STRAWBERRY (*Fragaria x ananassa* Duchesne) RESPONSE TO CLOPYRALID APPLIED DURING FRUITING STAGE

By

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To my parents

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HAT	Hours after treatment
IFAS	Institute of Food and Agricultural Sciences
LDPE	Low-density polyethylene
PHI	Pre-harvest interval
RH	Relative humidity
VIF	Virtually impermeable film
WAP	Weeks after planting
WAT	Weeks after treatment

Abstract of Thesis Presented to the Graduate School
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In Florida, strawberry (*Fragaria x ananassa* Duchesne) is considered to be one of the most economically important winter crops, with production occurring primarily within a 13 square mile area near Plant City, Florida. Florida is responsible for supplying approximately 15% of the total U.S. strawberry production. Within this15%, Florida produces a large percentage of the domestically-grown winter strawberry crop. In 2011 approximately 4,000 ha of strawberries were planted in Florida with an estimated value of $366 million. Florida's ability to produce strawberries at a time when others cannot is the main reason this crop has become economically important in the state. The Florida strawberry industry is currently facing many challenges, one of which being the loss of methyl bromide use as a soil fumigant, and the many challenges that must be addressed with the transition to a new integrated pest management approach and alternative fumigant system. Recent studies are showing that along with the new fumigant systems, separate but complementary herbicide applications throughout the growing season will also be a necessity for acceptable weed control. The purpose of the studies reported herein, were to evaluate the impacts of the herbicide clopyralid on

the fruit production of strawberry growing in an annual plasticulture cropping system. Greenhouse and open field trials were conducted, evaluating the application of varying rates of clopyralid either as a directed spray or drip application to well established, mature plants. Strawberry fruit size, shape, and number were evaluated to determine any loss of marketable yield due to the physiological and or phytotoxic herbicidal effects of clopyralid. Results from these studies demonstrated that when clopyralid was applied at the maximum labeled rate of 213 $g{\bullet}ha^{-1}$, less than 5% leaf malformation was observed and yield was estimated at approximately 104% of the non-treated control. Negative effects on plant growth and yield were observed when clopyralid application rates reached 628 $g{\bullet}ha^{-1}$.

CHAPTER 1
INTRODUCTION

Florida Strawberry Industry

During the winter of 2010-2011 strawberries (*Fragaria x ananassa* Duchesne) were planted in west central Florida on approximately 4,000 ha, with an estimated value of $366 million (NASS-USDA 2011). On an annual basis this production accounts for a large portion of the domestically produced winter crop, and approximately 10-15% of the total U.S. production. As of 2007, approximately 90% of the Florida strawberry production occurred within Hillsborough County, with the remainder of the crop scattered throughout west-central Florida (Census of Agriculture-USDA 2007). Advancements in breeding of short-day cultivars have given producers a unique market advantage; resulting in the ability of Florida producers to provide strawberries at times no other domestic producer is able to (Jones et al. 2003). This ability enables Florida producers to receive premium price for strawberries, resulting in the economic importance of the crop to the state (Santos et al. 2007a).

Fumigation

For over 50 years it has been repeatedly demonstrated that soil fumigation is necessary to obtain maximum plant growth and yields in strawberry production (Chandler et al. 2001; Fort et al.1996; Johnson et al. 1962; Yuen et al. 1991). Most fumigants are typically shank-injected either prior to or during bed formation. In addition to shank injections, drip-application (chemigation) of specially formulated fumigants has become a common practice in Florida strawberry.

Methyl Bromide

For more than 50 years strawberry producers in Florida have relied heavily on a preplant soil fumigation treatment with methyl bromide to alleviate pest pressures from weeds, nematodes, and soil borne pathogens (Chandler et al. 2001). Over 50 years ago the first successful control of Verticillium wilt (*Verticillium spp.*) in strawberry was documented in California with a preplant application of chloropicrin (Johnson et al. 1962). This sparked a new era of research in strawberry production with rapid advances in raised bed and field application technology, plastic mulch bed covers, and refinement of fumigant combinations. A combination of two-thirds methyl bromide and one-third chloropicrin was found to be the most effective at controlling pests and initially became the industry standard (Johnson et al. 1962). In more recent years, a combination consisting of methyl bromide and chloropicrin, in a 98:2 ratio, was the more popular choice among Florida producers.

In 1993 methyl bromide was classified as a class I ozone depleting substance under the provisions of an international treaty known as Montreal Protocol for substances that deplete the ozone. At this time, the Protocol recommended a phase out of the use and production of methyl bromide in the U.S. and other developed countries by the year 2010 (Honaganahalli and Seiber 1996). Subsequently the U.S. Environmental Protection Agency (EPA) acting under the Clean Air Act of 1990 initially set a much stricter phase out date of January 1, 2001 which was ultimately postponed until January 1, 2005 (Ferguson and Padula 1994; Honaganahalli and Seiber 1996; Noling et al. 2011). States have the ability to request the use of additional methyl bromide under a process referred to as a Critical Use Exemption (CUE). It requires a collaboration of university researchers and grower organizations to adequately describe and document the need for methyl bromide where “no technically or economically feasible alternative to methyl bromide is shown to exist” (Noling et al. 2011).

As methyl bromide reserves diminish and prices increase, many strawberry producers who have transitioned to alternative fumigants are now beginning to observe the shortcomings of the alternative fumigants. A recent strawberry grower survey conducted by the University of Florida Cooperative Extension Service revealed that the majority of respondents indicated that pest problems present in their fields were increasing (Snodgrass et al. 2011). In general, problems were reported to be increasing following use of alternative fumigants including metam potassium (Potassium N-methyldithiocarbamate), metam sodium (Sodium N-methyldithiocarbamate) and 1,3-Dichloropropene/ chloropicrin combinations commercially know as Pic-Clor 60® (39:59) and Telone C35® (63:35).

Methyl Bromide Alternatives

Pre-plant soil fumigation has historically been an essential part of weed control in plasticulture strawberry production and has been shown to be essential to maximize yields (Johnson et al. 1962). Extensive field research continues to evaluate methyl bromide alternative fumigants and fumigant systems for their herbicidal activity. Due to the overall lack of herbicidal activity associated with many of the alternatives, weed control is deemed one of the highest pest management priorities (Noling et al. 2011). Current alternative fumigants for strawberry production include chloropicrin (Pic), 1,3-dichloropropene (1,3-D), metam potassium or sodium (Metam), and newly registered dimethyl disulfide (DMDS). Pre-mix combinations of chloropicrin and 1,3-D are also available such as Telone C35® and PicClor 60®, containing 35 and 60% chloropicrin respectively. Among these fumigants, Noling (2010) reported the greatest strawberry yield was obtained with applications of Telone C-35® (325 L•ha^{-1}) and PicClor 60® (336 kg•ha^{-1}). Othman et al. (2010) reported yields similar to methyl bromide and chlorpicrin (57:43) at 336 kg•ha^{-1}with applications of DMDS+Pic (389/111 kg•ha^{-1}). Similar to the findings of Othman et al. (2010), Noling (2010) reported higher strawberry yields than that of the methyl bromide and chloropicrin standard with applications of DMDS+Pic (21% V/V) at 570 L•ha^{-1}. Currently the University of Florida/IFAS recommendation for a methyl bromide and chloropicrin alternative fumigant for sting nematode infested strawberry fields is a pre-bed application of Telone C35® at 327 L•ha^{-1} followed by a bed-top application of napropamide and oxyfluorfen at 4.45 and 0.54 kg•ha^{-1} respectively (Noling et al. 2011).

Strawberry Production

Unlike many of the northern strawberry producing regions which utilize the perennial matted-row production system, Florida strawberries are grown in an annual plasticulture cropping system much like that of many vegetables crops. This production system consists of raised beds covered with polyethylene mulch, soil fumigation, and the use of drip tubes in the bed for irrigation (Mossler 2010). Following an appropriate fumigant aeration period, bare-root strawberry plants are typically planted from mid-September through the end of October, depending on cultivar, in central Florida. Average strawberry yields range from approximately 7,780 to 10,750 3.6 kg flats per hectare (Maynard and Santos 2007; Santos et al. 2011).

Cultivars

Strawberry (*Fragaria x ananassa* Duchesne) is a commonly cultivated crop widely adapted to many geographic regions ranging from the low land tropics to higher altitude regions of the world with continental climate (Darnell 2003). *Fragaria. x ananassa* was first discovered in 1766 by the French botanist Antoine Nicholas Duchesne, who determined that *Fragaria. x ananassa* was a hybrid resulting from a cross between *Fragaria chiloensis* L. Mill. and *Fragaria virginiana* Duchesne. In comparison to other crops of major importance, the delay in cultivation of the strawberry is thought to have stemmed from the abundance in which they were available and ease in which they were collected from the wild (Hancock 1999).

The classification of strawberry cultivars is determined by the way in which they respond to the length of photoperiods (Darnell 2003). Long-day, or ever bearing, cultivars will initiate flower and fruit formation under daily photoperiods of light exceeding 12 hours. The flowering and fruiting of day-neutral cultivars is based on a cycle and therefore is independent of photoperiod. In Florida, the majority of strawberry cultivars grown are short-day cultivars, also referred to as June bearing. Short-day cultivars will initiate flower formation under photoperiods typically less than 14 hours of light (Hancock 1999; Darnell 2003). These short-day cultivars are necessary for producers in order to be able to produce a strawberry crop during the winter, which are times of the year favorable for strawberry growth in Florida. The majority of the strawberries grown in Florida are shipped as fresh fruit throughout the eastern U.S. In addition to the need for timely production of visually attractive and tasteful fruit, producers need varieties that provide fruit capable of withstanding the effects of handling and travel that come with long distance shipping (Chandler et al. 2000).

‘Strawberry Festival’ is short-day cultivar released by the University of Florida in 2000 and recognized by growers for its consistently firm and attractive fruit, and long shelf life (Chandler et al. 2000; Chandler 2004). Currently ‘Strawberry Festival’ is the most widely planted cultivar throughout the west central Florida producing region (Santos et al. 2009). ‘Strawberry Festival’ originated from a cross of the seed parent ‘Rosa Linda’, and the pollen parent ‘Oso Grande’ (Chandler 2004). One of the distinguishing characteristics of ‘Strawberry Festival’ is the abundance of runners it produces if planted in early October. ‘Strawberry Festival’ is reportedly moderately susceptible to anthracnose fruit rot (*C. acutatum*), Colletotrichum crown rot (*C. gloeosporodies*), and angular leaf spot (*X. fragarie*), however is more resistant to Botrytis fruit rot (*B. cinerea*) and powdery mildew (*S.* macularis) in comparison to other varieties (Chandler et al. 2000; Chandler 2004). During the 2007 and 2008 growing seasons, ‘Strawberry Festival’ was shown to yield an average of 14.05 t•ha^{-1} (Santos et al. 2009).

‘Treasure’ is a short-day cultivar released in 2000 by J&P Research. ‘Treasure’ originated from a 1997 cross between ‘A3’ and ‘Oso Grande’ (Chang 2002). The major distinguishing characteristic of ‘Treasure’ is its high yields of large and early-season fruit. Plants of ‘Treasure’ are typically ready for harvesting 7 weeks after planting in west central Florida production fields. ‘Treasure’ has a high tolerance for anthracnose crown rot (*C. gloeosporodies)* and is moderately tolerant to botrytis (*B. cinerea*) in comparison to other cultivars (Chang 2002). During the 2007 and 2008 growing seasons, ‘Treasure’ was shown to yield an average of 14 t•ha^{-1} (Santos et al. 2009).

‘Florida Radiance’ is a short-day cultivar released by the University of Florida in 2009 (Chandler 2009; Chandler et al. 2009). ‘Florida Radiance’ originated from a cross between the seed parent ‘Winter Dawn’ and the pollen parent FL 99-35 (Chandler 2009). It was developed to complement ‘Strawberry Festival’ in that ‘Florida Radiance’ was shown to provide earlier yields while still producing larger berries later in the season, times when the production of 'Strawberry Festival' was not prime (Chandler et al. 2009). 'Florida Radiance' has been shown to be moderately resistant to Botrytis fruit rot (*B. cinerea*) as well as anthracnose fruit rot (*C. acutatum*), however is suspectible to various crown rots thought to be caused by *C. gloeosporioides* and *Phytophthora spp.* (Chandler 2009; Chandler et al. 2009). During the 2007 and 2008 growing seasons, 'Florida Radiance' reportedly yielded an average of 12.8 $t \bullet ha^{-1}$ (Santos et al. 2009).

'Winterstar' is a short-day cultivar recently released by the University of Florida in 2011. Formally known as FL 05-107, 'Winterstar' originated from a cross between 'Florida Radiance' and 'Earlibrite'. Fruit size and shape is described as being between that of 'Strawberry Festival' and 'Florida Radiance' with a shelf life similar to 'Strawberry Festival' (Whitaker et al. 2012a; Whitaker et al. 2012b). 'Winterstar' has been reported as being susceptible to both anthracnose (*C.* acutatum) and Botrytis (*B. cinerea*) fruit rots (Seijo et al. 2011). 'Winterstar' has also been shown to be susceptible to root and crown rots when inoculated with *P. cactorum* (Whitaker et al. 2012a). While yields of 'Winterstar' have shown to be comparable to 'Strawberry Festival' and 'Florida Radiance', 'Winterstar' tends to show less post-freeze yield loss in comparison to 'Strawberry Festival' and 'Florida Radiance' (Whitaker et al. 2012b).

'Camino Real' is a short-day cultivar released by the University of California in 2001. 'Camino Real' resulted from a cross between Cal 89.230-7 and Cal 90.253-3, 2 unpatented advanced selections of the University of California. 'Camino Real' is characterized by its nature to fruit over an extended period of time. The fruiting pattern of 'Camino Real' is similar to that of 'Camarosa', another popular California variety.

‘Camino Real’ is moderately susceptible to both common leaf spot (*R.* tulasnei) and powdery mildew (*S.macularis*), however has shown resistance to Verticillium wilt (*V. dahlia*), and Phytophthora (*P. cactorum*) and Anthracnose (*C. acutatum*) crown rots (Shaw et al. 2002).

Irrigation and Fertilization

It is estimated that drip-irrigated vegetables and strawberries in Florida, total approximately 24,300 ha annually (Simonne and Hochmuth 2011). In Florida plasticulture strawberry production, irrigation is provided to strawberry plants via drip tubes placed in the bed, under the polyethylene mulch. Besides supplying water to the crop, the drip tubes offer the opportunity to deliver fertilizers, pesticides, and also fumigants to the planting beds and or strawberry crop being grown (Simonne et al. 2011). University of Florida/IFAS fertilizer recommendations include a broadcast application of all phosphorus and approximately 20% of the nitrogen and potassium season requirements prior to bed preparation. The remaining nitrogen and potassium is to be injected through the drip irrigation system at rates of 0.35 to 0.84 $kg \bullet ha^{-1} \bullet day^{-1}$, depending on the stage of the crop (Santos et al. 2011).

Overhead irrigation is also a necessity in common Florida strawberry production practices, including field preparation, plant establishment, and for freeze protection (Mossler 2010; Simonne et al. 2011). In Florida strawberry production fields, overhead irrigation systems are usually permanently installed on a grid with an approximate 14.5 m spacing within and between rows of sprinklers. Prior to bedding they are used to provide adequate moisture to the soil for proper bed formation and fumigant retention. Adequate soil moisture is crucial when forming beds to ensure a tight-fitting mulch application (Simonne and Hochmuth 2011). Overhead irrigation is also utilized during daylight hours for approximately 7 to 10 days after planting of bare-root transplants to aid in establishment. The intent of the overhead irrigation is to maintain moisture on the foliage of newly set transplants in order to reduce leaf temperature and water loss by way of transpiration.

Overhead irrigation in strawberry production is also extensively used for freeze protection (Simonne et al. 2011). Since strawberries are grown as a winter crop in Florida, the foliage, flowers, and fruit are periodically exposed to overnight temperatures at or below freezing. Water is continuously applied to plants to establish a protective coating of ice. In general, overhead irrigation systems are designed to provide approximately 0.65 cm of water per hour during freeze events. While this system at the estimated application rate can be an efficient means for freeze protection, the effectiveness of the protection at or below approximately -2°C to -3°C, or at wind speeds above 8 $km \cdot h^{-1}$, is reduced (Perry 1998; Simonne et al. 2011). The overhead application of water provides freeze protection mainly by the latent heat of fusion of the freezing water. Approximately 80 calories of heat energy is released for every 1g of water that freezes. This heat energy helps to replace the heat lost to the environment by the foliage, flowers, and fruit (Perry 1998). While there are many benefits of overhead irrigation for freeze protection, it must be managed properly to avoid applying excess water which could lead to water soaking fruit, cracking, and significant losses due to rot (Hochmuth et al. 1993). Another concern in past years has been the ramifications of overhead irrigation for freeze protection after repeated freeze events. Repeated ground water pumping has been linked to increased load bearing of soil surfaces from ponded and infiltrating water resulting in water shortages, well problems, and sinkholes (Bengtsson 1989).

Mulches

Polyethylene mulches provide many benefits to plasticulture cropping systems such as: increased early and overall yields, reduction of fertilizer leaching, retention of soil moisture, reduced weed growth and needs for tillage, and improving fumigant retention and efficacy (Hochmuth et al. 1988; Lament 1993; Olson 2007). Unlike other vegetable production systems in Florida, strawberry producers generally rely on one specific type and color of polyethylene mulch. In previous years a low density polyethylene (LDPE), was the mulch material of choice. However, a recent survey showed that the majority of respondents have opted to utilize a more fumigant impermeable film, known as virtually impermeable film (VIF) (Snodgrass et al. 2011). With reduced rate applications of methyl bromide: chloropicrin and other alternative fumigants, the economic benefits of utilizing the VIF mulch became more apparent as the industry moved further into the post-methyl bromide era. Studies have shown VIF mulch to have a much lower permeability to many fumigant gasses, resulting in a significantly higher retention rate of fumigant gasses in comparison to other plastics. Longer retention of soil gasses has generally resulted in enhanced pest control efficacy of alternative fumigant systems. Enhancement for pest control has been reported for weeds, nematodes, and soil borne pathogens (Nelson et al. 2001; Noling 2002; Santos et al. 2007a). Earlier prototypes of VIF mulches were not readily accepted by growers mainly due to their limited pliability, leading to problems in mulch installation. However, VIF mulch design has greatly improved and many growers are now integrating them into their production practices (Olson 2007). Black is the mulch color of choice for strawberry producers in Florida. Black polyethylene mulch is typically used in winter and early spring because it is more efficient at capturing and converting light energy to heat and transferring the heat to soil within the planting bed (Freeman and Gnayem 2005; Olson 2007). This microclimate manipulation creates warmer and more satisfactory growing conditions for strawberry plants. Winter soil temperatures under black polyethylene mulch during the daytime generally range from 1.7°c to 2.8°c higher than that of bare ground soil (Lament 1993).

Soil Borne Strawberry Pests

The soil borne pests of strawberry represent a large and diverse group of pests including diseases, nematodes, and weeds. All have the potential to negatively affect plant growth and crop yield. Soil fumigation with methyl bromide and chloropicrin has been the major control measure for soil borne pests for over 50 years (Chandler et al. 2001; Himelrick and Dozier 1991). Alternative fumigants to methyl bromide are available; however their spectrum of activity and efficacy is generally not as broad or as consistent as that of methyl bromide and chloropicrin (Noling et al. 2011).

Diseases

In Florida, soil borne root rot diseases have historically been rare, thought mainly to be attributed to the extensive use of pre plant fumigation. Fungal pathogens such as *Fusarium* spp., *Pythium* spp., and *Rhizoctonia* spp. are among the most commonly observed pathogens on roots of infected and or dead plants (Legard et al. 2003). *Phytophthora cactorum* is a fungal pathogen common to central Florida production regions with the capacity to cause serious plant injury (Peres 2011). *P. cactorum* is more commonly identified as the causal agent for crown rots in Florida strawberry (Hancock 1999; Legard et al. 2003). While *P. cactorum* has historically been associated with expression of Phytophthora crown rot; more recently, outbreaks of *P. citricola* in Florida have been attributed to the expression of Phytophthora crown rot (Legard et al. 2003). The selection and use of more resistant cultivars can aid in the management of soil borne diseases such as *P. cactorum*. Previously reported, 'Strawberry Festival' has been described as resistant to Phytophthora root rot while 'Florida Radiance' has been shown to be highly susceptible (Whitaker et al. 2012c). For susceptible cultivars, an application of mefenoxam (Ridomil Gold®) has been shown to provide good control of *Phytophthora* spp. and is recommended immediately following plant establishment to prevent their outbreaks (Legard et al. 2003).

The incidence of *Macrophomina phaseolina,* causal agent of charcoal rot, has recently been reported to be increasing in strawberry production fields in Florida. The first case was reported in 2001, with multiple cases reported every season since (Peres and Mertley 2007). Symptoms of plant injury caused by *M. phaseolina* are much like those of *Colletorichum* spp. and *Phytophthora* spp., making distinguishing the causal agent difficult in the field. The higher incidence of infected plants is thought to derive from field areas receiving inadequate fumigation or following use of less efficacious alternative fumigants. With time, Charcoal rot is expected to evolve into an emerging threat as producers transition to alternative fumigants (Peres and Mertely 2007; Mossler and Nesheim 2004). In a recent grower survey conducted by Snodgrass et al. (2011), respondents not only reported the presence of charcoal rot in the field, but indicated the severity (i.e. pressure) was increasing in the two year period following the use of methyl bromide alternatives in their fields.

Nematodes

Sting nematode *(Belonolaimus longicaudatus* Rau*)* has been reported as a key pest species affecting Florida strawberry production (Noling 2009). The sting nematode is a plant parasitic nematode native to Florida, confined to sandy soils (minimum 80% sand) typical of the southeastern United States (Noling 2009; Robbins and Barker 1974). Sting nematodes negatively affect plant growth by feeding directly on plant roots, reducing root density, and decreasing foraging and translocation efficiencies for water and nutrients, leaving plants low in vigor and often exhibiting symptoms of water and nutrient deficiency (Hancock 1999). In addition, the reduction in vigor caused by the nematode can leave plants more susceptible to infection from bacterial and fungal pathogens, further reducing yield (Noling 2011). Host range, population fitness, and reproduction have shown to differ among populations throughout the southeast US, suggesting multiple pathogenic races of the sting nematode (Abu-Gharbieh and Perry 1970; Bekal and Becker 2000; Robbins and Hirschmann 1974; Timper and Hanna 2005). Sting nematode reproduction is greatest at temperatures of 25-30°C (75-85°F), in sandy soils containing moderate moisture. Within these optimal conditions, the life cycle of the sting nematode is reported to be as little as 28 days. (Noling 2009).

Many different weed species have been classified as alternative hosts for reproduction of plant parasitic nematodes, making weed management an essential component of managing nematodes (Thomas et al. 2005). Many weeds such as ragweed (*Ambrosia artemissifolia* L.), wild carrot (*Daucus carota* L.), crabgrass (*Digitaria spp.*) and common bermuda (*Cynodon dactylon* L. Pers), are common in vegetable production fields throughout Florida and have been classified as good host for sting nematode (Noling 2009). In a recent survey of strawberry producers in Florida, respondents listed sting nematode as infesting an average of 21% of their production fields with a production loss estimated at 17% (Snodgrass et al. 2011).

Weeds

Given the long 6 to 7 month strawberry production season in Florida, shifts in predominant weed species during the cropping season are experienced (Stall 2008). Nutsedge (*Cyperus* spp.) has been described as one of the more problematic weeds facing strawberry growers with populations anticipated to increase as producers transition further to methyl bromide alternatives (MacRae 2010). Snodgrass et al. (2011) reported from a recent survey of grower respondents who estimated that nutsedge infested approximately 40% of the strawberry acreage.

Herbicide resistance is another problem facing strawberry growers. In select locations, goosegrass (*Eleusine indica* L.) growing in row-middles has been shown to have developed resistance to paraquat due to extensive use (Mossler 2010). Other weeds commonly found in strawberry production fields are Carolina geranium (*Geranium carolinianum* L.), black medic (*Medicago lupulina* L.), common purslane (*Portulaca oleracea* L.), Florida pusley (*Richardia scabra* L.), cutleaf evening-primrose (*Oenothera laciniata* Hill), and eclipta (*Eclipta prostrate* L.) (Mossler 2010).

Weed Competition in Strawberry

The occurrence of competition between 2 neighboring plants has been explained by Anderson (2007) as being evident when one or more of the factors necessary for growth and development does not meet the combined needs of the plants. Since the crop and weeds are both plants, they compete for many of the same factors needed for growth including water, nutrients, light, and space (Anderson 2007). During strawberry plant establishment and early growth, strawberry plants can be highly susceptible to weed competition (Fennimore et al. 2008). Research on weed competition with strawberries has mostly focused on perennial strawberry planted with matted-row production systems. While much of the weed competition literature pertains to alternate cropping systems, this information can be valuable in understanding the susceptibility of strawberry to the effects of weed competition. In a recent strawberry survey, grower respondents estimated their combined losses in production due to Carolina geranium and black medic to be approximately 11.5% (Snodgrass et al. 2011).

Pritts and Kelly (2001) conducted studies to examine the effects of early season weed competition in strawberries growing in a perennial matted row production system. These studies demonstrated that perennial strawberries are most sensitive to weed competition within the first 2 months after planting. The results from this work showed that competition for 1 and 2 months following planting resulted in a yield reduction of 20 and 65% respectively, the following year. Season-long weed competition had the ability to reduce yield by up to 90% in the matted-row strawberry production system. Weed biomass was found to be a strong predictor of yield with a reduction in yield of 5.5% for every 100g•m^2 of weed biomass (Pritts and Kelly 2001).

Pritts and Kelly (2004) have also examined the effects of selected periods of weed competition on mature plantings of strawberry grown in a matted-row production system. These studies showed that the yield of well-established strawberry plantings could possibly be unaffected by weed competition for up to 2 years. During the second and third year of the study, season-long weed competition was shown to reduce yield by 14 and 51% respectively, while no differences were seen among treatments in the first year with season long weed competition. Pritts and Kelly (2004) also noted that several of the treatments receiving minimal amounts of weed competition yielded higher than the weed-free control. It was speculated that constant disturbance of the strawberry roots, because of hand-weeding, possibly caused a reduction in yield of the weed-free control plots (Pritts and Kelly 2004).

Weed Control In Strawberry

Weed control in strawberries is concentrated in two areas, those being the soil beneath the mulch covered beds and the bare ground area between adjacent mulch covered beds, referred to as the row-middle (Mossler and Nesheim 2004). Soil fumigants in combination with herbicides are the two most common methods of weed control in commercial strawberry production (Fennimore et al. 2003). Hand-weeding is also common throughout much of the U.S. strawberry producing regions. There are numerous pre-emergent and post-emergent herbicides labeled for row-middle application in annual strawberry grown in Florida (Santos et al. 2011; Stall 2008). Historically, weed control within the planting bed has been achieved with the use of preplant fumigants and hand weeding. With the exception of nutsedge (*Cyperus spp.*), weed pressure within the planting bed comes from weeds germinating and growing out of the planting holes. Weeds growing in the planting holes typically begin to germinate within three to five weeks after planting and compete with the crop for light, nutrients, space, and water (Gilreath and Santos 2005). While many annual weeds can be controlled with fumigation, many dormant hard-seeded annual weeds such as Carolina geranium (*Geranium carolinianum* L.), black medic (*Medicago lupulina* L.), and cutleaf evening-primrose (*Oenothera laciniata* Hill) can survive fumigant treatment and become mid to late season problems (Mossler 2010; Stall 2008).

Herbicides

The strawberry production season typically lasts from 6 to 7 months depending on the market prices and strawberry production volume from other national and international producers, environmental conditions, and labor availability. Because the Florida production cycle takes place over such a protracted time period, shifts in weed species present in the field may occur multiple times during the growing season. These shifts occur due to the changing temperatures and photoperiod which may result in germination of weeds late in the growing season. Thus, no one weed control strategy could be expected to provide weed control the entire growing season. Instead, a combination of weed control measures should be implemented, including among these is the use of herbicides (Mossler 2010; Stall 2008).

Currently in Florida there are multiple herbicides labeled for row-middle application in strawberry (Mossler 2010). Post-emergent herbicide options in strawberry include acifluorfen, carfentrazone, clethodim, glyphosate, and paraquat. Pre-emergent tank mix partners are recommended for adequate residual control. Common pre-emergent herbicide options in Florida include flumioxazin and pendimethalin (Santos et al. 2011). All row-middle herbicide applications are applied using hooded or shielded sprayers in order to avoid herbicide contact with strawberry plants or plastic mulch bed tops.

Weed control within the raised bed has consistently been achieved with methyl bromide and chloropicrin combinations; however with forced transition to alternative fumigants, the reduction in levels of control has been considered by many to be the weakest link of the alternative fumigants (Mossler and Nesheim 2004). Augmenting the alternative fumigants with complementary in-bed herbicide applications during bed formation have recently become an area of intensive weed research interest. The herbicide terbacil is extensively used in perennial strawberry for post-emergent and residual control of seedling and germinating annuals (Rogers et al. 2001). While terbacil has shown to be a promising option, the pre-harvest interval (PHI) of 110 days, makes the product nearly useless due to the need to harvest the Florida crop during restricted periods (Mossler and Nesheim 2004). Napropamide and oxyfluorfen are two other herbicides that have been shown to be safe on annual strawberry and have efficacy on common weeds, when applied pre-transplant under the plastic mulch (Daugovish et al. 2008; Gilreath and Santos 2005).

Gilreath and Santos (2005) examined the effects of napropamide alone at 4.5 to 9 kg•ha^{-1}, oxyfluorfen alone at 0.57 kg•ha^{-1}, and a combination of napropamide and oxyfluorfen at 4.5 and 0.57 kg•ha^{-1}respectively, applied pre-transplant under the plastic mulch of annual strawberry. Herbicide applications were made 3 weeks prior to transplanting and no visual plant injury was reported for any of the treatments at 6 and 12 WAP. Furthermore, Gilreath and Santos (2005) reported that the highest marketable strawberry yield was achieved with the combination of napropamide and oxyfluorfen with an increase of approximately 13% in the number of fruit and approximately 20% in the total weight of fruit, in comparison to the non-treated control.

Currently napropamide, marketed under the trade name Devrinol 2E® and Devrinol 50DF®, is labeled for application under plastic mulch at rates from 2.2 to 4.4 kg•ha^{-1} (Anonymous 2009). Oxyfluorfen, marketed under the trade name Goal 2XL® and Goaltender®, is labeled for fallow bed application in strawberry at application rates from 0.27 to 0.56 kg•ha^{-1} (Anonymous 2011b). Research suggests that at labeled rates, the combination of napropamide and oxyfluorfen applied pre-transplant in annual strawberry is capable of providing adequate control of a variety of weed species including southern crabgrass (*Digitaria ciliaris* Retz. Koel), common ragweed (*Ambrosia artemisiifolia* L.), and cutleaf evening-primrose (*Oenothera laciniata* Hill) (Gilreath and Santos 2005).

Clopyralid

Clopyralid (3,6-dichloro-2-pyridinecarboxylic acid) is a member of the synthetic auxin family of herbicides. While their true mechanism of action and specific binding site are not well understood, they are known to affect cell wall plasticity at higher concentrations and interfere with nucleic acid biosynthesis at lower concentrations (Anderson 2007; Senseman 2007). Clopyralid is known to readily translocate within the phloem, with higher concentrations accumulating in regions of meristematic growth (Senseman 2007). Clopyralid is closely related to the herbicides picloram (4-amino-3,5,6-trichloro-2-pyridine-carboxylic acid), triclopyr ([(3,5,6-trichloro-2-pyridinyl)oxy]acetic acid), and aminopyralid (4-amino-3,6-dichloro-2-pyridinecarboxylic acid) because of the pyridine ring base they share. Clopyralid is one of two active ingredients is premixes such as Confront® (clopyralid + triclopyr) and Curtail® (clopyralid + 2,4-D) (Anderson 2007). Stinger®, the formulation used in most cropping situations, is formulated as a monoethanolamine salt containing 40.9% active ingredient (Anonymous 2010).

Stinger® is labeled for use in many non-cropland scenarios as well as barley, oats, wheat, stone fruit, sugar beets, tree plantations and permanent grass pastures at use rates of 68 to 567 $g{\bullet}ha^{-1}$ (Anonymous 2010). Stinger® also carries numerous 24c registrations throughout the U.S. for select fruit and vegetable crops such as cabbage (*Brassica oleracea* L.), blueberry (*Vaccinium corymbosum* L.), cranberry (*Vaccinium macrocarpon* Ait.), and strawberry (*Fragaria x ananassa* Duchesne). Clopyralid alone or in combination with other tank mix partners has shown to provide acceptable control of Canada thistle (*Cirsium arvense* L. Scop.), horseweed (*Conyza Canadensis* L. Cronq.), common ragweed (*Ambrosia artemisiifolia* L.), honey mesquite (*Prosopis glandulosa* Torr. var. glandulosa), common groundsel (*Senecio vulgaris* L.) and various vetch species (*Vicia spp.*) (Bovey and Meyer 1985; Figueroa and Doohan 2006; McMurray et al. 1996; Renner 1991). Suppression of species such as white clover (*Trifolium repens* L.) and black medic (*Medicago lupulina* L.) have also been shown with field application rates of clopyralid (MacRae et al. 2005; McMurray et al. 1996).

The field half-life of clopyralid has been reported as approximately 30 to 90 days (Pik et al. 1977). Clopyralid is degraded in the soil by microbial activity and the rate at which this occurs has been shown to be related to application rate, soil temperature, moisture, and texture of the soil (Senseman 2007; Pik et al. 1977). Since the rate at which degradation occurs is related to microbial activity it has also been shown that degradation rates are reduced under periods of cold, dry weather (Pik et al. 1977). From the section 3 federal label, Stinger® has extensive crop rotation restrictions, with up to an 18 month rotation interval for susceptible crops. It is recommended that a field bioassay be conducted before planting any non-labeled crop (Anonymous 2010). To avoid injury in Florida's intensive vegetable cropping rotations, major consideration must be given to the planting, double-cropping, or inter-cropping of susceptible crops following an application of clopyralid. In North Dakota on silty clay, clay loam, and silty clay loam soils it has been shown that yield of select dicot crops was not affected by clopyralid at rates up to 280 $g \cdot ha^{-1}$ when planted 11 months after application

(Thorsness and Messersmith 1991). Continued research is needed to investigate the potential for carryover injury in Florida growing conditions and cropping systems.

Clopyralid Use in Strawberry

Clopyralid has previously been shown to be a potential candidate for post-emergent broadleaf weed control in both perennial matted-row production as well as annual plasticulture production of strawberries (Clay and Andrews 1984; Figueroa and Doohan 2006; McMurray et al. 1996). While much of the literature is focused on the perennial matted-row production system and applications at or shortly after renovation, one study conducted by McMurray et al. (1996) sought to examine the effects of an early season clopyralid application on annual strawberry. Stinger® is labeled for use in perennial matted-row production in numerous states throughout the U.S. North Carolina and Florida are the only two states with a 24c registration for application in annual strawberry production (Anonymous 2006; Anonymous 2011b).

For perennial production systems, an application of 2,4-D is allowed at the time of postharvest renovation for control of broadleaf weeds (Figueroa and Doohan 2006). It was proposed by McMurray et al. (1996) that clopyralid could potentially serve as an alternative to 2,4-D because clopyralid provided equal or better control of problematic weeds, at the same time causing less injury to strawberry plants. In 2006, Figueroa and Doohan conducted trials with clopyralid application rates ranging from 25 to 400 $g•ha^{-1}$ applied as a post-harvest spray in a perennial strawberry production system. An 82% control of common groundsel (*Senecio vulgaris* L.) was achieved when clopyralid was applied at 200 $g•ha^{-1}$ without adversely affecting strawberry plant growth. While strawberry foliage growth was unaffected a significant yield increase was reported at 200 $g•ha^{-1}$. At the maximum application rate of 400 $g•ha^{-1}$ there was evidence of a significant reduction in strawberry yield as well as crop canopy (Figueroa and Doohan 2006).

A study conducted by McMurray et al. in 1996 investigated the effects of an early season application of clopyralid on an actively growing annual strawberry crop. Ninety percent control of Vetch (*Vicia spp.*) was achieved 4 weeks after treatment (4 WAT) with clopyralid rates from 140 to 200 g•ha^{-1}, while clopyralid at 70 g•ha^{-1} provided only 62% control 4WAT. Regardless of rate, 100% control of vetch was achieved 8 WAT. A rate response was observed for control of black medic (*Medicago lupulina* L.) with an increase in weed control as the rate of clopyralid was increased. Clopyralid at 70, 140, and 280 g•ha^{-1} provided 49, 63, and 83% control of black medic, respectively, 4 WAT. McMurray et al. (1996) hypothesized that the control of black medic was related directly to spray coverage. It was noted that black medic plants that survived were small and generally located under the canopy of the strawberry plants, ultimately leading to less than 100% control. Regardless of season, growth stage, or application rate, crop injury was reportedly less than 6% and confined to higher clopyralid rates (McMurray et al. 1996).

As strawberry producers transition to alternative fumigants the previously mentioned pest problems will possibly become unavoidable. The need for alternative weed management products and practices will be necessary to maintain the standard of quality expected of the industry. As indicated previously, the efficacy of clopyralid on select weeds in a variety of different cropping systems has proved to be adequate. Strawberry crop tolerance to clopyralid has also been shown to be acceptable. The objective of this project is to evaluate the herbicide clopyralid for use in annual plasticulture strawberry production in Florida, and to determine whether additional pest management practices need to be integrated with this herbicide into the Florida strawberry production system. More specifically, the scope of this work is to 1) quantify strawberry tolerance to clopyralid, when grown in an annual plasticulture cropping system during the typical Florida strawberry production season and 2) evaluate the efficacy of clopyralid on key problematic weeds typical of the production region.

CHAPTER 2
GREENHOUSE GROWN STRAWBERRY RESPONSE TO A SPRAY APPLICATION OF CLOPYRALID

Objectives

Two identical greenhouse trials were conducted during the winter of 2010-2011 at the University of Florida/IFAS Gulf Coast Research and Education Center in Balm, Florida. The purpose of these greenhouse studies was to initially examine the extent of injury to strawberry plants from a spray application of clopyralid, and to validate its possible use in Florida strawberry production. These greenhouse studies were also designed to evaluate any differences in herbicide tolerance among 4 popular strawberry cultivars currently grown in Florida. In other production areas of the U.S., clopyralid has been shown to be a promising option for weed control in strawberry (Figueroa and Doohan 2006; McMurray et al. 1996). Based on results of the previous research, we hypothesize that when clopyralid was applied at currently labeled rates, marketable yield would be unaffected. However, as application rates of clopyralid increased, marketable yield would decrease significantly in comparison with the non-treated control. It was also hypothesized that leaf malformation would be significant at all levels; however, it is predicted that this injury would not affect overall plant growth and development when clopyralid was applied at labeled rates. The objectives of these experiments was 1) to discover any differences in tolerance levels among 4 strawberry cultivars popular in the Florida production region, and 2) determine if clopyralid rate was positively correlated with leaf malformation and production, and whether this injury would translate into a reduction in marketable yield.

Materials and Methods

Rooted transplants of strawberry cultivars ‘Festival’, ‘Radiance’, ‘Treasure’, and ‘Winterstar’ were planted into black 3.8 L polyethylene pots filled with Fafard #2® potting media (Conrad Fafard Inc., Agawam, Massachusetts). Strawberry plants for the first of two trials were planted on October 25, 2010 with the remaining being planted on November 12, 2010 for the second trial. All plants were watered daily or as needed to maintain adequate media moisture.

Treatments consisted of a spray application of clopyralid at the rate of 0, 213, 426, 628, and 841 $g \cdot ha^{-1}$. Herbicide treatments were applied with a CO_2 pressurized backpack sprayer calibrated to deliver 280 $L \cdot ha^{-1}$ at 213 kPa, utilizing TeeJet® 11004 XR nozzles (TeeJet Technologies, Springfield, Illinois). Herbicide applications for the 2 trials were separated by one day with the first trial being treated on January 12th 2011, 11 weeks after planting (11 WAP), and the second following on January 13th 2011 (9 WAP). Following herbicide application, irrigation water was directed toward the base of the plant and away from foliage. Each treatment included 4 plants in separate pots per cultivar and was replicated 5 times, for a total of 400 plants per trial. All treatments were arranged in a randomized complete block design.

Initial leaf counts were taken on January 11th, 2011 and January 12th, 2011 for the first and second trial respectively. Following application, strawberry fruits were harvested from each potted plant and graded by recording the number and weight of marketable as well as malformed fruit. Harvest continued for 5 weeks with 2 harvests per week. Following the final harvest at 5 WAT, leaf counts were taken by recording the total numbers of leaves per plant in addition to the number of malformed leaves per plant. These numbers, along with the initial leaf count were used to calculate the number of news leaves formed since the time of application, including the percentage of new, malformed leaves.

The leaf count data was analyzed using the generalized linear mixed models conducted under the PROC GLIMMIX procedure in SAS version 9.2 (SAS Institute, Inc., Cary, NC) to investigate the effect of herbicide rate and strawberry cultivar on new leaf production, the percentage of malformed leaves, and yield of marketable fruit. The results of new leaf production are presented as the number of new leaves per plant formed during the 5 week period from the time of application until the time of rating (5WAT). The leaf malformation data is presented as the percentage of new leaves which become malformed during the 5 week post application period. Yield data are presented as the number of marketable fruit per plant. Cultivar, clopyralid rate, and cultivar by rate were treated as fixed effects while trial by replication were treated as the random effect in the model. The SLICE function was used to analyze the effect of rate at each level of cultivar, and treatment means were compared using Fisher's Protected LSD at $\alpha=0.05$. Curve fitting was performed using Sigmaplot 12.0 (Systat Software Inc., San Jose, CA).

Results

New leaf data as well as leaf malformation data were combined across trials, and are presented by cultivar in response to clopyralid rate. All leaf data was fitted using the treatment means and standard errors estimated from the generalized linear mixed model analysis to a quadratic equation indicated below, where f is the percent leaf malformation or number of new leaves, and x designates the clopyralid rate.

$$f = y0+a*x+b*x^2$$

No cultivar by rate interactions were observed for marketable yield and these data were combined across trials and cultivars and presented as marketable fruit per plant in response to clopyralid rate. Data were then best fit using treatment means and standard errors estimated from the generalized linear mixed model analysis to the linear equation indicated below where *f* represents marketable yield and *x* designates clopyralid rate.

$$f = y0+a*x$$

New Leaf Formation

For each cultivar, treatment means for new leaves formed and clopyralid rate were fit to a quadratic equation with coefficient of determination (r^2) values of 0.97, 0.93, 0.92, and 0.65 for the cultivars 'Winterstar', 'Treasure', 'Radiance', and 'Festival', respectively (Figure 2-1). Clopyralid at 92 and 190 g•ha^{-1} was estimated to decrease new leaf production 10 and 20%, respectively, for the cultivar 'Treasure'. Strawberry 'Radiance' was estimated to express a 10 and 20% reduction in new leaves at 110 and 262 g•ha^{-1} of clopyralid, respectively. 'Winterstar' was estimated to show a 10% reduction in new leaves at 230 g•ha^{-1}, while strawberry 'Festival' displayed an estimated 10% reduction at 340 g•ha^{-1} of clopyralid. A 20% reduction in new leaves of strawberry 'Festival' was estimated at 605 g•ha^{-1}. Since a biologically appropriate curve could not be fit to the data, predictions outside the measured parameters were not performed, leading to a 20% reduction in new leaves of 'Winterstar' estimated at greater than 840 g•ha^{-1} of clopyralid.

Leaf Malformation

For each cultivar, treatment means of percent malformed leaves and clopyralid rate were fit to a quadratic equation with coefficient of determination (r^2) values of 0.98, 0.98, 0.91, and 0.89 for the cultivars ‘Winterstar’, ‘Festival’, ‘Radiance’, and ‘Treasure’, respectively (Figure 2-2). A 10 and 20% level of leaf malformation was predicted for strawberry ‘Festival’ when clopyralid was applied at 200 and 411 g•ha^{-1}, respectively. For cultivars ‘Radiance’ and ‘Treasure’ a 10% level of leaf malformation was estimated at 339 and 501 g•ha^{-1}, while 20% leaf malformation was predicted at 603 and 741 g•ha^{-1}, respectively. Since a biologically appropriate curve could not be fit to the data, predictions outside of the measured parameters was not performed resulting in 10 and 20% leaf malformation estimated at greater than 841 g•ha^{-1} of clopyralid for ‘Winterstar’.

Marketable Yield

Marketable yield data exhibited substantial variability between replications within trials as well as within cultivars. Therefore, no interactions between cultivars were observed and marketable yield data was pooled across all cultivars and both trials for the analysis. Marketable yield and clopyralid rate was well described by a linear equation with a coefficient of determination (r^2) value of 0.96 (Figure 2-3). The well correlated linear relationship showed an increase in marketable strawberry yield (across all cultivars and both trials) as the rate of clopyralid was increased. Overall marketable yield was estimated to increase by 18 and 35% when clopyralid was applied at 213 and 426 g•ha^{-1}, respectively. Clopyralid applied at 628 and 841 g•ha^{-1} was predicted to increase yield by 52 and 70%, respectively.

Discussion

Significant cultivar by rate interactions for new and malformed leaves suggest differences in tolerance levels among the cultivars tested. Strawberry cultivars, even within a specific genotype (ie. short-day), can vary in their response to environmental factors such as temperature and photoperiod (Darnell 2003). These factors could contribute significantly to the differential expression of tolerance of these cultivars to clopyralid. ‘Winterstar’ was shown to produce the highest number of new leaves while also expressing the lowest percentage of malformed new leaves.

A positive linear relationship was observed between marketable yield and clopyralid rate. This trend was unexpected considering that higher rates translated into increased phytotoxicity in the form of leaf malformation. Explaining this trend has been difficult, but is thought to be related to inadequate pollination of strawberry flowers in the greenhouse, rather than clopyralid rate. It is possible that as leaf malformation increased, there was an overall reduction in canopy coverage resulting in more effective pollination of flowers from the small amount of wind provided by greenhouse fans. In general, primary means of pollination of strawberry flowers is by wind and gravity (McCandless and Korvak 2003). Fruit deformation and yield reduction can become problems in greenhouse grown strawberries when pollinators are not introduced (Kakutani 1993). For this reason, honeybees are one of the most common pollinators and recommended for greenhouse or protected culture of strawberries (Goodman and Oldroy 1988; Nye and Anderson 1974; Handcock 1999). The potential impacts of poor pollination in these results cannot be ignored.

It is not possible to completely rule out the potential cofounding effects of pollination masking the actual effect of clopyralid on marketable yield. Therefore, field studies are needed to further examine the effect of clopyralid on marketable yield in a typical open-field cropping situation. Leaf malformation results were shown to agree with the working hypothesis that malformation would increase with clopyralid rate. However, in comparison to previous literature, results from this study showed higher levels of leaf malformation at comparable rates. This variability between the expression of herbicide injury on plants grown in the greenhouse and open field grown plants was recently investigated by Riemens et al. (2008). Riemens et al. (2008) showed that among the 4 species tested, all were more sensitive to applied glufosinate ammonium when grown in the greenhouse compared to the field grown plants. While glufosinate has a much different mode of action in comparison to clopyralid, Riemens et al. (2008) hypothesized this difference among field and greenhouse plants was a function of environmental conditions that differentially promoted the rates of plant growth. If this environment effect of the greenhouse was causing increased vegetative growth of the strawberry plants, it would help explain the increase in leaf malformation due to the overall increase in total leaf production.

Kloppenburg and Hall (1990) showed that in Canada thistle (*Cirsium arvense* L. Scop.) the monoethanolamine salt formulation of clopyralid differed in the percentage of absorption and translocation under differing relative humidity (RH) regimes. At 65% RH, ^{14}C labeled clopyralid formulated as a monoethanolamine salt was shown to be absorbed and translocated at 80 and 55% of applied 72 hours after treatment (HAT), respectively. When RH was decreased to 35% ^{14}C labeled clopyralid absorption and translocation was decreased to 60 and 45% of applied 72 HAT, respectively. Findings from this study would suggest that clopyralid applied in a greenhouse situation, at higher RH in comparison to the field setting, would have greater absorption and translocation, and subsequently more injury to strawberry plants.

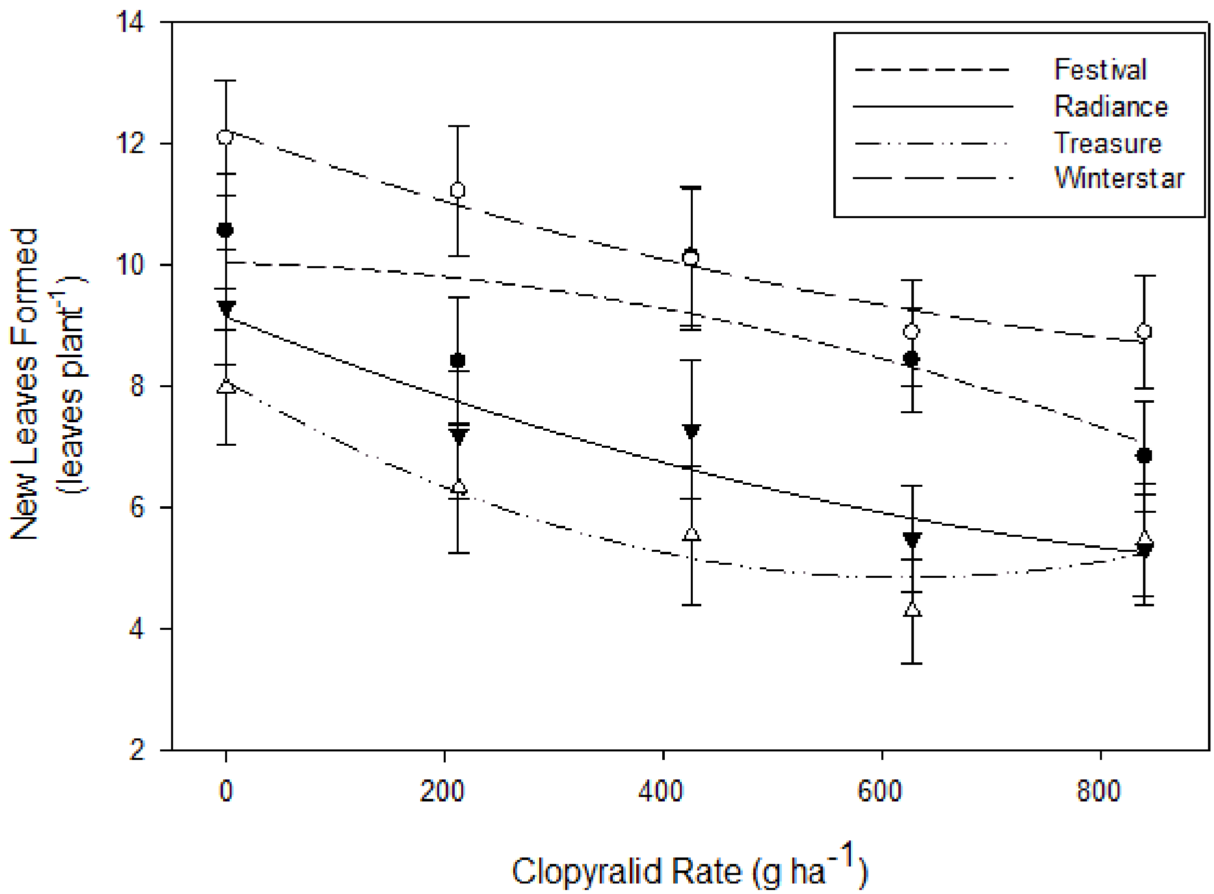

Figure 2-1. Number of new leaves formed for 4 greenhouse grown strawberry cultivars in response to 5 rates of clopyralid application 5 WAT. Data values represent means +/- standard error. Festival refers to the cultivar ‘Strawberry Festival’, and Radiance refers to the strawberry cultivar ‘Florida Radiance’. For each cultivar the regression model is f=y0+a*x+b*x^2. Winterstar: y0=12.2057, a=-0.0064, b=2.629E-6, x=rate, and r^2=0.97. Festival: y0=10.0203, a=-0.0004, b=-3.7524E-6, x=rate, and r^2=0.65. Radiance: y0=9.1408, a=-0.0073, b=3.1351E-6, x=rate, and r^2=0.92. Treasure: y0=8.0701, a=-0.0104, b=8.3601E-6, x=rate, and r^2=0.93.

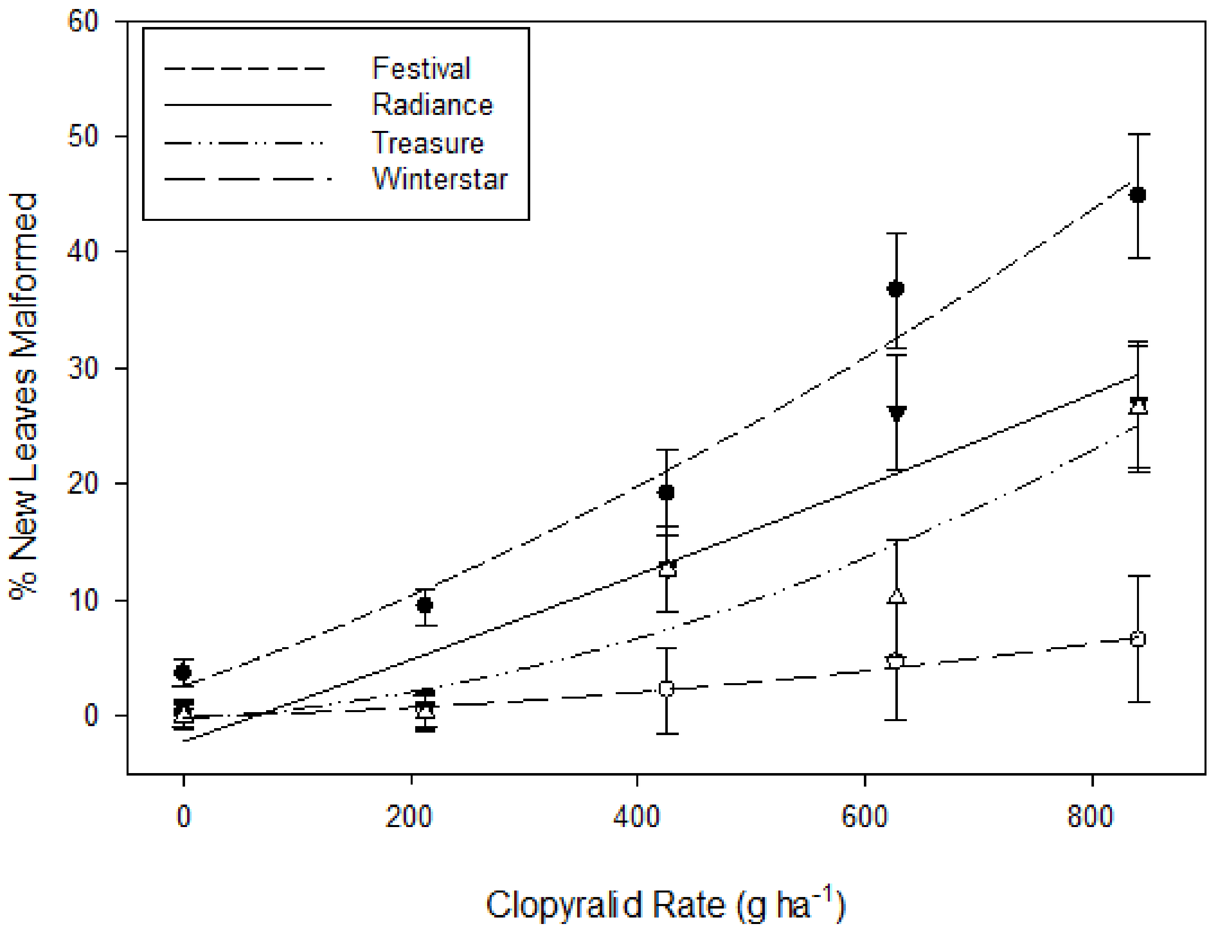

Figure 2-2. Percentage of new leaf malformation for 4 greenhouse grown strawberry cultivars in response to 5 rates of clopyralid application 5 WAT. Data values represent means +/- standard error. Festival refers to the cultivar ‘Strawberry Festival’, and Radiance refers to the strawberry cultivar ‘Florida Radiance’. For each cultivar the regression model is f=y0+a*x+b*x^2. Winterstar: y0=-0.0444, a=0.0027, b=6.4724E-6, x=rate, and r^2=0.98. Festival: y0=2.6423, a= 0.0346, b=2.0874E-5, x=rate, and r^2= 0.98. Radiance: y0=-2.1521, a=0.0345, b=3.6957E-6, x=rate, and r^2= 0.91. Treasure: y0=-0.2106, a=0.0059, b=2.8813E-5, x=rate, and r^2=0.89.

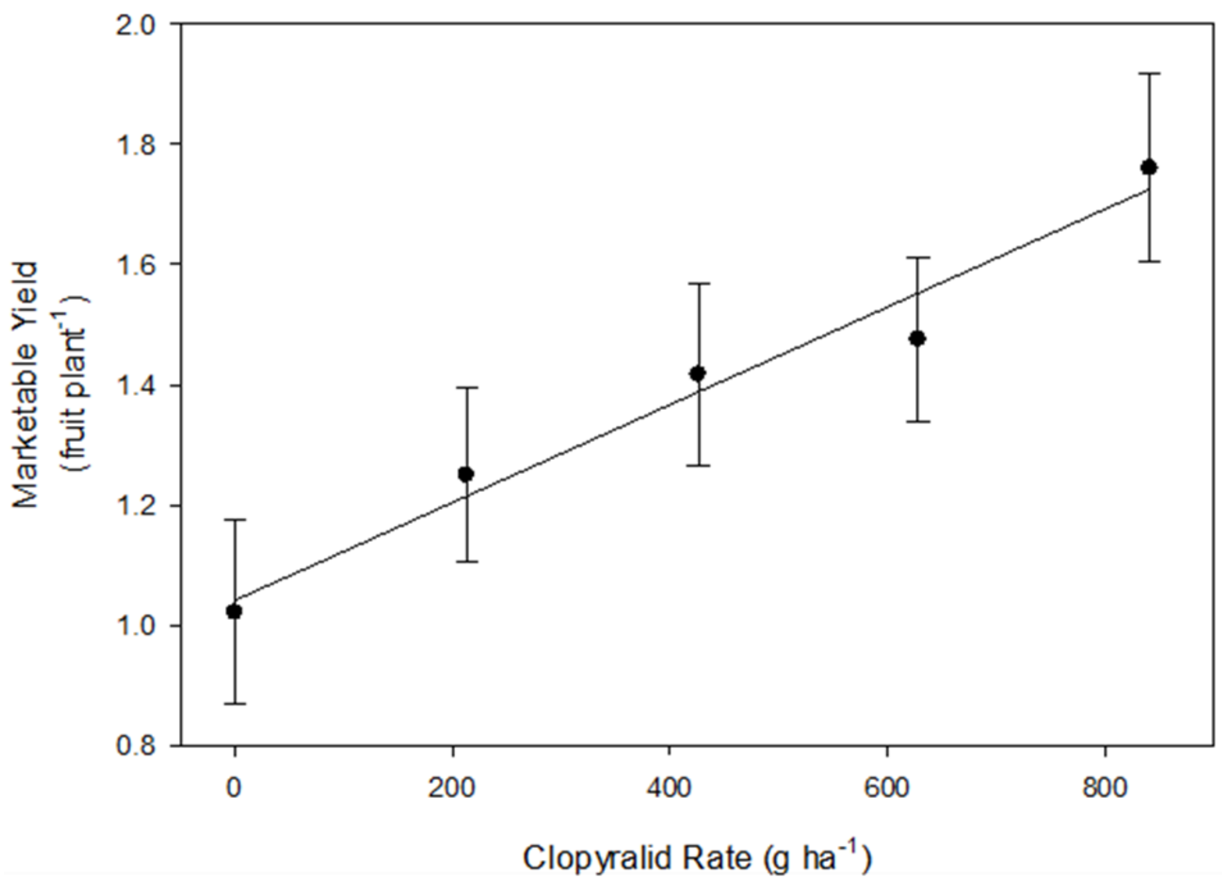

Figure 2-3. Marketable yield of 4 greenhouse grown strawberry cultivars in response to 5 rates of clopyralid (0, 213, 426, 628, 841 g•ha^{-1}), expressed as the number of marketable fruit per plant. The regression equation represents data pooled across 2 replicated greenhouse trials and 4 strawberry cultivars. Data values represent means +/- standard error. The regression model is f = y0+a*x, where y0=1.041, a=0.0008, x=rate, and r^2=0.96.

CHAPTER 3
FIELD GROWN STRAWBERRY RESPONSE TO CLOPYRALID APPLICATION

Objectives

As the Florida strawberry industry transitions further into the post methyl bromide era with alternative fumigants, new and additional measures of weed control are becoming a necessity. Post-transplant herbicide applications could be an integral part of the new weed management program. During the winter of 2011-2012 a number of field research trials were conducted at the University of Florida/IFAS Gulf Coast Research and Education Center in Balm, Florida to evaluate post-transplant herbicide applications. As a basis for further research, the working hypotheses which would be tested was that marketable yield would be unaffected by clopyralid at labeled rates of application. However, as application rates are increased above that of the maximum labeled rate, marketable strawberry yields would significantly decrease in comparison with the non-treated control. It was also hypothesized that leaf malformation would be significant at all rates of clopyralid application, however this injury would not affect overall plant growth, development, and yield at labeled rates. The objective of these studies was to determine the effects of a post-transplanted directed spray and drip-injected application of clopyralid at various rates on plant injury as well as on total and marketable yield of annual strawberry.

Materials and Methods

During the fall of 2011, fields at the University of Florida/ IFAS Gulf Coast Research and Education Center in Balm, Florida were prepared by means of conventional tillage and standard plasticulture bedding practices common throughout the producing region. Planting beds were formed and fumigated with PicClor 60® (Soil

Chemicals Corporation, Hollister, California), a combination of 39% 1,3-Dichloropropene and 59.6% chloropicrin, at 331 $kg \cdot ha^{-1}$. Following bed preparation and fumigation, beds were covered with a 1.2 mil polyethylene mulch (Blockade, Berry Plastics Corporation, Evansville, Indiana). Each bed received 2 drip tapes placed 20 cm apart and 3 cm deep, with the capacity to deliver 605 ml per hour per emitter, with emitters spaced 30.5 cm apart (Streamline Series, Netafim USA, Fresno, California).

Planting beds were established on 1.2 m centers with a height of 30.5 cm and a bed top width of 66 cm. Each trial consisted of 4 beds by 97.5 m long. Plots were 9 m long and consisted of 48 plants per plot. All trials were arranged in a randomized complete block design with each bed representing a block and each of the 6 treatments replicated 4 times.

Separate trials were conducted for each of the 3 different strawberry cultivars, including 'Treasure', 'Strawberry Festival', and 'Camino Real'. Following sufficient time for fumigant dissipation, planting holes were made utilizing a tractor mounted punch wheel which spaced planting holes in twin rows on 38 cm centers with 38 cm between rows. Bare root transplants of strawberry "Treasure" were planted on October 11th, 2011, while bare root transplants of strawberry 'Festival' and 'Camino Real' were planted on October 25th, 2011 and October 26th, 2011 respectively. Transplants received overhead watering for 10 days during daylight hours to aid in establishment. Plants were irrigated and received fertilizer injected through the drip irrigation system on a daily basis. All production and pest management practices were in accordance with common commercial practice and University of Florida/IFAS recommendations (Santos et al. 2011).

Spray Applications

Treatments consisted of a post-transplant spray application at 0, 146, 213, 426, 628, and 814 g•ha^{-1} of clopyralid. All post-transplant spray treatments were applied using a CO_2 pressurized backpack sprayer calibrated to deliver 280 L•ha^{-1} at 213 kPa, utilizing TeeJet® 11004 DG nozzles (TeeJet Technologies, Springfield, Illinois). Treatment timings varied between trials, with the strawberry cultivar 'Treasure' being the first to be treated on December 30th, 2011, 11 weeks after planting (WAP). Clopyralid applications were made on January 13th, 2012 (11 WAP) to the cultivar 'Festival' hereafter referred to 'Festival' A, followed by clopyralid application on January 18th, 2012 (12 WAP) to the strawberry cultivar 'Camino Real'. A final spray application of clopyralid was made on February 1st, 2012 (14 WAP) in another separate field trial using the strawberry cultivar 'Festival', hereafter referred to as 'Festival' B.

Drip-Injected Applications

For this method of clopyralid application, treatments consisted of a post-transplant drip injection application at 0, 146, 213, 426, 628, and 814 g•ha^{-1} of clopyralid. Treatments were applied using a CO_2 pressurized injection system consisting of a manifold constructed of polyvinyl chloride (PVC) pipe. Herbicide was combined with 30 L of water to form the stock solution, which was injected into the PVC manifold containing irrigation water. The flow rate of the herbicide solution into the irrigation water was controlled with a needle valve which ensured a constant flow of solution throughout the entire 2.5 hour injection period. The solution was then delivered to individual plots by way of a flexible low density polyethylene tubing (The Toro Company Irrigation Division, Riverside, California). The existing 2 drip tapes were used for the herbicide application and consideration was given to adequately flush all injection lines and drip tapes before the system was put back into use for irrigation. To determine the proper interval for application, a dye test was conducted by injecting a colored dye into the drip irrigation system in the same manner and time duration in which the herbicide was to be injected. The blue dye was injected for 2, 2.5, and 3 hours in order to identify the optimum injection time to maximize herbicide movement across the bed. Based on cross sectional imaging of dye movement, optimal injection scheduling was determined as a uniform and continuous injection over a 2.5 hour period followed by a 20 minute flush period meant to purge any herbicide residue from drip irrigation lines on the surface of the bed.

The dates in which the different treatments were applied varied between the 4 cultivar specific trials. In this regard, the strawberry cultivar 'Treasure' was the first to be drip treated on January 3rd, 2012 (12 WAP). The second drip application of clopyralid was made on January 13th, 2012 (12 WAP) to the cultivar 'Festival', hereafter referred to as 'Festival' A, followed by an application on January 19th, 2012 (12 WAP) to the cultivar 'Camino Real'. The final drip application was made on February 1st, 2012 (14 WAP) to another trial consisting of strawberry 'Festival' plants hereafter referred to as 'Festival' B. Application timings were meant to coincide with fruiting schedules resulting in earlier producing cultivars treated first, followed by mid and late season cultivars treated after.

Randomly selected subsamples of 10 plants from each plot were used to determine plant injury responses to the applied clopyralid, and an initial leaf count was taken of the subsample at the time of application. Leaf ratings were taken at 2 and 4 weeks after treatment (WAT), which consisted of a count of the total number of leaves and the total number of malformed leaves. These numbers were used to determine the number of new leaves formed since the time of clopyralid application, as well as the percentage of new leaves which might become malformed in response to its application. Mature strawberry fruit were harvested 1 to 2 times per week until the time of application. Following clopyralid application strawberries were then harvested by plot and graded for both marketable and malformed fruit. Harvest continued for 8 weeks after clopyralid application with strawberries being harvested on Monday and Thursday of each week, totaling 16 harvests; with the exception of the 'Festival' B trial in which harvest continued for 6 weeks for a total of 12 harvests.

For data analysis, the generalized linear mixed models conducted under the PROC GLIMMIX procedure in SAS version 9.2 (SAS Institute, Inc., Cary, NC) were used to investigate the effect of application method, herbicide rate, and strawberry cultivar on new leaf production, the percentage of leaf malformation, and yield of marketable fruit. New leaf production is presented as the number of new leaves formed per plant from the time of application until the time of rating (4 WAT). Leaf malformation is presented as the percentage of new leaves since the time of application becoming malformed (4 WAT). Marketable yield data was standardized and is presented as the percent of the total number or cumulative weight of fruit harvested from the untreated control. Application method, cultivar, and clopyralid rate were treated as fixed effects in the model. The SLICE function was used to analyze the effect of rate at each level of cultivar, and clopyralid treatment means were compared using Fisher's Protected LSD at α=0.05. Curve fitting was performed using Sigmaplot 12.0 (Systat Software Inc., San Jose, CA). New leaf and malformed leaf data for the 'Festival' B trial was analyzed separately from the other cultivars due to extreme variability. Yield data for 'Festival' B trial was not included in the analysis due to the difference in harvest schedules and subsequent extreme degrees of harvest yield variability.

Results

No interactions were observed in regards to application method for any of the measured parameters; therefore, new leaf formation was combined across clopyralid application methods and is presented by cultivar in response to clopyralid rate. Clopyralid rate treatment means within cultivars were compared using Fisher's Protected LSD at $\alpha=0.05$. Data for leaf malformation per plant was combined across application method and presented by cultivar in response to clopyralid rate. Using treatment means and standard errors determined from the generalized linear mixed model analysis, the data for leaf malformation was best fitted to the exponential growth (3 parameter) equation indicated below:

$$f = y0+a*exp(b*x)$$

Data for marketable yield was combined across cultivars and application methods and presented as percent of the untreated control in response to clopyralid rate. Using the treatment means and standard errors determined from the generalized linear mixed model analysis, the combined data for marketable yield data was then best fit to the Weibull peak (5 parameter) equation indicated below:

$$if(x<=x0-b*((c-1)/c)^{\wedge}(1/c),y0, y0+a*((c-1)/c)^{\wedge}((1-c)/c) * (abs((x-x0)/b+((c-1)/c)^{\wedge}(1/c))^{\wedge}(c-1)) * exp(-abs((x-x0)/b+((c-1)/c)^{\wedge}(1/c))^{\wedge}c+(c-1)/c))$$

New Leaf Formation

The number of newly forming leaves was variable within and between cultivars. With the exception of 'Festival' B which showed a decrease in new leaf production at 841 g•ha^{-1} of clopyralid, no differences were observed when compared to the non-treated control (Table 3-1).

Leaf Malformation

Nonlinear regression of leaf malformation and clopyralid rate was best described by an exponential growth (3 parameter) model, explaining 97, 98, and 91% of the variability for the 'Festival' A, 'Festival' B, and 'Treasure' cultivars, respectively (Figure 3-1). No meaningful relationship was found between malformed leaves and clopyralid rate for the 'Camino Real' cultivar. For the cultivar 'Festival' A, a 5 and 10% leaf malformation was estimated at 366 and 681 g•ha^{-1}, respectively. A 5 and 10% leaf malformation was estimated when clopyralid was applied at 578 and 790 g•ha^{-1} for the 'Treasure' cultivar, respectively. A 10 and 20% leaf malformation was estimated when clopyralid was applied at 529 and 698 g•ha^{-1} to the 'Festival' B cultivar. When clopyralid was applied at the maximum labeled rate (213 g•ha^{-1}) leaf malformation was predicted at 3.7, 3.6, and 3.3% for cultivars 'Festival' A, 'Festival' B, and 'Treasure', respectively. Applications of clopyralid at 426 g•ha^{-1} was predicted to cause 5.6, 6.8, and 3.8% malformation for the cultivars 'Festival' A, 'Festival' B, and 'Treasure', respectively. Based on the generalized linear mixed model analysis for the 'Camino Real' cultivar, leaf malformation of 4.3 and 6.3% were estimated when clopyralid was applied at 213 and 426 g•ha^{-1}, respectively. When clopyralid was applied at 841 g•ha^{-1}, leaf malformation was estimated at 12.3, 14.7, and 37.4% for the cultivars 'Treasure', 'Festival' A, and 'Festival' B, respectively.

Marketable Yield

Using treatment means and standard errors, nonlinear regression of strawberry yield and clopyralid rate was best fit to a Weibull peak (5 parameter) function with an coefficient of determination (r^2) value of 0.99 (Figure 3-2). When clopyralid was applied at the minimum and maximum labeled rates of146 and 213 $g{\bullet}ha^{-1}$, marketable yield was predicted at 103.6 and 104.1% of the non-treated control, respectively. A clopyralid application of 426 and 628 $g{\bullet}ha^{-1}$ was estimated to result in a marketable yield of 103.4 and 101% of the non-treated control. Marketable yield was estimated at 97.8%of the control when clopyralid was applied at 841 $g{\bullet}ha^{-1}$. No trend with clopyralid rate was found for malformed yield. All clopyralid treatments resulted in the production of malformed fruit within 1% of the non-treated control (data not shown).

Discussion

Significant cultivar by clopyralid rate interactions were observed for production of new and malformed leaves, suggesting differences in cultivar tolerance to clopyralid. While these interactions were deemed significant, other environmental factors which may also be as important as differences between cultivars seem to be present. The potential role of the environment in defining the effect of clopyralid can be described by comparison of the ‘Festival’ A trial and ‘Festival’ B trial. Both trials were planted with strawberry ‘Festival’ plants on the same day and had been subjected to the same cultural practices throughout the growing season. While these trials were treated the same, the ‘Festival’ B trial resulted in approximately 154% more leaf malformation at 841 $g{\bullet}ha^{-1}$ in comparison to the ‘Festival’ A. This is thought to have been caused by a interactions of plant genotypic and environmental factors. Darrow et al. (1930) described the optimum temperature for strawberry vegetative growth to be between 20 and 27°C, while the optimum temperature for flower bud initiation was described as between 14 and 18°C (Darnell 2003). As figure 3-4 shows, average air temperatures following the 'Festival' B clopyralid application were considerably higher, possibly leading to higher rates of vegetative growth and therefore more clopyralid being partitioned into the leaves. In contrast, following clopyralid application in the 'Festival' A trial a period of lower than average temperatures was experienced (Figure 3-4), possibly leading to less vegetative growth and more clopyralid partitioned into the strawberry fruits.

On closer examination of the fruiting cycles at the time of application (Figure 3-3) for the cultivars 'Camino', 'Treasure', and 'Festival' A, it is evident that clopyralid application occurred during an increase or plateau phase in fruit production. In comparison, clopyralid application for the 'Festival' B trial took place prior to a period of reduced fruit production (Figure 3-3d). As previously indicated, clopyralid has been reported to readily translocate within the phloem, with higher concentrations accumulating in regions of meristematic growth (Senseman 2007). Macias-Rodriguez et al. (2002) showed that during periods of vegetative growth, carbohydrates, also transported in the phloem, were shown to accumulate in the crown of strawberry plants. This accumulation was followed by the mobilization of the carbohydrates during flowering and fruiting with much of the carbohydrates being partitioned to the fruits, which have been described as the most competitive sink in the plant (Forney and Breen 1985; Macias-Rodriguez et al. 2002). If in fact clopyralid is mobilized and accumulated similar to that of carbohydrates, this could also help explain the excessive injury during times of vegetative growth due to high levels of clopyralid in strawberry crowns.

When an amino (NH_2) group is added to the clopyralid parent acid in the fourth position, a slightly different herbicidal compound is formed. Aminopyralid (4-amino-3,6-dichloro-2-pyridinecarboxylic acid) is similar in chemical make-up and in the way it is mobilized and acts within the plant (Senseman 2007). In a recent study Pfleeger et al. (2012) showed that at low concentrations, aminopyralid stimulated growth of bristly dogstail grass (*Cynosurus echinatus* L.). This stimulation response was seen in both field and greenhouse trials leading Pfleeger et al. (2012) to conclude that the increased growth was a definite response to the aminopyralid rather than other underlying factors. This response to low concentrations of a closely related growth regulating herbicide could help explain the trend for slight increases in marketable yield at low concentrations of clopyralid. Figueroa and Doohan (2006) saw a similar trend in perennial strawberry marketable yield, reporting a significantly higher yield when clopyralid was applied at 200 $g{\bullet}ha^{-1}$ in comparison to hand-weeded controls. However, they speculated that this increase might be attributed to strawberry competition with groundsel seedlings between weed removal events in the hand-weeded plots. For many years it has been known that substances lethal at higher doses can have beneficial or stimulating effects when applied at very low concentrations (Cedergreen 2008). This phenomenon was first known as the Arndt-Schulz law (Calabrese 2005). Since then, the term ‘hormesis’ has been used to describe this effect low levels of toxins can cause. Recently, Cedergreen (2008) reported a number of herbicides that stimulated growth and biomass accumulation of barley. These effects were seen when low concentrations of herbicides were applied to foliage as well as growing media. If this stimulation of growth is in fact present, it would help justify the observation of no detectable decreases in leaf production in comparison to the non-treated control, with the exception of the 'Festival' B trial (Table 3-1).

Table 3-1. Number of new leaves formed per plant for 3 strawberry cultivars in response to 6 rates of clopyralid application, 4 weeks after treatment (WAT).

Clopyralid rate	New leaf formation Cultivars 'Festival' A[b]	'Festival' B	'Camino'	'Treasure'
$g\cdot ha^{-1}$	new leaves•plant^{-1}			
0	16.60 ab[a]	12.12 a	18.25 a	13.94 ab
146	18.37 a	13.64 a	19.58 a	17.76 a
213	16.98 ab	11.91 a	16.52 a	13.51 b
426	17.57 ab	11.71 a	17.13 a	13.17 b
628	16.20 ab	11.02 a	16.60 a	15.35 ab
841	14.92 b	7.17 b	17.87 a	12.91 b

[a] Responses within columns with the same letter are not different according to Fisher's Protected LSD at p≤0.05.

[b] 'Festival' refers to the cultivar 'Strawberry Festival', 'Camino' refers to the strawberry cultivar 'Camino Real'. 'Festival' A refers to the first set of Festival trials treated while 'Festival' B refers to the latter treated festival trials.

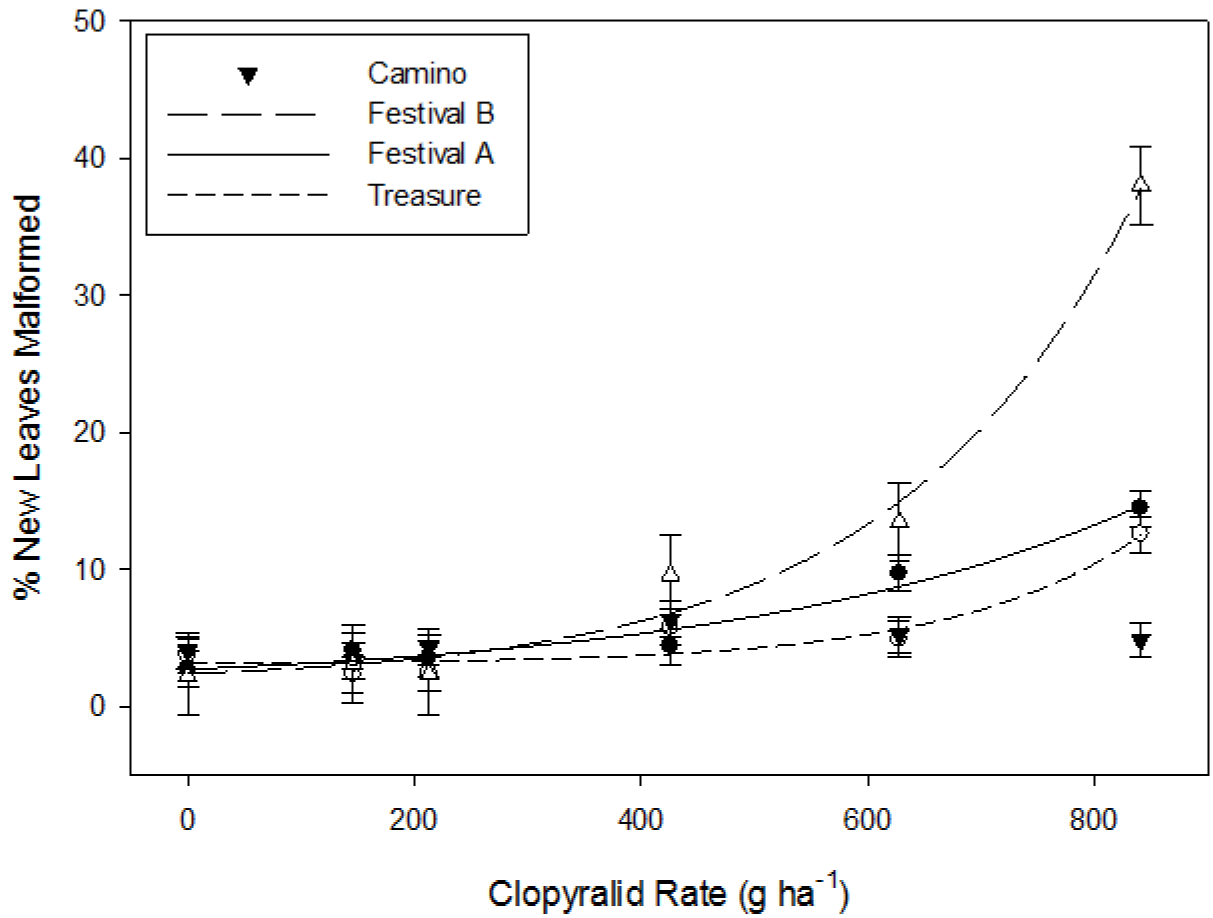

Figure 3-1. Leaf malformation expressed as the percentage of new leaves malformed in response to 6 rates of clopyralid application, 4weeks after treatment (WAT). Data values represent means +/- standard error. Camino refers to the strawberry cultivar 'Camino Real', Festival A refers to the first applied set of trials with the cultivar 'Strawberry Festival' while Festival B refers to the latter treated trials of the cultivar 'Strawberry Festival'. The regression model is f=y0+a*exp(b*x). Festival A: a=1.26, b=0.002, y0=1.47, and r^2=0.97. Festival B: a=0.68, b=0.004, y0=1.73, and r^2=0.98. Treasure: a=0.05, b=0.006, y0=3.11, and r^2=0.91. No significant dose response relationship between percent malformed leaves and clopyralid rate was detected within the strawberry cultivar 'Camino Real'.

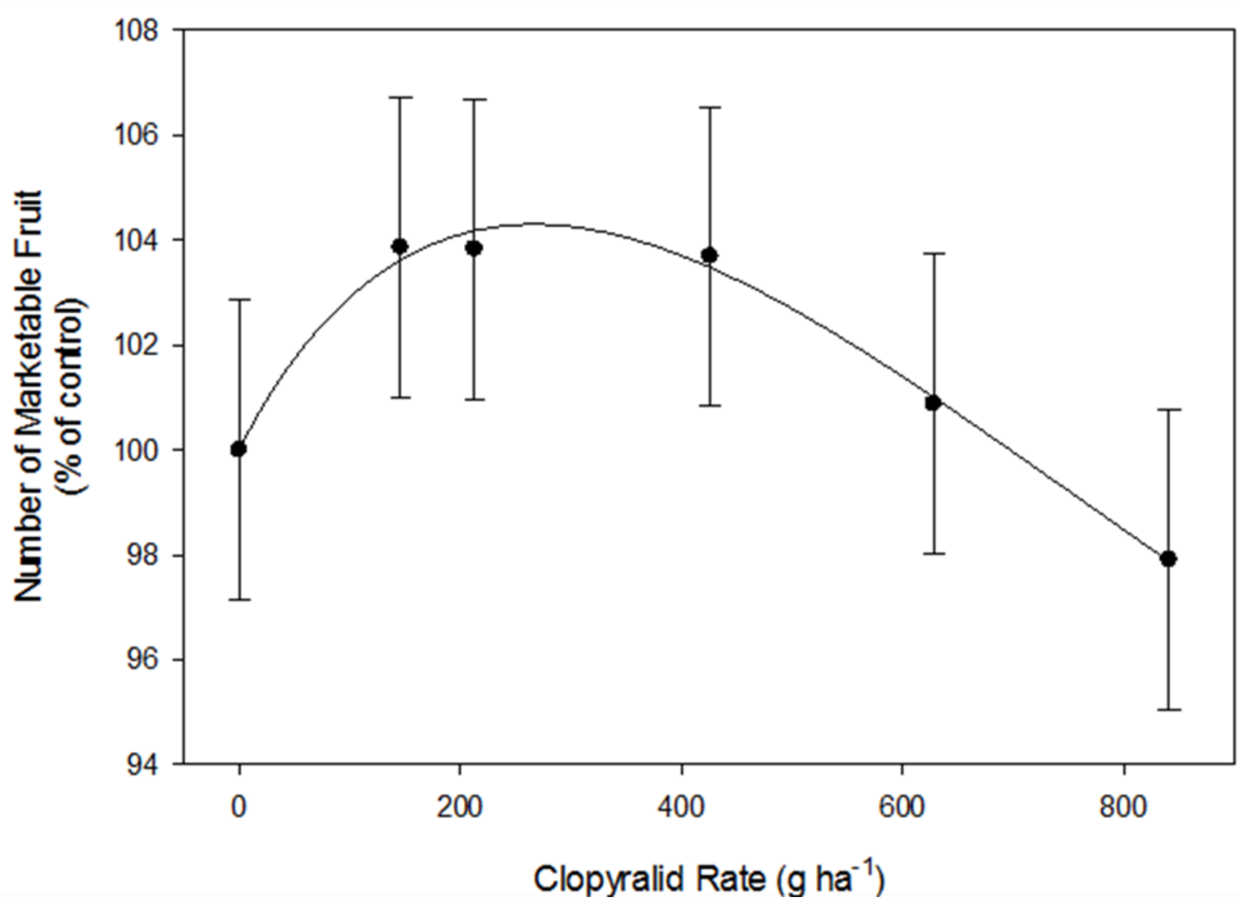

Figure 3-2. Number of marketable fruit expressed as a percent of the control in response to 6 rates of clopyralid application. The regression equation presented represents data pooled across field trials of 3 cultivars and 2 application methods. Data values represent means +/- standard error. The regression model is if(x<=x0-b*((c-1)/c)^(1/c),y0, y0+a*((c-1)/c)^((1-c)/c) * (abs((x-x0)/b+((c-1)/c)^(1/c))^(c-1)) * exp(-abs((x-x0)/b+((c-1)/c)^(1/c))^c+(c-1)/c)), where a=16.8, b=830.4, c=1.4, x0=266.3, y0=87.5, x=clopyralid rate, and r^2=0.99.

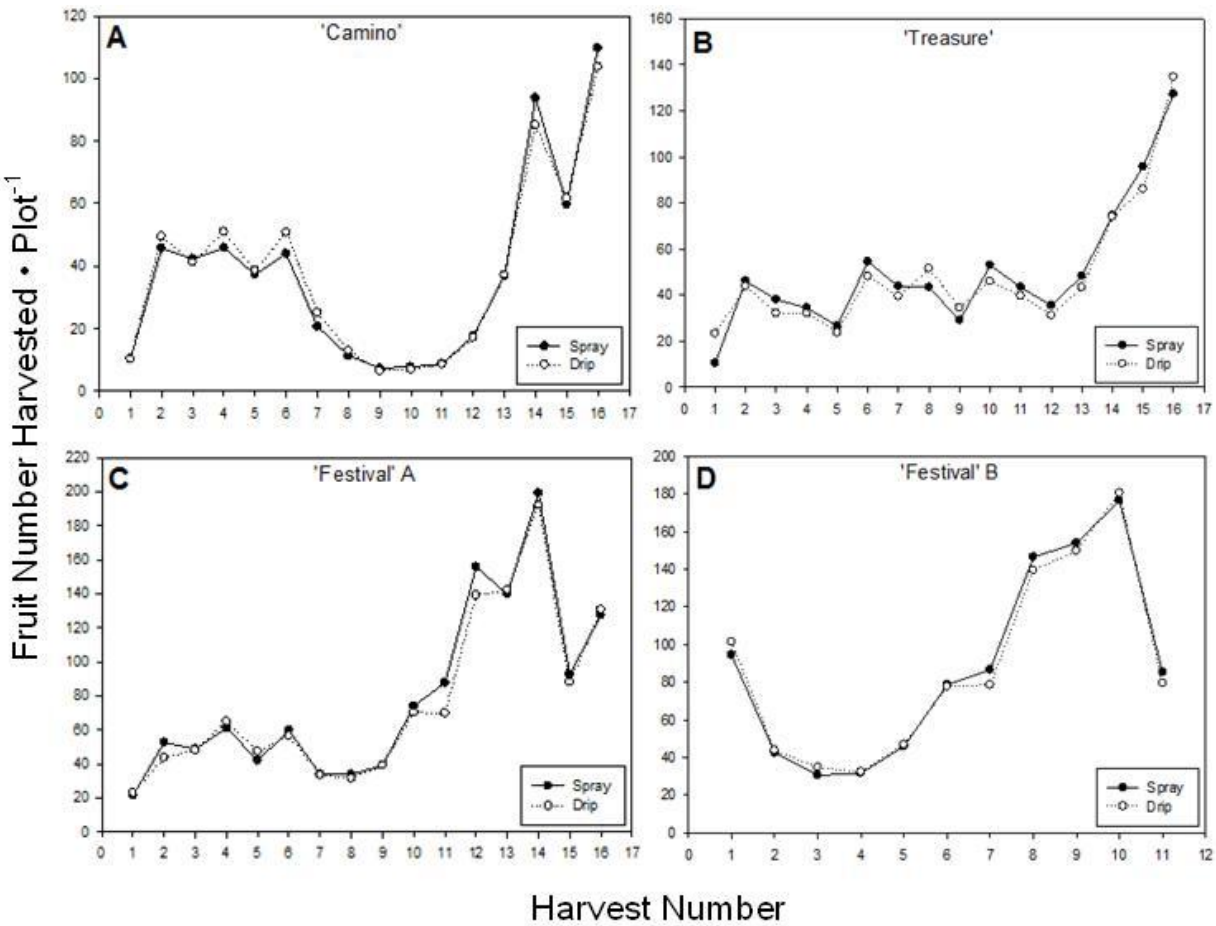

Figure 3-3. Fruiting cycles of 3 strawberry cultivars following clopyralid application as either a directed foliar spray or as a drip application to soil. Applications were made 2 days prior to harvest number 1. Camino refers to the strawberry cultivar ‘Camino Real’, Festival A refers to the first applied set of trials with the cultivar ‘Strawberry Festival’ while Festival B refers to the latter treated trials of the cultivar ‘Strawberry Festival’. Figure panels (A,B,C,D) are separated by cultivar with the solid line representing fruit production of plants receiving a directed foliar spray and the dashed line representing plants receiving drip applications of clopyralid. Note scales among panels differ in representing fruit production and harvest number.

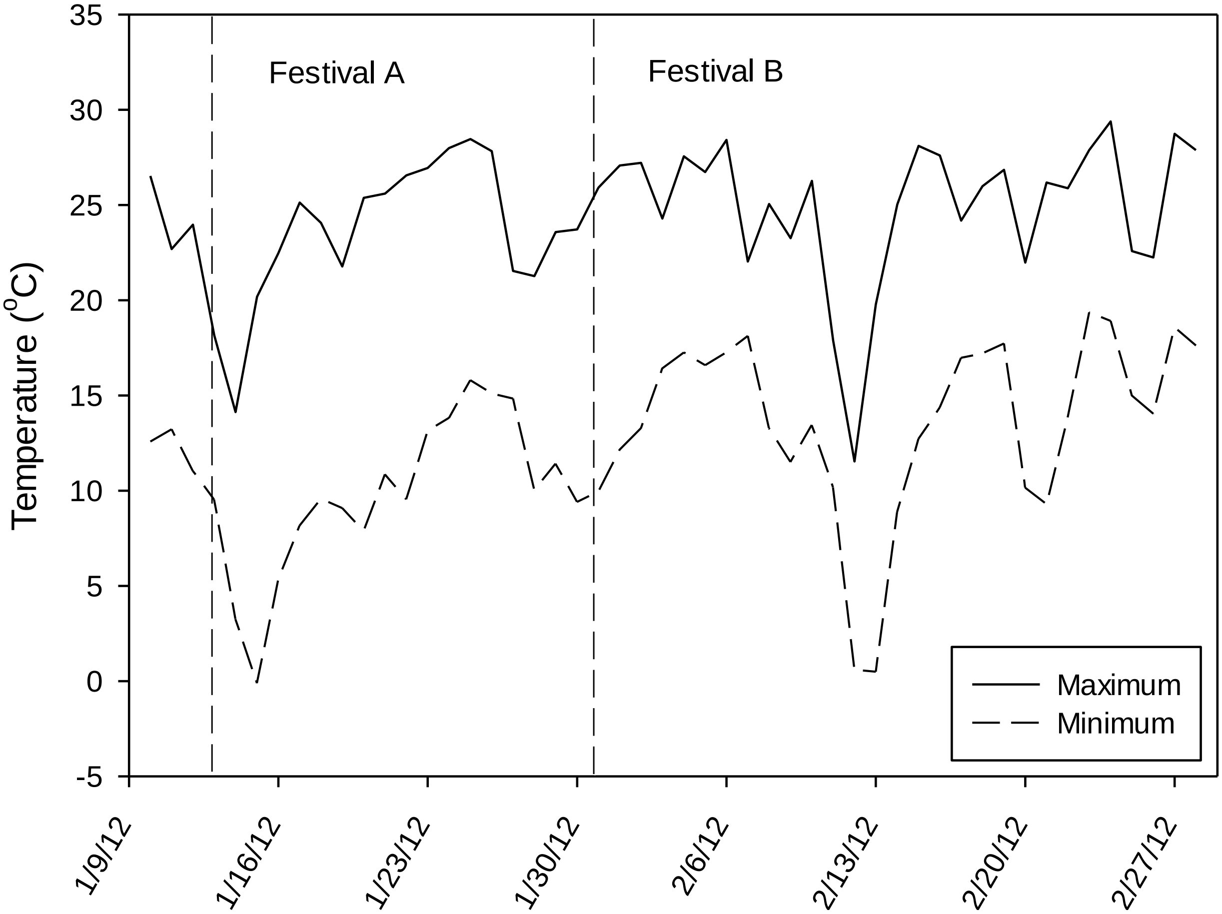

Figure 3-4. Minimum and maximum air temperatures following clopyralid application in 2 separate 'Festival' trials. Dashed line labeled as 'Festival A' represents application timing of the initial 'Strawberry Festival' trials. The dashed line labeled as 'Festival B' represents application timing of the later treated 'Strawberry Festival' trials.

CHAPTER 4
CONCLUSION

Results obtained from these studies indicate that the use of clopyralid in annual strawberry production in Florida is a promising tool for weed management. Strawberry plants showed good crop tolerance to clopyralid applied as a post-emergent spray as well as drip-injected application at labeled rates. At the maximum labeled rate of 213 $g \cdot ha^{-1}$, clopyralid caused less than 5% leaf malformation and resulted in a marketable yield of approximately 104% of the non-treated control. At rates above 628 $g \cdot ha^{-1}$, equivalent to three times the currently labeled rate, some plant injury effects were observed such as leaf malformation and reduced marketable yield. New leaf production was variable and thought to be attributed to differences between strawberry cultivars.

The results from this work suggest that other underlying genotypic and environmental interactions may contribute to the way in which strawberry plants tolerate clopyralid. While simple cultivar differences were more pronounced in the greenhouse trials, field trials showed differences within a cultivar that appear to be expressed with regards to crop stage and environmental factors. On further examination of leaf malformation results, ‘Festival’ B showed a much different trend for malformation in comparison to ‘Festival’ A. The only differences between these same cultivars in the 4 separate trials were the growth and flowering stage of the crop, and environmental conditions existing at the time of clopyralid application. For example, ‘Festival’ B was treated approximately 2 weeks after ‘Festival’ A, and average temperatures following the application of ‘Festival’ B was considerably higher than those following ‘Festival’ A. Figure 3-3 illustrates how applications for ‘Camino’, ‘Treasure’, and ‘Festival’ A were all made on either an increase or plateau of fruit production. With ‘Festival’ B, the fruiting cycle is shown to be decreasing to their lowest fruiting levels shortly after application (harvests 1-4). This would suggest that differences in yield and plant injury expressed between the same cultivars were attributable to differences in application timing and fruiting cycle. For ‘Festival’ A, application occurred during a peak period of fruit formation, and a greater portion of clopyralid absorbed by the strawberry plant could have been partitioned into the fruit, resulting in a decrease in leaf malformation. Corresponding to this period of increased fruit production is a decrease in leaf production, which suggests that the majority of clopyralid partitioned into the leaves at this time is being divided between fewer leaves than would have occurred during periods of intensive vegetative growth. A more accurate assessment of partitioning is not possible since this study did not quantify the extent to which the leaves were malformed, only the number of leaves per plant exhibiting any level of malformation.

In review of Figure 3-4, it is evident that following treatment application for ‘Festival’ A, the average air temperature was considerably lower than that of ‘Festival’ B. Higher air temperatures are known to encourage vegetative growth of strawberry, whereas lower temperatures encourage flower and fruit formation (Darrow et al. 1930; Darnell 2003). If the cooler temperatures did in fact cause a period of reduced vegetative growth, it would again support the hypothesis that clopyralid was differentially partitioned into fruit rather than among leaves being formed.

The increase in marketable yield observed in the greenhouse was thought to have been a direct response to pollination. As a result of higher leaf malformation following herbicide application, the reduction in canopy coverage, was thought to allow for better air flow and subsequently better pollination of clopyralid treated plants. While research documentation is limited, the trend for marketable yield in these field trials may have occurred in response to applications of low rates of a growth regulating herbicide; since the reduction in canopy coverage seen in the greenhouse was clearly not evident in field trials. In these trials, marketable yield weights were similar or closely correlated to that of fruit numbers suggesting no differences in fruit size or individual weight among herbicide treatments (data not shown).

The results from the late season clopyralid efficacy trials (Figure 5-1) showed promise for the control of black medic in Florida strawberry (Appendix). In this study, drip-injected applications of clopyralid at 213 $g{\bullet}ha^{-1}$ resulted in 68% control of black medic 3 WAT, while spray applications at 213 $g{\bullet}ha^{-1}$ resulted in 46% control of black medic 3 WAT. The late season efficacy trials consisted of applications to black medic plants larger than 5 leaf stage, which is the largest recommended size for application according to the label. Applications earlier in the season would be more typical and would be expected to result in better efficacy since the weeds would be of a small, more vulnerable size.

Previous research has demonstrated that strawberries can exhibit acceptable tolerance to clopyralid, without negatively effecting yield or plant growth and development. However, weighed consideration should be given to the timing of clopyralid application. Our results and recommendation would be to proceed with applications only during times of reduced vegetative growth in order to minimize leaf malformation, since our results show no deleterious effects on fruit production with labeled rates of clopyralid. Our research also demonstrates that it is possible to suppress the larger stages of black medic with labeled rates of clopyralid. These studies support the registration of clopyralid in Florida for strawberry production at use rates of 146 to 213 g•ha^{-1}. However, further research is needed to increase our understanding of the interaction of clopyralid within the plants and its ensuing environmental conditions at the time of application. The focus of this research would be to identify safe and appropriate times of application which minimize potential for plant injury. Complementary research should focus on early season spray and drip-injected applications of clopyralid and their efficacy for control of black medic.

APPENDIX
EFFICACY OF CLOPYRALID FOR THE CONTROL OF BLACK MEDIC

Materials and Methods

Trials were conducted during the winter of 2011-2012 in the Plant City/ Dover area of Florida to evaluate the efficacy of clopyralid for the control of black medic (*Medicago lupulina*) in annual strawberry. Two sites with adequate black medic populations present were selected for the trials. 'Site 1' consisted of 3 trials, while 'site 2' consisted of only one trial. At 'site 1', 3 methods of application were evaluated including drip-injected, post-emergent over-the-crop, and row middle spray applications. For each method of application at 'site 1', clopyralid was applied at 146, 213, and 426 $g{\bullet}ha^{-1}$, equivalent to field rates of 0.33, 0.5, and 1 $pt{\bullet}A^{-1}$ respectively. The trial at 'site 2' consisted of a row-middle spray trial of the application rates previously mentioned. Plot size for all spray applications and rates were 15 m in length by 1 bed or row middle. Plots receiving the drip-injected application method were 30.5 m in length by one bed. Treatments within each trial were arranged in a randomized complete block design and replicated 4 times.

Spray treatments were applied with a CO_2 pressurized backpack sprayer calibrated to deliver 280 $L{\bullet}ha^{-1}$ at 213 kPa, utilizing TeeJet® 11004 DG nozzles (TeeJet Technologies, Springfield, Illinois). Injection period for the drip-injected treatments was approximately 90 minutes with adequate time before and after injection for drip tape pressurization and flush. Treatments were applied using a polyvinyl chloride (PVC) injection manifold consisting of the necessary components to ensure a uniform concentration in the injection water and even distribution of the herbicide solution over the entire injection period and plot length. A single drip tape, located in the center of the bed, was used. Weed control ratings for 'site 1' were taken 3 weeks after treatment (WAT), while ratings for 'site 2' were taken 2 WAT. Weed control ratings were recorded as visual assessments of percent control of black medic, with 0 being no control and 100 being complete plant death. Each trial was analyzed separately using generalized linear mixed models conducted under the PROC GLIMMIX procedure in SAS version 9.2 (SAS Institute, Inc., Cary, NC) which investigated the effect of clopyralid rate on percent control of black medic. Treatment means were compared using Fisher's Protected LSD at $\alpha=0.05$.

Results and Discussion

A significant dose response relationship was observed for all trial sites and methods of application, with percent control of black medic increasing with clopyralid rate (Figure 4-1). When clopyralid was applied by drip-injection at 146, 213, and 426 $g \cdot ha^{-1}$, black medic control increased from 60, 68, to 80% respectively 3 WAT (Figure 4-1a). At 'site 1' no differences in black medic control were observed when clopyralid was applied as a post-emergent over-the-crop spray at 146 and 213 $g \cdot ha^{-1}$ 3 WAT achieving 40 and 46% control, respectively. At 'site 1' black medic control significantly increased to 66% 3 WAT when clopyralid was applied as a post-emergent spray at 426 $g \cdot ha^{-1}$ (Figure 4-1b). For the row-middle spray trial at 'site 1', black medic control increased from 52 to 72 to 91% at 146, 213, and 426 $g \cdot ha^{-1}$ of clopyralid, respectively 3 WAT (Figure 4-1c). At 'site 2', clopyralid at both 146 and 213 $g \cdot ha^{-1}$ was found similar with control estimates of 42 and 55%, respectively, 2 WAT. Clopyralid at 426 $g \cdot ha^{-1}$ was estimated to provide 75% control of black medic at 'site 2' 2 WAT (Figure 4-1d).

Many factors could have played a part in the variability in the control of black medic between application methods. In general, superior control was achieved with the drip-injected trials in comparison to the over the top foliar spray trial. Improvements in weed control are probably best explained by the incomplete coverage of the bed top due to plant foliage in the over-the-crop spray trials. In a similar study, McMurray et al. (1996) hypothesized that the control of black medic in strawberry with clopyralid was directly related to the spray coverage, suggesting the strawberry plants were shielding the emerged black medic growing from the planting holes. The observed trend for an increase in percent control compared to other treatments with the row middle applications at ‘site 1’ can be explained by the size of black medic. At ‘site 1’ black medic was slightly larger in size (approximately 25-30 cm in diameter) in the beds when compared to those plants growing in the row-middles (approximately 20-25 cm in diameter). The smaller plant sizes suggests an early season suppression of black medic in the row-middles due to a pre-emergent herbicide application, and possibly more satisfactory growing conditions in the bed in comparison to the row middle. The overall lack of complete control among all sites, methods of application, and rates is most likely related to the prevalence of the larger more tolerant and difficult to kill plant sizes of black medic present at the end of the growing season. In these trials, black medic ranged in size from 20 to 35 cm in diameter which is significantly larger than 5 leaf stage, the maximum size recommended on the product label to achieve adequate control. Applications earlier in the season to smaller black medic plants would be more typical and would be expected to achieve better control.

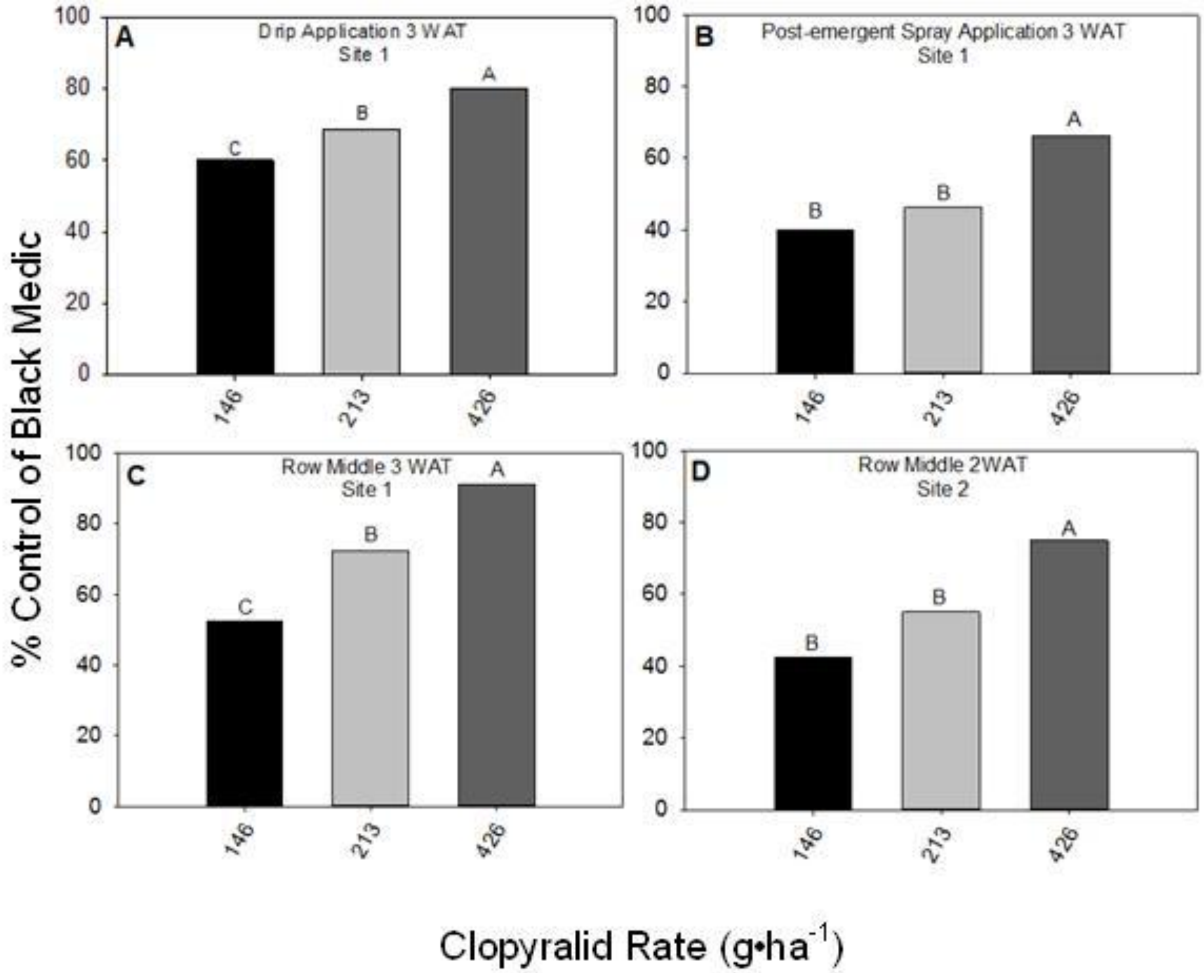

Figure A-1. Percent control of black medic (*Medicago lupulina*) with 3 application rates of clopyralid and 3 methods of clopyralid application. 'Site 1' refers to an experiment location containing 3 trials, while 'site 2' refers to a separate experiment location containing only 1 trial. Panels A and B express percent control of black medic growing in the planting bed while panels C and D express percent control of black medic growing in the row middles. Bars within a single graph with the same letter are not different according to Fisher's Protected LSD at $p \le 0.05$.

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BIOGRAPHICAL SKETCH

Clinton Hunnicutt grew up in Avon Park, Florida where he graduated from Avon Park High School in 2006. The following fall he began courses at South Florida Community College where he completed him Associates in Arts degree. In the fall of 2008, Clinton began his course work at Florida Southern College where he graduated with a Bachelor of Science in Horticulture. The following August he began coursework at the University of Florida to pursue a Master of Science degree in Horticulture Science.