incubation period, germination percentages were recorded for the vegetables. Seeds were considered germinated when radicles reached 2 mm. Radicles of both vegetables and weeds were then separated from the shoot. Then, they were floated in distilled water in a clear plastic tray and scanned using a HP Scanjet 8200 scanner. The images were analyzed for length using WinRHIZO® 2003b (Regent Insturments Inc., Ste-Foy, Que. Canada).

Field Equivalent Concentration Estimates

Field equivalent concentrations were estimated based on the extract retrieved per unit of area harvested. These estimates were made using the assumptions of 1) 15 cm of cover crop incorporation, 2) simultaneous release of the extracted materials, and 3) 2.5 ml

of aqueous extract are placed in each Petri dish, equating to 39.1 L per cubic meter of soil. The following equation was used to estimate field equivalent rates of the extracts:

$$FER = \frac{E(g) * P(cm^3)}{A(cm^2) * D(cm) * W(L)} \quad [1]$$

Where *FER* is the estimated extract field equivalent rate, *E* is the total extract dry weight retrieved, *A* is the cover crop area harvested, *P* is the Petri dish volume (64 cm^3), *D* is the hypothetical depth of cover crop incorporation in the field (15 cm), and W is the volume of extract solution added to the Petri dish during the bioassay tests (2.5 ml, 3.0 ml for corm). Because the extracted materials are likely released over time, and not simultaneously, these extract field rate estimates are likely maximum rates. Under true field conditions the concentrations are likely lower.

Statistical Analysis

All data were subjected to analysis of variance to test the differences among treatments and between experiments. When no experiment-by-treatment interaction was observed, data from corresponding experiments were combined. Germination and radicle elongation parameters were analyzed using SAS PROC GLM (Version 8, SAS 2001). Normality was checked using ANOVA. Means were separated using Fisher's Protected LSD. A p-value of <0.05 was used to indicate significance. Regression analyses were performed using TableCurve™ 2D (Version 4, AISN Software, Inc., 1996).

To allow for comparison among species, data for radicle length were converted to percent of the control for each replicate in each species.

Data on radicle inhibition were fitted to the logistic dose response equation:

$$RL(x) = a + \frac{b}{\left(1 + \left(\frac{x}{c}\right)^{d}\right)} \qquad [2]$$

where *RL(x)* is the radicle length (as a percent of the control) at extract concentration x, *x* is the extract concentration and *a, b, c,* and *d,* are regression coefficients. A separate model was used to describe the responses when a strong initial radicle stimulation was observed (Norsworthy and Meehan 2005). The model had the following form:

$$RL(x) = a * \exp\left(-0.5 * \left[\{x - b\ \}/c\right]^2\right) \qquad [3]$$

where *RL(x)* is radicle length (as a percent of the control) at extract concentration x , *x* is extract concentration, *a* is maximum radicle length (as a percent of the control), *b* is extract concentration at maximum length, and *c* is a constant. The regression equations were used to estimate the extract concentration required to cause 25% (IC_{25}), 50% (IC_{50}), and 75% (IC_{75}) inhibitions of radicle growth.

RESULTS

Extract Yield

The fresh biomass of cowpea per unit area was higher and yielded about 45% more extract per kilogram of fresh biomass than hairy vetch (Table 2). The hairy vetch and cowpea harvest yielded 44.98 and 107.71 $g \cdot m^{-2}$, respectively. This equated to 22.13 and 32.12 g of extract per kilogram of fresh biomass for hairy vetch and cowpea, respectively.

Germination

Hairy vetch. The germination rates of carrot, corn, and onion were significantly reduced by the hairy vetch extracts (Table 3). Germination percentages of 42.5, 66.3 and 71.3% were observed for carrot, corn, and onion, respectively, which were exposed to the 8 $g \cdot L^{-1}$extract. All other species germination percentages were non-responsive. Lettuce was a special case due to an experiment-by-treatment interaction. Experiment one showed no effect of the hairy vetch extract on germination, while experiment two displayed significantly reduced germination percentage and concentration increased.

Cowpea. Carrot and corn germination percentages were significantly affected by the presence of increasing concentrations of cowpea extracts (Table 3). Percentages were 30 and 83% at 8 $g \cdot L^{-1}$ treatment for carrot and corn, respectively. The germination rates of the other crop species examined were not affected. In the case of tomato, there was an experiment-by-treatment interaction. Germination in experiment two was negatively affected by increasing cowpea extract concentrations, while in experiment one it was not.

Radicle Elongation

The radicle lengths of all vegetable crops and all weed species examined were significantly impacted by increasing concentrations of both hairy vetch and cowpea extracts (Fig. 1, 2, and 3). The responses were adequately described by the logistic dose response (Eq. [2]) or Eq. [3] (Tables 4 and 5). The r^2 values ranged from 0.82 to 0.99 for vegetables and 0.42 to 0.99 for weeds. The one exception was common milkweed, which was not significantly affected by the cowpea water extract and therefore did not fit either equation. Experiment-by-treatment interactions are shown individually, by experiment.

Estimated inhibitory concentrations (IC) resulting in 25, 50, and 75% decreases in radicle length varied with cover crop and test species (Figs. 2 and 3).

Hairy vetch extract. The radicle growth of carrot (Experiment 2) and pepper were stimulated by low concentrations of hairy vetch water extract (Fig. 2). Carrot radicles reached 130 % of the control at 0.5 $g \cdot L^{-1}$ and pepper was stimulated to 153% at 4 $g \cdot L^{-1}$. The radicle elongation of the rest of the vegetables was steadily reduced by all concentrations of the hairy vetch water extract. From least to most sensitive at 8 $g \cdot L^{-1}$ the vegetables fall in the following order: pepper < cucumber < corn < onion < tomato < carrot < lettuce. Corresponding reductions in growth ranged from 16 to 79% of the controls.

Low concentrations of the hairy vetch water extract stimulated the radicle growth of barnyardgrass, common milkweed, and velvetleaf, with maximum lengths of 247 (at 0.5 $g \cdot L^{-1}$), 132 (at 1 $g \cdot L^{-1}$), and 127% (at 1 $g \cdot L^{-1}$), respectively. However, common chickweed, redroot pigweed, and wild carrot all experienced a rapid decline in radicle elongation with increasing concentrations of the hairy vetch water extract was observed. From least to most sensitive, the weeds fell in the following order: common milkweed < barnyardgrass 1 < velvetleaf < barnyardgrass 2 < common chickweed < redroot pigweed for the 8 $g \cdot L^{-1}$ concentration. Growth reductions ranged from 37 to 82% of the controls.

Cowpea extract. Concentrations of the cowpea water extract between 0.25 and 0.5 $g \cdot L^{-1}$ stimulated the radicle growth of corn. A maximum stimulation of 137% of the control was observed at the 0.5 $g \cdot L^{-1}$ concentration. All other vegetables tested showed a decline in radicle growth with increasing concentrations of the cowpea water extract. At 8 $g \cdot L^{-1}$ the crops from least to most sensitive fell in the following order: onion < carrot <

cucumber < pepper < corn < lettuce < tomato. The reductions compared with the controls ranged from 50 to 86%.

In a trend similar to the hairy vetch water extract, the cowpea water extract stimulated the growth of barnyardgrass and velvetleaf radicles at low concentrations, while common chickweed, redroot pigweed, and wild carrot were inhibited by all concentrations. Common milkweed was not found to be significantly affected by the tested concentrations. At 8 $g \cdot L^{-1}$ the order of weed species from least to most sensitive is: common milkweed < barnyardgrass < velvetleaf < wild carrot < redroot pigweed < common chickweed. Radicle length reductions ranged from 0.4 to 87% of the controls.

Inhibitory concentrations. Radicle elongation of all species examined was significantly impacted by the presence of the two cover crop extracts at varying concentrations (with the exception of common milkweed with cowpea). The IC rates predicted by the regression analyses for 25, 50, and 75% radicle growth reductions provided a better separation among the species tested (Table 6).

IC_{25}. At IC_{25}, corn (0.6 $g \cdot L^{-1}$) and common chickweed (0.002 $g \cdot L^{-1}$) were the most sensitive crop and weed species to hairy vetch, while pepper (8.4 $g \cdot L^{-1}$) and velvetleaf (4.35 $g \cdot L^{-1}$) were the least susceptible. For cowpea at IC_{25}, tomato (0.2 $g \cdot L^{-1}$) and common chickweed (0.14 $g \cdot L^{-1}$) were the most sensitive and cucumber (2.1 $g \cdot L^{-1}$) and velvetleaf (5.18 $g \cdot L^{-1}$) were the least affected.

IC_{50}. Similarly, corn and common chickweed were the most sensitive to both cover crops (3.3 and 0.30 g·L-1 for hairy vetch and 1.1 and 0.38 $g \cdot L^{-1}$ for cowpea) at IC_{50}; while cucumber (25.1 $g \cdot L^{-1}$) and velvetleaf (6.48 $g \cdot L^{-1}$) were the least responsive to hairy

vetch and onion (10.2 $g \cdot L^{-1}$) and wild carrot (3.38 $g \cdot L^{-1}$) were the least affected by cowpea at IC_{50}.

IC_{75}. Finally, for hairy vetch at IC_{75}, lettuce (7.6 $g \cdot L^{-1}$) and wild carrot (4.32 $g \cdot L^{-1}$) were the most sensitive, while cucumber (162.0 $g \cdot L^{-1}$) and common chickweed (11.88 $g \cdot L^{-1}$) were the least. For cowpea, tomato (5.1 $g \cdot L^{-1}$) and common chickweed (1.30 $g \cdot L^{-1}$) were the most susceptible and onion (33.6 $g \cdot L^{-1}$) and wild carrot (14.02 $g \cdot L^{-1}$) were the least susceptible.

Overall, corn and lettuce were the most susceptible vegetable crops to hairy vetch, while corn and tomato were the most sensitive to cowpea. Common chickweed was the most susceptible weed to both hairy vetch and cowpea. Cucumber and onion appear to be the least sensitive vegetables to hairy vetch and cowpea, respectively. Velvetleaf appeared to be the most tolerant weed species to water extracts from both cover crops. The predicted asymptotes reached by carrot, barnyardgrass, and common milkweed for hairy vetch and corn, barnyardgrass, and velvetleaf for cowpea indicate that these species may be more tolerant to higher concentrations of the extracts than the other species examined.

DISCUSSION

Previous reports of the inhibitory effects of hairy vetch and cowpea residues and extracts on the germination and radicle elongation of vegetable and weed species have lead to inquiries regarding allelopathy (Hoffman et al. 1993; Hutchinson and McGiffen 2000; Ngouajio et al. 2003; Ngouajio and Mennan 2005; Schroeder et al. 1998; Teasdale and Daughtry 1993; Wang et al. 2003; White et al. 1989). This study allowed us to

further understand the allelopathic impact of cowpea and hairy vetch cover crop on the growth of vegetables and weed species under controlled conditions.

Seed Germination

In the present study, the germination rates of only three crops were consistently reduced in the presence of the cover crops extracts (carrot and corn for cowpea and hairy vetch, and onion for hairy vetch). The effect of plant extracts and allelochemicals on seed germination has been shown to vary with both the donor species (source of the extract) and the test species (Kadioglu et al. 2005; Kim et al. 2005; Pennacchio et al. 2005). Neutral, stimulatory, and inhibitory effects of plant extracts on seed germination are documented in the literature. Kadioglu et al. (2005) observed 23% inhibition of chick pea seed germination by chamomile (*Matricaria chamomilla*) extracts and over 90% stimulation of the same species by licorice (*Glycyrrhiza glabra*), johnsongrass (*Sorghum halepense*), and yellow mignonette (*Reseda lutea*) extracts. The large variability of seed response to allelochemicals has made seed germination a rather inaccurate assessment of the presence of allelochemicals. Lettuce has been proposed as one of the efficient bioassay species but has also been shown to be less responsive to some allelochemicals. More recently, Pennacchio et al. (2005) have shown that *Arabidopsis thaliana* seed germination could be more sensitive to the presence of allelochemicals than the species previously reported in the literature. Lack of germination responses by most species tested in this study to hairy vetch or cowpea extract could simply be due to a low sensitivity of the species to the extracts. If that was proven to be the case, our results would be interesting to growers as this would reduce the risks associated with these cover

crops in the field. However, success of a species (both crop and weed) in cropping systems would depend on additional factors, including, radicle growth and seedling establishment.

Radicle elongation

Early radicle growth has been shown to be more responsive to the presence of allelochemicals than seed germination (Leather and Einhellig 1986). This effect is also species dependant. In this study, a strong stimulatory effect of low rates of hairy vetch extracts was observed on some crops (carrot and pepper) and weeds (barnyardgrass, common milkweed, and velvetleaf). Stimulatory effects of low concentrations of allelochemicals have been reported in many studies (Sinkkonen 2003). Norsworthy and Meehan (2005) found that at low concentrations, isothiocyanates commonly produced by Brassicaceae species stimulate radicle growth of many weed species. Also, barley extracts (*Hordeum vulgare*) were shown to stimulate durum wheat (*Triticum durum*) seedling growth (Ben-Hammouda et al. 2001). The stimulatory effect of allelochemicals on crop seedling growth could be exploited in cropping systems to enhance early seedling establishment and to improve competitiveness.

Apart from the species listed above, both hairy vetch and cowpea generally inhibited radicle growth of the weed and crops tested. This is in agreement with observations previously reported by other investigators. Results by Mohler and Teasdale (1993), Ngouajio and Mennan (2005), Teasdale and Daughtry (1993), Teasdale (1996) Teasdale et al. (2005) have shown reduced weed populations in systems with hairy vetch residue. Based on the reduced radicle elongation in cucumber found in response to the

hairy vetch extract in this study, the low cucumber stand and growth reported by Ngouajio and Mennan (2005) after a hairy vetch cover crop could be associated with the release of these extract components from the residue. Moreover, White et al. (1989) reported high sensitivity of corn to hairy vetch residue extracts. In their study, extracts made from dry plant material equivalent to 6.9 $g \cdot L^{-1}$, showed a 44% inhibition of corn germination and 61% reduction in radicle elongation compared to the control. In the present study, we found 38% reduction in radicle elongation at 8 $g \cdot L^{-1}$ of hairy vetch water extract. Our study used extracts made from fresh plant material, which may explain the differences between the two reports. Results of the present study strongly suggest that those initial observations could be due to allelochemicals released from the decomposed or hydrolyzed natural products. The reduced radicle lengths of barnyardgrass, common chickweed, and redroot pigweed in response to increasing concentrations of hairy vetch water extract correspond with previous field observations of reduced emergence in the presence of increasing hairy vetch residue rates in the field (Mohler and Teasdale 1993).

Based on the amount of extract yielded per unit of fresh weight per unit of area, we estimated the maximum field rates for hairy vetch and cowpea extracts to be 7.78 $g \cdot L^{-1}$ and 18.38 $g \cdot L^{-1}$, respectively (the field rates for corn are 6.38 $g \cdot L^{-1}$ and 15.31 $g \cdot L^{-1}$ due to the increased volume of water required). This assumed that residues were incorporated 15 cm into the soil and that the plant material breaks down simultaneously. Under field conditions, the residues will break down gradually over time. Decomposition rates are dependent on both environmental conditions and biological conditions (Kuo et al. 1997; Weston 1996; White et al. 1989). Therefore, the concentrations produced by the residues are likely to be lower, perhaps even more so under conditions of high rainfall or frequent

irrigation. Under low soil moisture, however, allelochemicals from hairy vetch or cowpea residue could accumulate, reaching concentrations high enough to affect weeds and potentially some crops.

Future studies should examine the relationships between the germination and radicle elongation of vegetable and weed seeds and cover crop water extracts in a greenhouse and field setting. Only then will we have a better view of the potential advantages or dangers when using these two legume cover crops. Finally, to confirm that the observed effects are due to allelochemicals released from the residue, further studies should identify, isolate, and test the allelochemical(s) from both of these cover crops. Understanding the chemistry of allelochemicals in hairy vetch and cowpea water extracts will then facilitate the exploration of their potential as natural herbicides.

Table 1. Vegetable and weed species used in the bioassays of hairy vetch and cowpea water-soluble extracts. Incubation temperatures separated by a slash indicate the 16/8 hour fluctuations of the growth chambers.

Common name	Scientific Name	Variety/Bayer code	Incubation Temperature (°C)	Incubation Time (Days)
Crop				
Carrot	*Daucus carota*	Delmar	21	7
Cucumber	*Cucumis sativus*	Vlaspik	21	4
Lettuce	*Lactuca sativa*	Ithaca	21	4
Onion	*Allium cepa*	Sweet Sandwich	21	5
Pepper	*Capsicum annuum*	Jalapeno	21	7
Sweet corn	*Zea mays*	Jubilee	21	5
Tomato	*Lycopersicon esculentum*	Mountain Spring	21	5
Weed				
Barnyardgrass	*Echinochloa crus-galli*	ECHCG	30/25	7
Common chickweed	*Stellaria media*	STEME	21	11
Common milkweed	*Asclepias syriaca*	ASCSY	30/25	4
Redroot pigweed	*Amaranthus retroflexus*	AMARE	30/25	4
Velvetleaf	*Abutilon theophrasti*	ABUTH	21	4
Wild carrot	*Daucus carota*	DAUCA	30/25	7

Table 2. Hairy vetch and cowpea harvest and water extract data.

Cover crop	Area harvested	Total fresh biomass	Extract dry weight	Ratios		Extract field rates*
				Extract/area harvested	Extract/fresh biomass	
	—m^2—	—kg—	—g—	—$g \cdot m^{-2}$—	—$g \cdot kg^{-1}$—	—$g \cdot L^{-1}$—
Hairy vetch	12.54	25.49	564.00	44.98	22.13	7.68
Cowpea	9.29	31.15	1000.67	107.71	32.12	18.38

*Estimated extract field equivalent rate calculation (Eq. [1])

Table 3. Germination percentages of seven vegetable crops resulting from exposure to seven extract concentrations of hairy vetch and cowpea water extracts.

Cover Crop	Vegetable Crop	Extract Concentration ($g \cdot L^{-1}$)							p<0.05
		0	0.25	0.5	1	2	4	8	
Hairy Vetch	Carrot	63.8	77.1	67.5	63.8	61.4	72.5	42.5	*
	Corn	97.5	100.0	98.8	98.8	86.3	73.8	66.3	*
	Cucumber	85.0	78.8	86.3	86.3	85.0	82.9	83.8	NS
	Lettuce 1	95.0	97.5	95.0	95.0	100.0	95.0	95.0	NS
	Lettuce 2	100.0	97.5	97.5	100.0	97.5	95.0	80.0	*
	Onion	96.3	93.8	81.3	87.5	85.7	82.5	71.3	*
	Pepper	97.5	92.9	96.3	93.8	88.8	92.3	86.3	NS
	Tomato	97.5	85.0	93.8	92.5	92.5	86.3	85.0	NS
Cowpea	Carrot	73.8	62.5	66.3	67.5	65.0	52.5	30.0	*
	Corn	95.0	100.0	98.8	95.0	88.8	91.4	82.5	*
	Cucumber	76.3	83.8	74.3	83.8	86.3	72.5	73.8	NS
	Lettuce	96.3	97.5	92.5	96.3	96.3	93.8	98.8	NS
	Onion	90.0	87.5	88.8	83.8	91.3	88.8	76.3	NS
	Pepper	91.3	90.0	95.0	93. 8	92.5	87.5	90.0	NS
	Tomato 1	95.0	95.0	100.0	97.5	100.0	97.5	92.5	NS
	Tomato 2	95.0	100.0	97.5	90.0	90.0	87.5	65.0	*

*= Significant, NS= Not Significant

Table 4. Regression parameters for the logistic dose response equation fitted to data on radicle elongation of vegetable crops exposed to water extracts of hairy vetch and cowpea.

		Logistic Dose Response $y=a+b/(1+(x/c)^d)$				
Cover Crop	**Vegetable Crop**	**r^2**	**a**	**b**	**c**	**d**
Hairy Vetch	Carrot 1	0.82	-264.55	364.85	193.27	0.44
	Carrot 2	0.91	30.51	85.02	1.80	3.23
	Corn	0.98	10.80	89.26	2.37	0.72
	Cucumber	0.93	-54.11	153.61	190.78	0.37
	Lettuce	0.94	-7293.79	7384.66	989.09	0.97
	Onion	0.95	-18.86	113.79	7.12	1.52
	Pepper †	0.98	153.65	3.71	-3.91	-
	Tomato	0.92	-2890.48	2982.72	1140.14	0.78
Cowpea	Carrot	0.93	28.03	73.01	2.28	1.11
	Corn	0.90	46.51	74.58	0.99	26.69
	Cucumber	0.96	-81.07	182.77	20.31	0.77
	Lettuce	0.99	22.48	77.22	1.77	1.59
	Onion	0.90	-166.33	266.04	280.18	0.44
	Pepper	0.98	-21.26	121.07	7.71	0.54
	Tomato	0.97	-197.99	297.30	96.02	0.37

† Pepper exposed to hairy vetch extract concentrations did not fit a logistic dose response relationship due to sustained stimulation from 0.25-4.0 $g \cdot L^{-1}$. Those data were fitted to the following equation, $y=a*\exp(0.5((x-b)/c)^2)$.

Table 5. Regression parameters for the logistic dose response equation fitted to data on radicle elongation of weed species exposed to water extracts of hairy vetch and cowpea.

		Logistic Dose Response $y=a+b/(1+(x/c)^d)$				
Cover Crop	**Vegetable Crop**	**r^2**	**a**	**b**	**c**	**d**
Hairy Vetch	Barnyardgrass 1	0.71	40.32	107.03	1.95	27.30
	Barnyardgrass 2	0.56	55.52	142.42	3.56	3.40
	C. chickweed	0.97	-43.43	143.83	7.20	0.19
	C. milkweed	0.86	42.90	72.25	4.35	16.60
	Redroot pigweed	0.96	-107.47	207.84	27.16	0.40
	Velvetleaf	0.75	-7.55	115.53	6.46	2.32
	Wild carrot	0.99	7.88	84.08	2.97	3.64
Cowpea	Barnyardgrass	0.42	78.01	21.91	1.59	6.64
	C. chickweed	0.99	10.44	89.30	0.31	1.15
	C. milkweed †	-	-	-	-	-
	Redroot pigweed	0.99	16.89	83.00	0.39	1.19
	Velvetleaf	0.65	130.51	-68.53	4.93	-29.58
	Wild carrot	0.96	-130.40	230.16	91.19	0.39

† Common milkweed did not show a significant response for cowpea and did not fit either of the equations presented.

Table 6. Inhibitory concentrations of hairy vetch and cowpea extracts that reduced vegetable and weed radicle lengths by 25 (IC_{25}), 50 (IC_{50}), and 75% (IC_{75}). These rates are based on the derived regression analyses for each species. The ∞ signs signify that an asymptote was reached prior to the given reduction.

	Hairy vetch extract ($g \cdot L^{-1}$)			Cowpea extract ($g \cdot L^{-1}$)		
Species	**IC_{25}**	**IC_{50}**	**IC_{75}**	**IC_{25}**	**IC_{50}**	**IC_{75}**
Carrot	0.55	3.88	9.24	1.33	4.88	∞
Carrot 2*	1.75	2.62	∞	NA	NA	NA
Corn	0.64	3.34	24.24	1.01	1.11	∞
Cucumber	2.05	25.10	161.98	2.06	6.08	13.34
Lettuce	1.74	4.65	7.64	1.10	2.57	14.86
Onion	2.56	5.37	9.68	1.64	10.15	33.57
Pepper	8.40	9.57	11.17	0.61	3.95	18.92
Tomato	1.62	5.12	9.36	0.15	1.28	5.08
Barnyardgrass	2.00	2.12	∞	∞	∞	∞
Barnyardgrass 2*	6.12	∞	∞	NA	NA	NA
C. chickweed	0.00	0.29	13.34	0.14	0.38	1.30
Redroot pigweed	0.19	1.55	6.58	0.19	0.54	2.34
Velvetleaf	4.35	6.48	9.67	5.18	∞	∞
Wild carrot	2.03	2.97	4.32	0.41	3.38	14.02

* The species with a "2" beside them indicate that for the hairy vetch and/or cowpea extract experiments there was a treatment-by-experiment interaction. For that reason the IC values are presented by experiment.

NA signifies that the first and second experiments were able to be combines, hence there is no second set of predicted inhibitory concentrations.

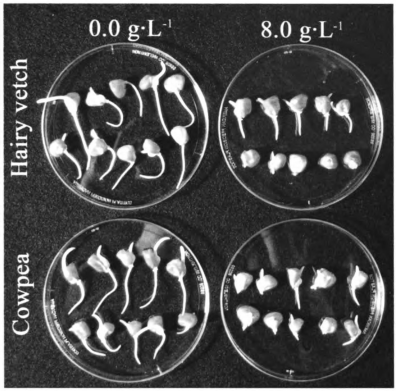

Figure 1. Corn exposed to hairy vetch (top) and cowpea (bottom) water extracts at 0.0 $g \cdot L^{-1}$ (left) and 8.0 $g \cdot L^{-1}$ (right) for 5 days. Images in this thesis are presented in color.

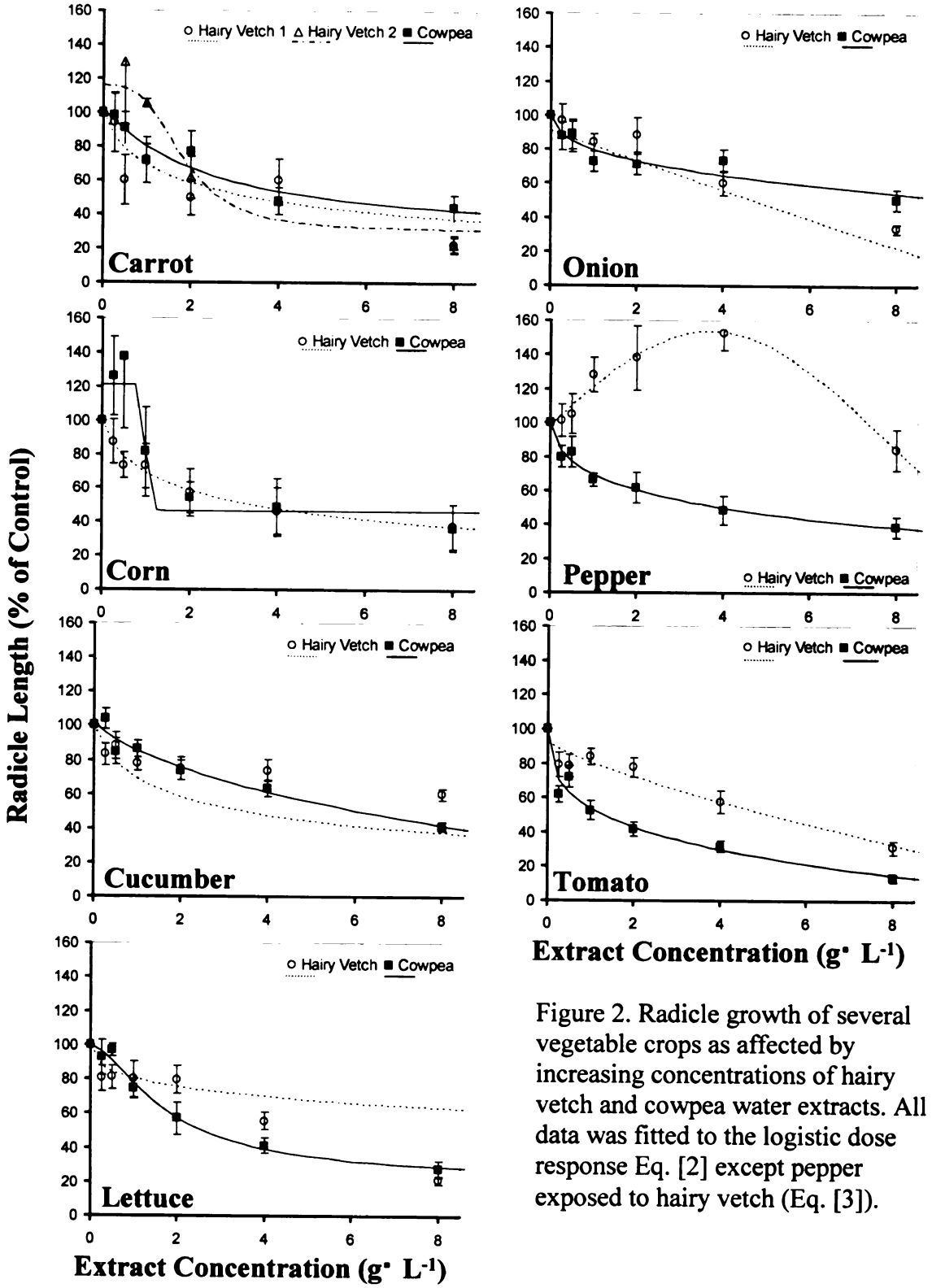

Figure 2. Radicle growth of several vegetable crops as affected by increasing concentrations of hairy vetch and cowpea water extracts. All data was fitted to the logistic dose response Eq. [2] except pepper exposed to hairy vetch (Eq. [3]).

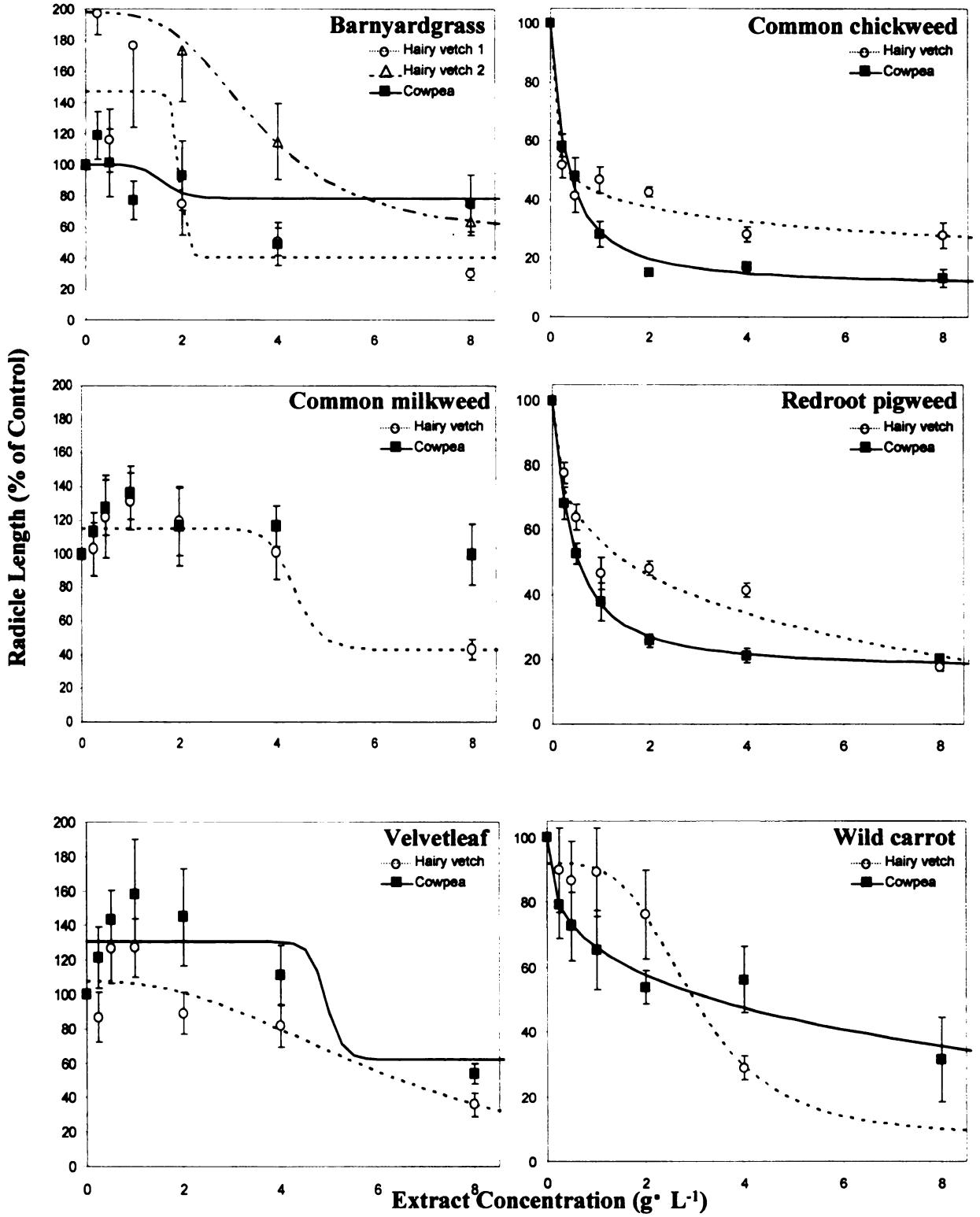

Figure 3. Radicle growth of several weeds as affected by increasing concentrations of hairy vetch and cowpea water extracts. All data was fitted to the logistic dose response Eq. [2] except common milkweed exposed to cowpea, which did not fit either of our equations.

CHAPTER 5: Effects of Hairy Vetch (*Vicia villosa*) and Cowpea (*Vigna unguiculata*) Methanol and Ethyl Acetate Extracts on Select Vegetable Crops and Weeds

CHAPTER 5: Effects of Hairy Vetch (*Vicia villosa*) and Cowpea (*Vigna unguiculata*) Methanol and Ethyl Acetate Extracts on Select Vegetable Crops and Weeds

ABSTRACT

The residues and water extracts of two leguminous cover crops, hairy vetch (*Vicia villosa* Roth) and cowpea (*Vigna unguiculata* (L.) Walp), have been shown to injure vegetables crops and weeds in the field and in the laboratory. To test a broader spectrum of extracted material with potential allelopathic properties, a completely randomized laboratory experiment was conducted to determine the phytotoxicity of hairy vetch and cowpea residues methanol and ethyl acetate extracts on the germination and radicle elongation of three vegetable crops and three weed species. The methanol and ethyl acetate extracts of both species were dissolved in methanol to yield seven concentrations: 0, 0.25, 0.5, 1, 2, 4, and 8 $g \cdot L^{-1}$. Each treatment consisted of 10 seeds and had four replicates. The experiment was repeated once. The seed germination percentages of the three weeds studied were adversely affected by the presence of increasing concentrations of all of the extracts, whereas the vegetable crops were less affected. The radicle growth of most species, with the exception of corn and cucumber, was reduced by the methanol and ethyl acetate extracts of both cover crops. Corn and cucumber radicle elongation was stimulated at low concentrations of the extracts; however these observations were not significantly different among treatments. Overall, the predicted inhibitory concentrations (IC) for each extract show that redroot pigweed and tomato were the most sensitive species tested. This study demonstrates that each of the extracts examined contains allelopathic compounds and that their phytotoxicity is species specific. Future studies

should focus on the identification and isolation of the allelochemical(s) found in the methanol and ethyl acetate extracts of the hairy vetch and cowpea residues.

INTRODUCTION

Integrating cover crops into vegetable production systems has been shown to reduce erosion and nutrient leaching, increase the populations of beneficial organisms, and suppress weeds among other benefits (Malik et al. 2000; Teasdale and Daughtry 1993; Teasdale 1996). Weed suppression has been in some cases attributed to physical competition (Samarajeewa et al. 2005; Teasdale 1996) and in others to chemical competition (Caamal-Maldonado et al. 2001; Mohler and Teasdale 1993; Singh et al. 2003; Weston 1996; Weston and Duke 2003). Competition and stimulation resulting from the release of secondary plant compounds is termed allelopathy (Molisch 1937; Weston and Duke 2003). Allelopathy also includes plant-derived compounds that possess allelopathic properties after microbial transformation. On the occasion that allelochemicals are released into the soil, either by root exudation or residue decomposition, they have the potential to influence neighboring plant life, depending on the quantity and persistence of the chemical (Putnam 1988). Allelochemicals are being explored as alternatives to synthetic herbicides for weed suppression as they could be safer for the biotic environment (Bhowmik and Inderjit 2003).

Allelopathic legume cover crops are of particular interest to growers because, in addition to the previously mentioned benefits of cover crops, they provide nitrogen to the system through symbiotic fixation. Hairy vetch (*Vicia villosa*) and cowpea (*Vigna unguiculata*) are two leguminous cover crops that have been observed in the field and the

laboratory to reduce weed populations and to injure some vegetable crops (Hoffman et al. 1993; Hutchinson and McGiffen 2000; Ngouajio et al. 2003; Ngouajio and Mennan 2005; Schroeder et al. 1998; Teasdale and Daughtry 1993; Wang et al. 2003; White et al. 1989).

Thus far, laboratory studies mainly focused on the abilities of water soluble compounds from hairy vetch to affect crop and weed growth (White et al. 1989); the effects of cowpea have not been studied in a controlled setting. Though water is the "solvent of extraction in nature" and has been shown to remove more compounds from cover crops than 50% methanol (Barnes et al. 1986), there remains a broader spectrum of chemicals in plants that could exhibit allelopathic properties. Solvents such as ethyl acetate, ethyl ether, hexane, and methanol are commonly used to perform plant extractions aimed at examining allelopathic potential or isolating the responsible allelochemical(s) (Barnes et al. 1986; Beninger and Hall 2005; Chon et al. 2003; Chon et al. 2005; Djurdjevic et al. 2004, Jefferson and Pennacchio 2003; Kato-Noguchi and Tanaka 2004; Kong et al 2004; Rimando et al. 2001). Though there may be smaller quantities of these compounds and they may not be as mobile in the soil as those found in water extracts, there is evidence that in some cases alternative solvent extracts are more potent than those in which water was the solvent (Barnes et al. 1986; Chon et al. 2005).

To our knowledge no extracts of hairy vetch and cowpea, that were derived using organic solvents, have been used in laboratory bioassays. This study intends to examine the germination and radicle response of select vegetable crops and weed species to methanol and ethyl acetate extracts of both hairy vetch and cowpea. Understanding the potential of these compounds could lead to the development of new herbicides and a better overall understanding of the allelopathic abilities of these two cover crops.

MATERIALS AND METHODS

Plant Material Extraction

Hairy vetch was planted on September 3, 2003 and harvested on May 12, 2004 at the Horticulture Research and Teaching Center on the campus of Michigan State University in East Lansing, Mich. Cowpea was planted at the same location on June 17, 2004 and harvested on August 27, September 10, and September 28, 2004; delayed harvests were needed due to the time required to perform the initial extraction. The areas harvested for each cover crop were recorded in order to calculate biomass and extract production per unit area. All plant material was rinsed with reverse osmosis (RO) water and allowed to air dry prior to being weighed. The fresh plants were blended with water and successively extracted with water, methanol, and ethyl acetate. Details of the water extraction procedure are presented in the previous chapter (Chapter 3). The residue retrieved after water extraction was frozen at -20 °C until organic solvent extraction. The frozen residue of each cover crop was placed into an 8 L column, which was plugged with 4 layers of cheese cloth and cotton batting above the stopcock, and then filled with 4-5 L of methanol and allowed to stand for a minimum of 24 h. The column was then drained and the resulting extract was evaporated to dryness using a rotary evaporator (Rotavapor R110, Büchi Labortechnik AG, Flawil, Switzerland) at 32 °C. The resulting solid was the desired methanol extract. This extraction was repeated twice and the combined extracts were stored at -20 °C. The residue from the methanol extraction was then extracted with 4-5 L of ethyl acetate three times. This process was also completed

three times. After extraction, the solids resulting from the ethyl acetate extraction were stored at -20 °C until use in the bioassays.

Germinations and Radicle Elongation Assays

The vegetable crops examined were corn (*Zea mays*), cucumber (*Cucumis sativus*), and tomato (*Lycopersicon esculentum*), while the weed species tested were common chickweed (*Stellaria media*), redroot pigweed (*Amaranthus retroflexus*), and wild carrot (*Dauca carota*) (Table 1). The experiment consisted of a randomized complete block design with seven extract concentrations, six species, and four replications with ten seeds each. The entire experiment was repeated. The methanol and ethyl acetate extracts of both cover crops were dissolved in methanol, affording concentrations of 0, 0.25, 0.5, 1, 2, 4, and 8 $g \cdot L^{-1}$ by serially diluting a stock solution of 10 $g \cdot L^{-1}$. These treatments were then applied to 90 mm Whatman No.1 filter paper in 100 mm plastic Petri dishes at 2.5 ml per dish (3.0 ml per dish for corn). The methanol was allowed to evaporate prior to seed placement, leaving behind the methanol soluble extract. The seeds of each species tested were sterilized in a 1% sodium hypochlorite solution for 10 min. They were then rinsed three times using RO water and placed 10 at a time on the dried filter papers. The weed seeds were soaked in RO water for 24 h, after sterilization to increase germination rates. Once the seeds were in place, each dish received 2.5 ml of water (corn needed 3.0 ml for imbibition). Petri dishes were subsequently sealed using Parafilm® and incubated in the dark for 4 to 11 days at temperatures specified in Table 1. Preliminary trials were conducted to determine the optimum germination times and incubation temperatures for each species. Data loggers

(Watch Dog 100-Temp 2K, Spectrum Technologies, Plainfield Ill.) were used to record the temperature in each incubation chamber every 15 min. After the incubation period, germination percentages were recorded. Seeds were considered to have germinated when radicles protruded greater than 2 mm. Radicles from each dish were separated from the shoot and floated in RO water in a clear plastic tray and scanned using a HP Scanjet 8200 scanner. Images were analyzed for radicle length using WinRHIZO® 2-3b (Regent Instruments Inc., Ste-Foy, Que. Canada).

Field Equivalent Concentration Estimates

Field equivalent concentrations were estimated based on the extract retrieved per unit of area harvested. These estimates were made using the assumptions of 1) 15 cm of cover crop incorporation, 2) simultaneous release of the extracted materials, and 3) 2.5 ml of aqueous extract are placed in each Petri dish, equating to 39.1 L per cubic meter of soil. The following equation was used to estimate field equivalent rates of the extracts:

$$FER = \frac{E(g) * P(cm^3)}{A(cm^2) * D(cm) * W(L)} \qquad [1]$$

Where FER is the estimated extract field equivalent rate, E is the total extract dry weight retrieved, A is the cover crop area harvested, P is the Petri dish volume (64 cm^3), D is the hypothetical depth of cover crop incorporation in the field (15 cm), and W is the volume of extract solution added to the Petri dish during the bioassay tests (2.5 ml, 3.0 ml for corm). Because the extracted materials are likely released over time, and not simultaneously, these extract field rate estimates are likely maximum rates. Under true field conditions the concentrations are likely lower.

Statistical Analysis

All data were subjected to analysis of variance (ANOVA) to test of differences among treatments and between experiments. Residuals were examined for normality and outliers were removed. When no experiment-by-treatment interactions were observed, data from the experiments were combined. Germination and radicle elongation parameters were analyzed using Proc GLM in SAS (Version 8, SAS 2001). Means were separated using Fisher's Protected LSD. Significance was indicated by a p-value of <0.05. All regression analyses were performed using TableCurve™2D (Version 4, AISN Software, Inc., 1996).

Prior to analysis, all radicle elongation data were converted to a percentage of the corresponding control to allow for comparisons among species. Data on radicle elongation were fitted to the logistic dose response equation:

$$RL(x) = a + \frac{b}{\left(1 + \left(\frac{x}{c}\right)^{d}\right)} \qquad [2]$$

where *RL(x)* is the radicle length (as a percent of the control) at extract concentration x, *x* is the extract concentration and *a, b, c,* and *d,* are regression coefficients. When stimulation of radicle growth was observed at low extract concentrations, a separate model was used (Norsworthy and Meehan, 2005). The model was as follows:

$$RL(x) = a * \exp\left(-0.5 * \left[\{x - b\ \}/c\right]^2\right) \qquad [3]$$

where *RL(x)* is radicle length (as a percent of the control) at extract concentration x , *x* is extract concentration, *a* is maximum radicle length (as a percent of the control), *b* is extract concentration at maximum length, and *c* is a constant. Fitted regression equations were used to estimate the extract concentrations required to cause 25% (IC_{25}), 50% (IC_{50}), and 75% (IC_{75}) inhibitions in radicle growth.

RESULTS

Extract Yield

Cowpea yielded more fresh biomass per unit area than hairy vetch and resulted in approximately 56% more methanol extract per kilogram of fresh plant material (Table 2). However, the hairy vetch produced about 5% more ethyl acetate extract per kilogram of fresh plant material than cowpea. Hairy vetch produced 9.77 and 1.48 $g \cdot m^{-2}$ of the methanol and ethyl acetate extracts, respectively. Cowpea yielded 28.84 and 0.86 $g \cdot m^{-2}$ of the methanol and ethyl acetate extracts, respectively.

Germination

Effects of methanol extracts. Cucumber, along with common chickweed, redroot pigweed, and wild carrot all showed a decrease in germination percentage as they were exposed to higher concentrations of the hairy vetch methanol extract, particularly in the 4 and 8 $g \cdot L^{-1}$ treatments (Table 3). Sixty-two and 90% reductions in germination at 8 $g \cdot L^{-1}$ were observed for common chickweed and wild carrot, respectively. Corn and tomato germination percentages were not significantly impacted among treatments.

Tomato, common chickweed, redroot pigweed, and wild carrot germination percentages were all reduced by increasing concentrations of the cowpea methanol extract (Table 3). Wild carrot germination was reduced to zero when exposed to the 8 $g \cdot L^{-1}$ concentration and tomato and common chickweed germination percentages were reduced by 52 and 75%, respectively.

Effects of ethyl acetate extracts. Hairy vetch extracted with ethyl acetate negatively affected the germination percentages of all species tested except corn (Exp. 2) and tomato (Table 4). Again, the greatest decrease in germination was seen in the 4 and/or the 8 $g \cdot L^{-1}$ treatments. Wild carrot germination was reduced by 80% in the 8 $g \cdot L^{-1}$ treatment compared with the control.

Common chickweed and wild carrot germination percentages were significantly reduced as cowpea ethyl acetate extract concentration increased (Table 4). Maximum reductions of 32 and 84% were observed, respectively, for those two species at the 8 $g \cdot L^{-1}$ concentration. The germination of all the other species was not affected.

Radicle Elongation

The radicle lengths of all species tested were significantly reduced when exposed to the increasing concentrations of methanol and ethyl acetate extracts of hairy vetch (Fig. 1). The cowpea extracts inhibited radicle growth of all species except corn and cucumber (Fig. 2). These responses were described using the logistic dose response equation (Eq. [2]). Corn and cucumber radicle growth was stimulated by the cowpea ethyl acetate extract, though not significantly, and was best described by Eq. [3]. R^2 values for all species tested were generally at 0.90 or above (Tables 5 and 6). One

exception occurred with cucumber that was exposed to the ethyl acetate extract of hairy vetch. Cucumber radicle elongation showed no significant response to the increasing concentrations of the hairy vetch ethyl acetate extract. The few experiment-by-treatment interactions that occurred are listed by experiment. Estimated inhibitory concentrations (IC) that reduced radicle length by 25, 50, and 75% varied by cover crop, organic solvent, and species tested (Table 7).

Effects of methanol extracts. The radicle lengths of all species tested were reduced as the hairy vetch methanol extract concentration increased (Table 5, Fig. 1). The greatest reductions in radicle length compared with the control, occurred in redroot pigweed (18%) and tomato (20%) exposed to the 8 $g \cdot L^{-1}$ hairy vetch methanol extract treatment. The order of species sensitivity, from most sensitive to least, at 8 $g \cdot L^{-1}$: was as follows: redroot pigweed > tomato > common chickweed > wild carrot > corn > cucumber.

The cowpea methanol extract caused a radicle response similar to that of the hairy vetch methanol extract by reducing radicle lengths of all species tested with increasing concentrations (Table 5, Fig.1). The two most sensitive species were again redroot pigweed and tomato, each reduced to 15% of their respective controls in the 8 $g \cdot L^{-1}$ treatment. Species sensitivity to the cowpea methanol extract was as follows: redroot pigweed > tomato > common chickweed > cucumber > wild carrot > corn.

Effects of ethyl acetate extracts. The hairy vetch ethyl acetate extract stimulated the radicle growth of corn at the 0.25 to 4 $g \cdot L^{-1}$ rates (Table 6, Fig. 2). Maximum stimulation of 121% of the control was reached in corn in the 1 $g \cdot L^{-1}$ treatment. Cucumber growth was not affected at any of the tested concentrations, hovering around

100% of the control in all treatments. Tomato and all of the weed species tested were negatively affected by the hairy vetch ethyl acetate extract. Tomato and redroot pigweed were the most sensitive species with radicle lengths reduced to 22 and 29%, respectively, in the 8 $g \cdot L^{-1}$ treatment. Species sensitivity occurred in the following order (most sensitive to least): tomato > redroot pigweed > wild carrot > common chickweed > cucumber > corn in the 8 $g \cdot L^{-1}$ treatment.

Corn and cucumber radicle growth was stimulated by the cowpea ethyl acetate extract at all concentrations (Table 6, Fig. 2). Maximum stimulation of 136% of the control was observed for cucumber at 4 $g \cdot L^{-1}$ and 174% for corn at 2 $g \cdot L^{-1}$. The radicle lengths of all other species tested were significantly reduced by increasing concentrations of cowpea ethyl acetate extract. Redroot pigweed and tomato experienced the greatest reductions in radicle growth at 19 and 23% of their controls at 8 $g \cdot L^{-1}$. Sensitivity of the test species fell in this order: redroot pigweed > tomato > common chickweed > wild carrot > cucumber > corn.

Estimated inhibitory concentrations (IC). Radicle elongation of all species other than corn and cucumber were significantly impacted by the presence of both the methanol and ethyl acetate extracts of both hairy vetch and cowpea. Corn and cucumber were only significantly affected by the cowpea methanol extract. The IC rates, as predicted by the regression analyses, for 25, 50, and 75% reduction in radicle growth provide better separations among the species tested. The equations for some species reached asymptotes prior to the tested percentage reduction. In these cases the radicles would theoretically never be reduced by that percentage, no matter the extract concentration.

Methanol IC. When examining the methanol extract quantities needed to reduce radicle growth by 25, 50, and 75% of the control, it is apparent that redroot pigweed and tomato were the most sensitive species to both the hairy vetch and the cowpea extracts (Table 7). Common chickweed was also highly sensitive to the cowpea methanol extract, needing only a concentration of 1.2 $g \cdot L^{-1}$ to reduce radicle length by 75%.

Ethyl acetate IC. Overall, tomato (Ex. 1) and redroot pigweed were the most sensitive species to both the hairy vetch and cowpea ethyl acetate extracts as judged by the extract concentrations found by IC_{25}, IC_{50}, and IC_{75} (Table 7).

DISSCUSION

This study allowed for exploration beyond the previous field and water extract studies of hairy vetch and cowpea. Using methanol and ethyl acetate as solvents likely broadened the extract spectrum and thus allowed for further allelopathic investigation.

Seed Germination

The germination percentages of all weed species studied were significantly reduced by the methanol and ethyl acetate extracts of both hairy vetch and cowpea, with the exception of redroot pigweed, which was not sensitive to the ethyl acetate extract of cowpea. The germination percentages of corn and tomato were the least effected by the studied extracts, showing susceptibility only to the hairy vetch ethyl acetate and the cowpea methanol extracts, respectively. Cucumber germination was reduced by both hairy vetch extracts but was not affected by either of the cowpea extracts. In general, germination of the weed species was more susceptible to the presence of the tested

extracts than were the vegetable species. This finding concurs with the idea that seed size may be a factor in susceptibility to allelochemicals, with smaller seeds being more sensitive (Mohler and Teasdale 1993; Putnam and DeFrank 1983). Larger seeds have increased nutrient reserves which allow them to withstand harsh environmental conditions better than smaller seeds (Leishman 2001). Perhaps the heightened susceptibility of weed seed germination versus vegetable seeds could be of great advantage if the responsible allelochemicals can be isolated and formulated into a preemergence herbicide.

Radicle Elongation

Stimulation of corn and cucumber radicle lengths was observed for low concentrations of the ethyl acetate extracts, though significant differences among treatments were not found. In our previous study, corn was also stimulated at low concentrations of the cowpea water extract. Many other studies have also reported growth stimulations by low concentrations of plant extracts and residues (Ben-Hammouda et al. 2001; Mohler and Teasdale 1993; Norsworthy and Meenan; Sinkkonen 2003; Teasdale 1996). Perhaps the responsible allelochemicals are nitrogenous and stimulatory at sublethal levels, similar the stimulation of isothiocyanates observed by Norsworthy and Meehan (2005).

In all other species examined there was a significant decrease in radicle elongation with increasing concentrations of the methanol and ethyl acetate extracts of both cover crops. This finding was similar to that of our study on the water extracts of

these two cover crops. On the whole, redroot pigweed, tomato, and common chickweed were the species most inhibited by the extracts tested.

Tomato was one of the most sensitive species in terms of radicle length, though tomato germination was not vulnerable to the extracts. This supports the findings of Leather and Einhellig (1986) who observed that radicle elongation was a more sensitive parameter to measure than seed germination when looking for allelochemical responses in some species. However, wild carrot is an example of an exception. Though the germination of wild carrot was highly sensitive to the extracts (no seeds germinated in the presence at 8 $g \cdot L^{-1}$ of the cowpea methanol extract), radicle length was overall not as susceptible as that in redroot pigweed and tomato. These two conflicting points supports the idea that sensitivities to plant extracts and allelochemicals are species specific.

In order to put this study into prospective, it is important to know the amount of extract produced in the field by both hairy vetch and cowpea. Also, it is critical to know where the field equivalent rates of the extracts would fit in the range of concentrations tested in this study. Field equivalent rates for the methanol and ethyl acetate extracts of hairy vetch and cowpea were estimated based on extract yield per unit of fresh biomass per unit of area (Table 2). Maximum rates of 1.67 $g \cdot L^{-1}$ and 0.25 $g \cdot L^{-1}$ were found for the methanol and ethyl acetate extracts of hairy vetch. While 4.92 $g \cdot L^{-1}$ for methanol and 0.29 $g \cdot L^{-1}$ for ethyl acetate were calculated for cowpea. These values were within the range of concentrations tested in this study. The calculations assumed that the residues of the cover crops were incorporated 15 cm into the soil profile and that all plant material decomposed simultaneously. The amount of time required for residue breakdown under field conditions is influenced by environmental and biological conditions (Kuo et al.

1997; Weston 1996; White et al. 1989). As a result, the concentrations produced in the field are likely to be lower. Overall, at these estimated maximum field rates, we would expect to see reduced germination percentages and radicle lengths for cowpea and to a lesser degree for hairy vetch.

When comparing the activity of the extracts of the two cover crops (using the IC estimates), it appears that the compounds in cowpea are more inhibitory than those in hairy vetch. Of the two cowpea extracts, the methanol extract showed the greatest potential to reduce germination and radicle growth, warranting further isolation.

CONCLUSION

From this study it is evident that the extracts of hairy vetch and cowpea derived using methanol and ethyl acetate exhibited allelopathic effects on the tested vegetable crops and weed species germination and radical elongation. The next step should be to isolate, identify, and assay the specific allelochemicals and examine their potential for practical use in vegetable production systems.

Table 1. Vegetable and weed species used in the bioassays of hairy vetch and cowpea methanol and ethyl acetate extracts.

Common name	Scientific Name	Variety/Bayer code	Incubation Temperature[a] (°C)	Incubation Time (Days)
Crop				
Cucumber	*Cucumis sativus*	Vlaspik	21	4
Sweet corn	*Zea mays*	Jubilee	21	5
Tomato	*Lycopersicon esculentum*	Mountain Spring	21	5
Weed				
Common chickweed	*Stellaria media*	STEME	21	11
Redroot pigweed	*Amaranthus retroflexus*	AMARE	30/25	4
Wild carrot	*Daucus carota*	DAUCA	30/25	7

[a] Incubation temperatures separated by a slash indicate the 16/8 hour fluctuations of the growth chambers.

Table 2. Hairy vetch and cowpea harvest data and methanol and ethyl acetate extract data.

Cover crop	Area harvested	Total fresh biomass	MeOH		EtOAc	
			Extract dry weight	Extract field rates*	Extract dry weight	Extract field rates*
	—m^2—	—kg—	——g——	—$g \cdot L^{-1}$—	——g——	—$g \cdot L^{-1}$—
Hairy vetch	12.54	25.49	122.50	1.67	18.60	0.25
Cowpea	9.29	31.15	267.90	4.92	16.00	0.29

*Extract field equivalent rates (Eq. [1])

Table 3. Germination percentages of test species resulting from exposure to seven concentrations of hairy vetch and cowpea methanol extracts.

Cover Crop	Vegetable Crop	Extract Concentration ($g \cdot L^{-1}$)							LSD
		0	0.25	0.5	1	2	4	8	
Hairy Vetch	C. chickweed	81.3	88.8	91.3	91.3	80.0	60.0	35.0	16.5
	Corn	98.8	98.8	100.0	98.8	96.3	88.8	91.3	NS
	Cucumber	85.0	90.0	75.0	75.0	71.3	67.5	77.5	13.5
	Redroot pigweed	83.8	86.3	80.0	73.8	82.5	68.8	57.5	13.9
	Tomato	91.3	93.8	90.0	82.9	91.3	86.3	86.3	NS
	Wild carrot	51.3	46.3	50.0	50.0	38.8	17.5	5.0	11.6
Cowpea	C. chickweed	81.3	63.8	77.5	77.5	66.3	61.3	20.0	18.1
	Corn	97.5	95.0	95.0	92.5	95.0	98.3	82.5	NS
	Cucumber	83.8	77.5	88.8	86.3	85.0	88.8	81.3	NS
	Redroot pigweed	87.5	91.3	90.0	85.0	91.7	71.3	57.5	13.1
	Tomato	93.8	96.3	93.8	90.0	85.0	86.3	45.0	11.4
	Wild carrot	52.5	36.3	46.3	30.0	21.3	1.3	0.0	13.8

NS=Not Significant ($p>0.05$)

Table 4. Germination percentages of test species resulting from exposure to seven concentrations of hairy vetch and cowpea ethyl acetate extracts.

Cover Crop	Vegetable Crop	Extract Concentration ($g \cdot L^{-1}$)							LSD
		0	0.25	0.5	1	2	4	8	
Hairy Vetch	Common chickweed	85.0	91.3	87.5	92.5	91.3	77.5	65.0	11.8
	Corn Ex.1	100.0	95.0	95.0	97.5	100.0	85.0	95.0	9.2
	Corn Ex.2	87.5	97.5	100.0	100.0	100.0	100.0	90.0	6.0
	Cucumber	81.3	93.8	82.5	82.5	81.3	77.5	77.5	10.3
	Redroot pigweed	78.8	80.0	72.5	78.8	61.3	68.8	70.0	16.7
	Tomato	97.5	97.5	91.3	91.3	91.3	93.8	87.5	NS
	Wild carrot	55.8	56.3	56.3	56.3	42.5	27.5	11.3	17.8
Cowpea	Common chickweed	90.0	91.3	80.0	82.5	87.5	90.0	61.3	13.4
	Corn	93.8	95.0	96.3	100.0	96.3	100.0	100.0	NS
	Cucumber	81.3	85.0	80.0	83.8	83.8	82.5	83.8	NS
	Redroot pigweed	85.0	76.3	78.8	81.3	72.5	70.0	71.3	NS
	Tomato	93.8	88.8	85.0	96.3	93.8	90.0	93.8	NS
	Wild carrot	71.3	60.0	55.0	52.5	50.0	12.5	11.3	15.5

NS=Not Significant ($p>0.05$)

Table 5. Regression parameters for the logistic dose response equation fitted to data on radicle elongation of test species exposed to methanol extracts of hairy vetch and cowpea.

		Logistic Dose Response $y=a+b/(1+(x/c)^d)$				
Cover Crop	**Crop/Weed**	**r^2**	**a**	**b**	**c**	**d**
Hairy Vetch	Corn	0.90	-148.14	246.88	899.12	0.38
	Common chickweed	0.99	23.86	75.90	0.91	1.25
	Cucumber	0.86	66.78	33.18	0.59	0.45
	Redroot pigweed	0.99	18.55	81.81	0.53	1.69
	Tomato	0.99	14.46	85.72	1.01	1.40
	Wild carrot	0.98	32.26	73.54	1.20	2.04
Cowpea	Corn	0.91	13.48	86.56	6.26	0.60
	Common chickweed	0.99	16.77	83.12	0.25	1.41
	Cucumber	0.97	-153.49	252.80	210.70	0.33
	Redroot pigweed	0.99	10.27	89.71	0.29	0.83
	Tomato	0.99	-0.39	100.29	0.65	0.67
	Wild carrot	0.96	37.90	62.30	0.17	1.36

Table 6. Regression parameters for the logistic dose response equation fitted to data on radicle elongation of test species exposed to ethyl acetate extracts of hairy vetch and cowpea.

		Logistic Dose Response $y=a+b/(1+(x/c)^d)$				
Cover Crop	**Crop/Weed**	**r^2**	**a**	**b**	**c**	**d**
Hairy Vetch	Corn †	0.77	124.37	-0.30	-22.47	-
	C. chickweed Ex.1	0.98	37.61	58.30	1.95	2.06
	C. chickweed Ex.2	0.93	40.26	58.03	3.31	2.32
	Cucumber ‡	-	-	-	-	-
	Redroot pigweed	0.99	14.11	86.28	2.44	1.29
	Tomato Ex.1	0.99	-51.29	150.94	6.81	0.55
	Tomato Ex.2	0.94	27.05	76.40	2.67	1.04
	Wild carrot	0.98	-0.37	100.91	4.04	0.78
Cowpea	Corn	0.99	8.51	89.95	1.27	1.25
	Common chickweed	0.99	20.68	78.96	0.52	1.12
	Cucumber †	0.77	134.08	-0.46	-40.80	-
	Redroot pigweed	0.99	8.51	89.95	1.27	1.25
	Tomato	0.99	-17.03	116.70	2.44	0.59
	Wild carrot	0.98	33.43	66.19	0.37	3.20

† Ethyl acetate extracts of hairy vetch and cowpea stimulated the growth of corn and cucumber, respectively, therefore the data were best fit to equation 1 ($y=a*\exp(0.5((x-b)/c)^2)$).

‡ Cucumber did not show a significant response for the ethyl acetate hairy vetch extract and did not fit either of the equations presented.

Table 7. Inhibitory concentrations of the methanol and ethyl acetate extracts of hairy vetch and cowpea that reduced vegetable and weed radicle lengths by 25 (IC_{25}), 50 (IC_{50}), and 75% (IC_{75}). These rates are based on the derived regression analyses for each species. The ∞ signs signify that an asymptote was reached prior to the given reduction.

		Hairy vetch extract ($g \cdot L^{-1}$)			Cowpea extract ($g \cdot L^{-1}$)		
	Species	IC_{25}	IC_{50}	IC_{75}	IC_{25}	IC_{50}	IC_{75}
Methanol	C. chickweed	0.51	1.52	25.81	0.14	0.33	1.20
	Corn	2.45	22.32	94.82	1.40	10.98	141.51
	Cucumber	7.10	∞	∞	0.23	2.84	14.73
	R. pigweed	0.33	0.70	2.72	0.09	0.38	2.03
	Tomato	0.54	1.30	4.12	0.13	0.64	3.24
	Wild carrot	1.02	2.10	∞	0.12	0.47	∞
Ethyl Acetate	C. chickweed	1.47	3.68	∞	0.25	0.83	6.56
	C. chickweed 2*	2.78	6.58	∞	NA	NA	NA
	Corn	12.83	15.88	18.35	16.47	18.07	18.12
	Cucumber †	-	-	-	11.32	13.52	15.45
	R. pigweed	1.24	3.18	10.91	0.55	1.44	4.17
	Tomato	0.35	1.86	6.55	0.26	1.47	6.43
	Tomato 2*	1.62	6.04	∞	NA	NA	NA
	Wild carrot	1.02	4.06	16.31	0.31	0.52	∞

* The species with a "2" beside them indicate that for the hairy vetch and/or cowpea extract experiments there was a treatment-by-experiment interaction. For that reason the IC values are presented by experiment.

†Cucumber did not fit either of the tested regression equations for the ethyl acetate extract of hairy vetch, therefore no IC values could be predicted.

NA signifies that the data from experiments 1 and 2 were able to be combined; hence there is only one set of estimated inhibitory concentrations.

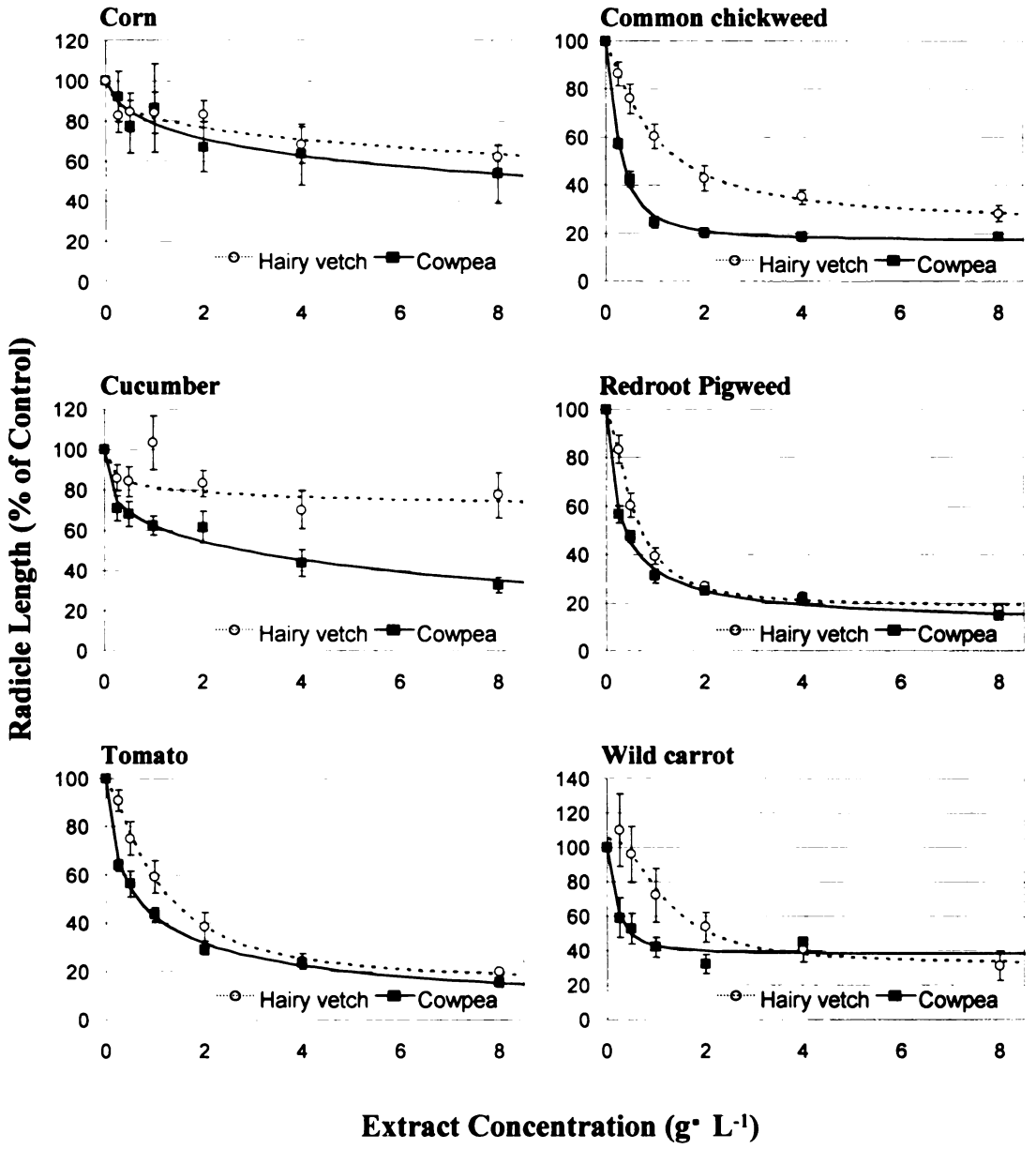

Figure 1. Radicle growth of vegetable crops and weeds as affected by increasing concentrations of hairy vetch and cowpea methanol extracts. All data were fitted to the logistic dose response equation (Eq. [2]).

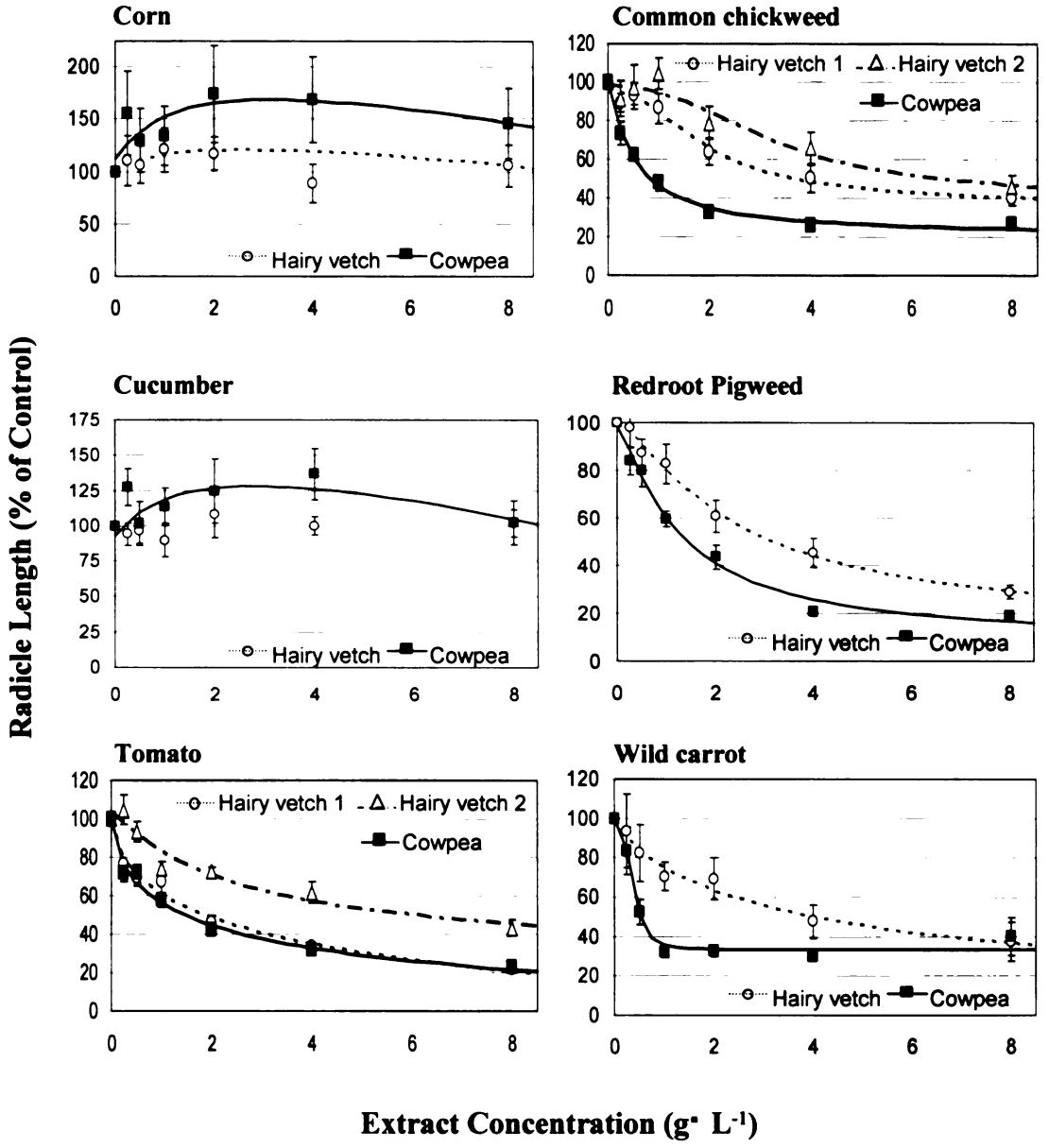

Figure 2. Radicle growth of several vegetable crops and weeds as affected by increasing concentrations of hairy vetch and cowpea ethyl acetate extracts. All data were fitted to the logistic dose response Eq. [2] except corn and cucumber (Eq. [3]). Cucumber exposed to hairy vetch did not fit either equation.

CHAPTER 6: Conclusions and Future Work

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Overall, these studies provided evidence to support the potential presence of allelochemicals in both hairy vetch and cowpea. The field study showed a shift in weed species composition in the hairy vetch system; even though overall weed biomass was not affected. Particularly noticeable was the transition from a quackgrass dominated weed community in the no cover treatments to common purslane dominated community in the hairy vetch treatments. Therefore, the relative importance of individual weed species should be taken into account if a hairy vetch cover crop is used as a part of an integrated weed management program. In all cases, additional management strategies should be used to achieve adequate weed suppression.

Cucumber benefited from the hairy vetch residues. All planting dates yielded at or above their no cover compliments. The best yields were achieved when 3 to 4 weeks were allowed between hairy vetch incorporation and cucumber planting.

In the laboratory studies examining the effects of hairy vetch and cowpea water, methanol, and ethyl acetate extracts, there was strong evidence of allelopathy by all extracts to most vegetables and weeds tested. Overall, germination and radicle elongation of the weed species were more sensitive to the extracts than the vegetable species. At 8 $g \cdot L^{-1}$, the water and methanol extracts of cowpea were most inhibitory when compared with the cowpea ethyl acetate extract and all hairy vetch extracts. Not only were these cowpea extracts more inhibitory than those of hairy vetch, but cowpea produced more of the extracts per unit of fresh biomass than hairy vetch; meaning that the allelochemical(s)

in cowpea are potentially more potent and more abundant than those in hairy vetch under field conditions.

This study has contributed significantly to the better understanding of the effects of hairy vetch and cowpea on cropping systems. We have demonstrated a significant benefit of integrating hairy vetch into pickling cucumber cropping systems. Cucumber yield was consistently higher in the hairy vetch treatments as a result of better nutrient cycling and other growth conditions (e.g. soil quality, soil ecology, etc.). We have shown that short term weed population shifts may occur under a hairy vetch cover crop. This information is essential in predicting changes in weed populations over time and for designing integrated weed management programs. Laboratory studies strongly suggest that allelopathy was the process underlying some of the weed population changes observed in the field. A screening of a large number of vegetables and weeds against extracts of hairy vetch and cowpea showed strong inhibitory effects at high rates and differential sensitivity of the species studied. The variance in sensitivity of the species helped explain why, under field conditions, weed species composition was more sensitive than total weed biomass to hairy vetch.

Despite the large amount of information collected, several questions remain unanswered at the conclusion of this research. The answer to those questions will require further studies in the laboratory, greenhouse, and field. The following are some of the directions that those studies could take.

Laboratory directions:

- Isolation and identification of the allelochemicals in hairy vetch and cowpea
- Testing of hairy vetch extract and/or allelochemical bioassays with quackgrass and common purslane to confirm population shifts observed in the field
- Exploration of extraction methods that are organic-friendly (i.e. no chemical solvents) for potential use of the extracts in certified organic systems

Greenhouse directions:

- Assessment of quackgrass, common purslane, and cucumber growth and development in the presence of hairy vetch residues at different concentrations
- Evaluation of hairy vetch and cowpea extract potentials for use as bioherbicides applied either pre or post emergence

Field directions:

- Extension of the study over more than two years to confirm the stability of the results
- Examination of weed biomass per species
- Search for synergistic relationships using hairy vetch with other means of weed control
- Determination of allelochemical release rates in the field (after allelochemical identification)
- Assessment of nitrogen release rates of hairy vetch residues under different soil types

- Comparison of weed suppression of hairy vetch when used as a green manure (i.e. entirely a surface residue) compared with as an incorporated residue

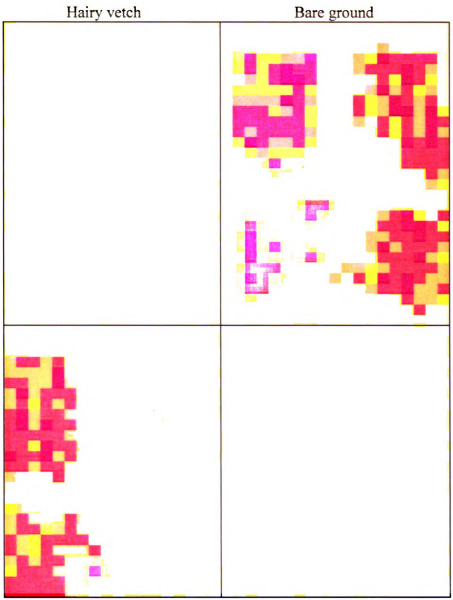
These studies will provide additional information that will improve sustainability of cucumber production by enhancing nutrient management and weed biosuppression by allelopathic interactions.

Appendix A: 2005 Quackgrass Mapping Prior to Hairy Vetch Incorporation

Appendix A: 2005 Quackgrass Mapping Prior to Hairy Vetch Incorporation

Due to its observed prominence in 2004, estimates of quackgrass (*Elytrigia repens*) canopy cover were taken in the research plot used for Chapter 2 prior to hairy vetch incorporation in 2005. Hairy vetch was planted on September 13, 2004 and incorporated on June 1, 2005 in two of the four blocks. On May 18, 2005 (14 days prior to hairy vetch incorporation), the entire field was setup with a grid system, consisting of 2,035 sampling areas each 91 by 91 cm. Quackgrass canopy cover was estimated visually using rating 0-5, with 0= no quackgrass, 1= 1-20% quackgrass canopy cover, 2= 21-40% cover, 3= 41-60% cover, 4= 61-80% cover, and 5= 81-100% quackgrass canopy cover. The resulting data were color coded to create a map of the plot in terms of quackgrass canopy cover (Figure 1).

Because quackgrass was so concentrated in the bare ground areas and not in the hairy vetch areas, it is possible that physical competition is deterring quackgrass from the hairy vetch plots prior to allelochemical release. Continued field observations, along with laboratory and greenhouse studies, would help to determine if the suppression of quackgrass caused by hairy vetch is due to physical competition, allelopathy, or a combination of the two factors.



Percent Cover	0	1-20	21-40	41-60	61-80	81-100
Color Code						

Figure 1. Map of quackgrass canopy cover prior to hairy vetch incorporation. Canopy cover is expressed as a range of percentages 0, 1-20, 21-40, 410-60, 61-80, and 81-100%. Each square represents an area of 91 by 91 cm. Images in this thesis are presented in color.

LITERATURE CITED

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