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Title: Early Detection of Weed Resistance: Pattern-Thinking and Rapid Microcalorimetric Assay

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Weed resistance is usually diagnosed after a weed control practice has lost efficacy and weed populations begin to increase rapidly. Prediction and validation in the field at a very early stage of resistance development is a promising method for preventing an uncontrollable problem. Pattern-thinking helped individuals connect their experience of weed infestations with development of weed resistance. Field representatives connected early verification with immediate management to reduce the potential problem of weed resistance.

A method for rapid detection of weed resistance by microcalorimetry was developed to distinguish between herbicide resistant and susceptible biotypes. This general method was used to test three different weed species and three different herbicide modes of action. Heat evolution as a product of plant respiration by samples

of meristematic tissue was compared between resistant and susceptible biotypes. The procedure readily distinguished between biotypes.

Since microcalorimetry provided quick and accurate results, all field representatives stated that the combination of pattern-thinking and rapid assay would improve management of weed resistant populations. The combination would improve visual detection based on the standard growth and development model for weed resistance and population growth. Also, biological verification using microcalorimetry provides immediate feedback and validation of weed resistance. Thus, early detection of weed resistance is a very important tool which will assist farmers in dynamically managing weed infestations.

Early Detection of Weed Resistance: Pattern-Thinking and Rapid Microcalorimetric Assay

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Duangporn Suwanagul, Author

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CHAPTER I

INTRODUCTION

Weed resistance is an important problem in weed management. Repeated use of any weed control practice can develop populations of certain weed biotypes that tolerate the practice. Repeated mowing in turf often selects for prostrate weed species or biotypes of a particular species. Repeated cultivation favors survival and population increases of deep-rooted perennial weeds. Since the first report of simazine resistant common groundsel (*Senicio vulgaris* L.) by Ryan in 1970, at least 57 species of weeds (40 broadleaf and 17 grasses) have been reported to be resistant to several herbicides (LeBaron, 1991). Prompt, preventative action is essential for managing weed resistance. Early detection of herbicide resistant weeds before they become serious problems would enhance preventative decisions and management.

Atrazine resistant *A. powellii* was first reported in Oregon in 1970 (LeBaron, 1991). The first study of simazine resistant *Senicio vulgaris* by Radosevich (1972) clearly exhibited the differential sensitivity of resistant and susceptible biotypes in response to several triazine herbicides. Since then, weed scientists have tried to educate growers about ways to prevent the development of herbicide resistance. In spite of these educational efforts, the problem began to appear in corn fields in the mid 1980's. With continued use of herbicides in Oregon, at least four weeds in Oregon have

become resistant to three herbicide modes of action. Triazine resistant *Bromus tectorum* was the first grass weed found in 1978 (LeBaron, 1991). Diclofop-methyl resistant Italian ryegrass (*Lolium multiflorum*) was first reported in 1989 (Stanger and Appleby 1989). In 1992, sulfonylurea resistant prickly lettuce (*Lactuca serriola*) as well as kochia (*Kochia vulgaris*) were found in winter wheat fields in eastern Oregon (Ball and Walenta, Unpublished data). Diclofop-methyl resistant wild oat (*Avena fatua*) was recently discovered by Canadian weed scientists (Joseph et al., 1990 and Heap et al., 1993). There is high probability that diclofop-methyl resistant wild oat, as well as other grass weeds, will become a problem in Oregon; however, there is currently no reported evidence.

Weed resistance has caused many herbicides to fail to control weed populations. The loss of these tools as effective components of crop management programs has forced growers to seek alternatives, often increasing costs of weed control. Because solving the problems associated with the management of herbicide resistance in weeds relates to human activity, the study of weed resistance should include social considerations. A procedure for rapidly detecting herbicide resistant weeds at the beginning of an infestation would be an integral part of an efficient management system. It was hypothesized that early detection of weed resistance including both microcalorimetric detection assay and pattern-thinking might delay weed resistance through preventative practices.

The study of field representatives from two processing plants in the Willamette Valley of Oregon shows that they are an important key to management of weed resistance in field situations since each fieldperson contacted represented at least ten or more grower points of view. Field representatives were asked a series of questions to assess pattern-thinking related to early detection of weed resistance. Several standard patterns or archetypes have been developed that seem to describe many diverse situations (Senge, 1990). The "limits to growth" pattern was selected because development of weed resistance follows exactly the same pattern as a standard growth curved. By connecting similarities between patterns, people can make an informed decision based on experience with other situations.

Even though a pattern of herbicide resistance in weeds has been suspected, early detection of this resistance is necessary to effectively manage and prevent wide spread infestations in crop lands. A method based on microcalorimetry was developed to detect herbicide resistance as a general method, one which would have broad scale application to many herbicides and weed species. In this study, heat evolution was measured to discriminate between resistant and susceptible biotypes of three weeds: atrazine resistant *Amaranthus powellii* (pigweed), metsulforon-methyl resistant *Lactuca serriola* (prickly lettuce) and diclofop-methyl resistant *Avena fatua* (wild oat). Characterization of heat evolution rate can serve as a rapid method to discriminate between resistant and susceptible biotypes collected from fields. Because a susceptible plant's metabolism is affected by an applied herbicide, it was hypothesized that

resistant and susceptible biotypes would respond differently to the herbicide. This difference in metabolism could be measured by microcalorimetry.

The combination of pattern-thinking and microcalorimetry designed to rapidly detect weed resistance is an important part of this study. It is the combination of these approaches that may improve weed control management by preventing the problem from being established in the crop field.

CHAPTER II

LITERATURE REVIEW

1. Weed Resistance

1.1. Definitions

A weed biotype is a plant or group of plants within a given species that exhibits an observed characteristic. It has a slightly different, but distinct genetic makeup. Hence, a weed biotype that has evolved resistance to a particular weed control practice is called a resistant weed biotype (Bair, 1990). Weed resistance is a result of selection pressure from repeated weed control practices. Some weed scientists argue that only herbicides could cause the selection pressure that will result in the development of resistance in weeds. To be considered as such, resistance has to be inherited. Repeated use of a chemical practice induces a susceptible weed population through selection to become a herbicide resistant population (Bair, 1990). However, some agree that selection pressure could be from either a cultural or chemical practice. Constant cultivation results in development of populations of deep-rooted perennial weeds. Repeated mowing causes annual bluegrass to shift from an upright form to a prostrate growth habit. Continuous hand weeding in rice paddy fields gave rise to a new biotype of barnyard grass that mimics the crop in its morphology. This type of resistance is similar to adaptation of an ecotype to a specific environment. Hence, plant species adapt to new environments for their survival (Appleby, 1992).

Several terms are used to describe weed populations as they relate to weed resistance. Weed tolerance refers to the plant's ability to tolerate weed control practices even before they are used. For instance, if a plant has the ability to tolerate a herbicide at its use rate due to a natural characteristic of the species, it is considered to be a herbicide tolerant weed. A weed species that is suppressed by any weed control practice is defined as a susceptible weed biotype. For example, a weed species that is controlled by the use rate of a herbicide is considered to be herbicide susceptible (Bair, 1990).

Several weed species have evolved resistance to more than one class of herbicides. If a weed has developed resistance to herbicides from different classes and only one herbicide mechanism, the weed is considered to be herbicide cross resistant. However, if it is resistant to more than one class of herbicides and has many distinct resistance mechanisms, it is said to have multiple resistance (Hall, et al., 1994).

1.2. Herbicide Resistance and Its Mechanism

Triazine resistant common groundsel (*Senicio vulgaris*) was the first reported herbicide resistant weed species. It was discovered in 1968 after simazine and atrazine failed to control common groundsel (Ryan, 1970).

Triazines are PS II inhibitors that compete for a common binding site on a 32 kDa protein in thylakoid membranes (Tischer and Strotmann, 1977). PS II inhibitors block photosynthetic electron transport and prevent the reduction of $NADP^+$ required for CO_2 fixation. However, the activity of the herbicide is not due to the interruption

of photosynthesis, but is due to oxidative stress when electron transport is blocked. The blocking of electron transport results in the destruction of the PS II reaction center and the photooxidation of lipid and chlorophyll molecules (Pellett and Dodge, 1980; Barry et al., 1990).

Radosevich et al. (1979) indicated that resistant biotypes of common groundsel were resistant to all *s*-triazine herbicides due to a mutation of the chloroplast gene, psbA gene, that encodes the binding site of the herbicide in photosystem II. A mutation of the psbA gene resulted in a substitution of Gly for Ser at residue 264 (Trebst, 1991). This mutation greatly reduces the affinity of atrazine at the Q_B-binding site. The mutation of the psbA gene (Ser 264 to Gly) reduces the rate of electron transfer between Q_A and Q_B (Bowes et al., 1980; Herishberg and McIntosh, 1980). Hence, it causes a significant reduction in ecological fitness of plants (Conrad and Radosevich, 1979; Holt, 1988; Ahrens and Stoller, 1983).

After the first discovery of a triazine-resistant weed, about 30 weed biotypes have evolved resistance, mostly in North America (Bandeen, et al. 1982) and Western Europe (Gressel, et al., 1982). At least 57 weed species, 40 broad-leaved and 17 grasses, have developed resistance to triazine herbicides (LeBaron, 1991). The area infested by these resistant weeds has continued to increase. Biotypes of *Amaranthus* spp., *Chenopodium* spp. and *Kochia* spp. are the most predominant and important triazine-resistant weeds in the US (LeBaron, 1991).

At least 50 weed biotypes have developed resistance to 14 herbicide classes other than triazines (Lebaron, 1991). ALS/AHAS (acetolactate synthase/ acetohydroxyacid synthase) and ACCase (acetyl-CoA carboxylase) inhibitors are the types of resistance of greatest concern. ALS herbicides have become an extremely important new tool in agricultural production. Their popularity is due to relatively low use rates, low mammalian toxicity, wide crop selectivity and high efficacy. These characteristics result in rapid selection of resistant biotypes (Saari, et al., 1994). Meanwhile, ACCase inhibitors are used over a large area of agricultural land since they can selectively control grass weeds in cereal and dicot crops (LeBaron, 1991).

Sulfonylurea herbicides were introduced to control broadleaf weed populations in wheat in 1982. After 5 years of repeated use, they failed to control many broadleaf weeds (Mallory-Smith, et al.,1990). This evidence strongly suggested that the herbicide selection pressure for resistance was very high. Prickly lettuce was the first weed reported to be resistant to a sulfonylurea in the US in 1988 (Mallory-Smith, et al., 1990).

ALS is the first enzyme involved in the biosynthesis of the branched-chain amino acids valine, leucine, and isoleucine. Herbicides in the ALS inhibitor group include sulfonylureas and imidazolinones. Genetic evidence suggests that these herbicides act directly upon ALS, thereby inhibiting the enzyme's activity and consequently blocking the biosynthesis of the branched-chain amino acids (Saari, et al., 1994).

Studies involving transformation of sensitive plants by encoding an ALS insensitive gene resulted in lack of activity by ALS/AHAS inhibiting herbicides. First, the binding site was altered. Second, the herbicides can no longer block the branched-chain amino acid synthesis which resulted in weed resistance (Haughn et al., 1988; Sathasivan, et al., 1991).

Aryloxyphenoxypropanoate herbicides are postemergence herbicides that control monocotyledonous weeds in grass and dicot crops. This group of herbicides includes diclofop-methyl, fluazifop-butyl, haloxyfop-methyl and chlorazifop-propynyl (Devine and Shimabukuro, 1994).

Two mechanisms have been suggested for the aryloxyphenoxypropanoate herbicide mode of action; a biochemical mechanism involving the inhibition of acetyl CoA carboxylase and subsequent fatty acid synthesis in plastids (Burton et al., 1987; Kobek et al., 1988; Rendina and Felts, 1988; Secor and Cseke, 1988 and Walker et al., 1988) and a biophysical mechanism involving the perturbation of the transmembrane proton gradient across the plasma membrane (Shimaburu and Hoffer, 1990; Serrano, 1985; Sze, Uribe and Luttge, 1985). Even though ACCase is well characterized as a herbicide target site, the link between ACCase inhibition and its total effects on lipid biosynthesis is not clear (Devine and Shimabukuro, 1994).

The mechanism of resistance to ACCase herbicides is complicated since the mode of action of these herbicides is not yet clearly understood. The fact that resistance to aryloxyphenoxypropanoate is not correlated with reduced ACCase sensitivity in

some weeds supports the idea of a mechanism of differential response within the plasma membrane in resistant- and susceptible biotypes. To date, there is no satisfactory explanation for the finding that these biotypes have a herbicide sensitive ACCase but are not killed by the herbicides (Devine and Shimabukuro, 1994).

Diclofop-methyl was registered in Oregon to control grass weeds in wheat in 1980. In 1987, Italian ryegrass was the first weed reported to be resistant to diclofop. Fortunately, there was no evidence of any cross or multiple resistance for this weed to other herbicide classes (Stanger and Appleby, 1989). Wild oat was another weed showing evidence of diclofop resistance. It was reported to resist diclofop in many wheat fields in south-central Saskatchewan and north-western Manitoba, Canada (Joseph, et al., 1990; Heap, et al., 1993). A study by Heap et al. (1993) suggested that some wild oat resistant biotypes exhibited cross resistance to five different herbicides including diclofop-methyl, fenoxaprop-ethyl, sethoxydim, tralkoxydim and quizalofop-ethyl.

1.3. Detection of Herbicide Resistance

1.3.1. Field and Greenhouse Testing with Intact Plants

Treating intact plants with herbicides and observing the results is a simple method used by many weed scientists to identify herbicide resistance. It can be used with any type of herbicide resistance and can be conducted with relatively unskilled labor. However, principal disadvantages in the use of these field and greenhouse

techniques include the duration of the experiments and the need for large numbers of plants (Truelove and Hensley, 1982).

Ryan (1970) collected the seeds of *S. vulgaris* from suspected resistant plants in the nursery, and at a location where triazines had not been used. His experiments were conducted in the greenhouse. The seeds were treated preemergence with simazine and atrazine at different levels. He demonstrated that seedlings from seeds collected from the nursery where triazine had been in continuous use were not affected by either atrazine or simazine at a rate of 18 kg/ha. In contrast, seedlings collected from a non-treated control location were killed at a rate of 2.2 kg/ha.

The modified greenhouse method to overcome the problem of an uneven distribution of herbicide within pots of soil involves germination of seeds on absorbent paper. Roots of the young seedlings were transferred to aerated nutrient solution containing ^{14}C labeled herbicide (Radosevich and Appleby, 1973; Radosevich, 1979). The relative uptake of herbicide from these solutions by the different biotypes and the fate of the labeled herbicide within the plants can be determined. It can be established whether resistance is due to physiological differences between plants.

1.3.2. Laboratory Techniques

Laboratory techniques used to identify herbicide resistance are usually specific for the target site of the herbicide in the plant. They usually can not be applied to other

herbicides since both the procedure and equipment are different for a particular herbicide resistance.

Triazine resistance is detected using chlorophyll fluorescence at the primary target site of action. When electron transport on the reducing side of PS II is inhibited, chlorophyll absorbed radiance is reemitted as fluorescence. Detection of fluorescence levels at the leaf surface is the key to differentiating between resistant and susceptible biotypes. Hence, a low level of fluorescence indicated no inhibition of electron transport in PS II as would be characteristic of a herbicide resistant plant, while a high level of fluorescence indicated inhibition of electron transport (Ahrens et al., 1981; Truelove, 1982)

The sinking leaf disk technique is another detection method for photosynthetic inhibition. This method involves floating leaf disks on a surfactant containing phosphate buffer under photosynthetic conditions. When photosynthesis is inhibited due to herbicide action, the leaf disk will lose its buoyancy and sink (Truelove, et al., 1974; Gawronski, et al.,1977).

Effects of photosynthetic inhibiting herbicides on whole plants or leaf sections can be detected by direct measurements in the rate of photosynthesis. CO_2 assimilation by plants can be detected by following the rate of CO_2 uptake with infrared gas analysis or by monitoring either $^{14}CO_2$ or O_2 evolution. This method can distinguish triazine resistant weeds, but can not define the nature of resistance. These procedures are

complicated and require considerable technical skills as well as expensive equipment (Truelove, 1982).

Measurement of nitrite reductase in leaf disks was introduced as a detection method. It was found that lower enzyme activity would increase nitrite concentration in green plant tissue treated with a photosynthetic inhibiting herbicide. Nitrite reductase is located within chloroplasts and has a requirement for the reduced form of ferridoxin. The reduction of ferridoxin appears in PS I following the transference of electrons between the structural complexes of PS II and PS I. Thus, inhibition of electron transport in PS II decreases the production of the reduced ferridoxin required for reduction of nitrite (Klepper, 1975). Finke et al., (1977) demonstrated that nitrite reductase assay with leaf disks could distinguish between triazine resistant and susceptible biotypes. Hence, the reduction of nitrite in susceptible *Amaranthus* sp. was completely inhibited, but there was no effect on enzyme activity in the resistant biotype.

Hill reaction measurements monitor the rate of reduction of an electron acceptor from isolated chloroplasts. This technique can distinguish between triazine resistance due to innate differences in chloroplast and resistance from the detoxification mechanism (Machado, et al.,1978)

Photoacoustic spectroscopy is used to measure the difference in response to heat stress on photochemistry. Changes in oxygen and photothermal effects on photochemical fluorescence quenching are compared. Hence, the oxygen component of

the photoacoustic signal is decreased in a susceptible biotype, but a resistant biotype is not changed. It was suggested pulse oxygen emitted by leaves could be used to detect weed resistance to photosynthetic inhibitors (Fuks, et al., 1992).

A method to detect sulfonylureas was proposed by Gervik et al. (1993). This technique is based on accumulation of acetoin in branched chain amino acid biosynthesis. Acetoin accumulation is induced by inhibition of ketol-acid reductoisomerase (KARI). Inhibition of ALS/AHAS prevents the accumulation of acetoin, thereby distinguishing between resistant and susceptible biotypes.

HPLC also has been used to quantify the levels of branched chain amino acids in sulfonylurea resistant and susceptible biotypes. It was suggested that levels of valine and isoluecine were higher in a resistant biotype than in a susceptible one (Dyer, et al.,1993).

Methods to detect ACCase inhibitor resistant weeds were proposed by Hubbard and Whitwell (1991). They concluded that measurement of net photosynthesis is consistent only with the youngest fully expanded leaf of *Calamagrostis arundinacea* but is not consistent with all herbicides within the same mode of action. Chlorophyll A fluorescence was ineffective in distinguishing between a tolerant and susceptible biotypes. Solute leakage from leaf discs treated with herbicides indicated that susceptible *Sorghum halepense* exhibited greater leakage than tolerant types.

2. Microcalorimetry

2.1. General Concepts

Calorimetry is the measurement of energy in the form of heat. Measurement of heat evolution from plant tissue provides a sensitive, non-invasive method to measure metabolic activity and estimate the relative efficiency of the system at a particular stage of development or under a determined set of environmental conditions (Loike, et al., 1981). The main principal behind calorimetric method is that when plant material undergoes treatment that causes any physical or chemical change in tissue, heat is either absorbed or released (McNaughton and Mortimer, 1979). During photosynthesis, heat energy is lost and CO_2 is evolved as a product of plant respiration. (Salisbury and Ross, 1992).

One output from calorimetric measurements on living plant tissues is the rate of heat production as a function of time and temperature. The values of heat per mass of tissue are proportional to metabolic rates and can be compared for different plants, tissues, growth states, environmental conditions, etc. Most rapidly growing plant tissues produce heat evolution rates of about 10 to 30 μW/mg dry weight or 1 to 3 μW/mg fresh weight when measured at 25 C (Criddle, et al. 1991).

As the precision of detection has improved, microcalorimeter methods have been developed for plant studies including simple isothermal calorimetry and differential scanning calorimetry (DSC). Isothermal microcalorimetry can be used to

examine metabolic rates of plant samples and to study the effect of a wide variety of natural or artificial treatments.

Differential scanning calorimetry is useful for defining temperature sensitivities and discovering temperature related plant responses. In addition, it provides an understanding of the mechanisms of the plant responses over a temperature range, at a predetermined rate of temperature change (Criddle, et al.,1991).

2.2. Applications of Microcalorimetry in Plant Respiration

Isothermal calorimetry can be used to predict relative growth rates. It has been applied to determine the effect of temperature and O_2 depletion on metabolic heat rate of tomato and carrot (Criddle et al., 1988). Germplasm screening by isothermal calorimetry provided an accurate prediction of growth rates of intact plants (Criddle, et al., 1990). This technique has been used to define suitable storage conditions to avoid spoilage of fresh cut pineapple (Iversen, et al., 1989), evaluate the physical state of water in pea and soy been cotyledons (Vertucci, 1990), and measure aerobic cell metabolism in unstirred cell cultures (Fontana, et al., 1990).

Simultaneous measurements of metabolic heat production, O_2 consumption, and CO_2 evolution were proposed by Criddle et al. (1990) to determine the efficiency of plant metabolism. Differential scanning calorimetry has been used to measure the heat evolution rate of dormant and developing buds and predict long term growth rate of larch clones (Hansen, et al., 1989). Moreno-Siunovic (1993) studied the respiratory

metabolism of peach seeds during stratification to seedling stage. He concluded that heat evolution rate as well as CO_2 evolution rate increased with stratification time. Gardea, et al. (1994) found by using differential scanning calorimetry that the pattern of plant respiration changed during dormancy and development of 'Pinot noir' primary grape buds through the broken bud stage. They illustrated that metabolic heat rates decreased during endodormancy and increased during ectodormancy and bud development.

The study of plant physiological response to natural stress and artificial conditions can be achieved with isothermal heat conduction calorimetry. Criddle, et al. (1990) found that barley root tips exhibited two levels of inhibition of heat evolution rate with increasing concentrations of NaCl. No significant difference was found among cultivars at salinity concentrations up to 150 μM. However, differences started to be detectable at concentration levels above 150 μM. Bower (1990) studied the effect of atmospheric pollutants on plant activities. The author found that metabolic heat rates of ponderosa and jeffrey pine needles were correlated to the level of ozone. A study of time and temperature response of tomato cells by David et al., (1991) demonstrated a simultaneous shift in metabolic heat rate, O_2 consumption rates, and CO_2 evolution rates. Calorimetric procedures have been used to define the effect of high and low temperature stresses on the metabolic activities of plant tissues and cell cultures. Inactivation of metabolism was found to be a complex function of both temperature and time of exposure to thermal extremes (Breidenbach, et al., 1990; Rank, et al., 1991).

3. System Approaches in Agricultural Inquiry

3.1. What is a System?

For three centuries, scientists believed that the best way to learn about something was to take it apart and find out what it was made of (Checkland, 1981). This concept may be suitable for some subjects such as biology, chemistry, and physics, but it often fails to understand the system as a whole. The word "system" in systems thinking is similar to "system" in everyday sense, such as a cooling system or nervous system (Kauffman, 1980). System thinking embodies the ideas of a set of elements connected together to form a whole, rather than properties of its component parts. For instance, the taste of water is a property of the substance water; not for the substance or taste of hydrogen and oxygen that combine to form water (Checkland, 1989).

In the real world, problem situations are very complex ranging from the technical and organizational to the social and political. Modern problematic situations embrace concerns about the environment, the framework of society, the role of corporations, and the motivation of individuals (Checkland, 1980).

Systems thinking offers a way to improve our abilities to deal with complexity. Systems thinking is a discipline for understanding the structures and patterns that underlie complex situations as a whole. It involves interrelationships as well as studying parts within the framework of reductionism; it enhances our ability to see patterns of

change rather than static pictures. It concerns a shift of the mind from seeing parts to seeing wholes (Senge, 1990).

3.2. The "Limits to Growth" Archetype

The key to seeing reality is searching for patterns and relationships within the system. In systems, the whole is different from the sum of its part. That difference is the emergent property which uniquely pertains to that systems (Wilson and Morren, 1990). Senge (1990) portrays these patterns as circles or casual loop diagrams. Every circle tells a story. The practice of systems thinking starts from understanding a simple concept called feedback. Feedback shows how actions can reinforce or counterbalance each other. By tracing the flows of influence, patterns will repeat themselves, time after time, making complex situations better or worse.

The "limits to growth" pattern or archetype consists of two related circles. Reinforcing or amplifying feedback loops represent a growth process, while a balancing or stabilizing feedback loop dampens the system. The "limits to growth" structure is useful for understanding all situations where growth bumps up against limits (Senge, 1990; Kim, 1994). For example, one herbicide is effective and will control weed populations for a while. Growers are pleased, so they repeat use of the herbicide (reinforcing circle). When the herbicide loses its efficacy, weed populations flourish, while growers become upset and must find a new herbicide or new method of control (balancing circle). Growers usually recognize weed problems after populations begin to

increase rapidly. Time delays cause weed problems to become difficult to manage. Hence, when weeds appear to escape a particular weed control practice, growers may wonder why this patch of weeds survive while weeds in most of the field are controlled. Either feedback or feedforward delays are impossible to assess. For example, a feedback delay might involve verifying that a treatment skip during application was the cause while a feedforward delay requires waiting one or more years to repeat the herbicide treatment on the same area. Therefore, the problem is rarely solved because people lose interest or forget. This implies that in order to accomplish improvement, a way of dealing with systemic relationships and delays must be found.

System archetypes have been used to predict behavior and improve management in business (Kim, 1994). He stated that accurate forecasts came from understanding the underlying structures within the system. The more we understand the structure of the system, the better we can predict future behavior of the system. Linking system archetypes with a specific set of behavioral patterns (for instance, population growth over time) can help predict future management decisions before they become problems. For example, the historical data of sales showed rapid growth and then plateauing that suggests a "limits to growth" structure. Behavior over time helps identify the particular limits affecting sales growth. Charting these factors over time can offer insight into particular balancing processes that need to be addressed to eliminate potential limits to growth before they affect future sales.

A "limits to growth" archetype can be used as a planning tool (Kim, 1993). Any successful product begins with a plan for achieving success. The "limits to growth" archetype shows that being successful can be dangerous. It helps show the actions, either intentional or unintentional that may reinforce themselves. It also helps recognize organizational barriers that relate to the growth. Kim (1993) suggested a seven-step process for using "limits to growth" to help identify success. He suggested that mapping these "limits to growth" structures in advance can anticipate future problems and eliminate them before they become threatening.

There are several archetypes proposed by Senge (1990). A "balancing process with delay" archetype is a pattern of a person or group acting toward a goal with adjustments for behavior in response to delayed feedback. A "shifting the burden" archetype describes a pattern of short-term solution with seemingly immediate improvement, usually resulting in dependency or addition. If dependency or addiction is evident, you must focus on other alternatives that may lead to fundamental improvement. An "escalation" archetype explains two people or organizations that compete with each other. When one side gets ahead, the other side acts more aggressively to establish its advantage. This results in a buildup that goes beyond either's desire. A "success to the successful" archetype describes a situation when two activities compete for limited support or resources. The more successful one becomes, the more support it gains, thereby, starving the other. A "tragedy of the commons" archetype is a pattern when individuals use a limited resource solely on the basis of

individual need. Eventually, the resource produces diminishing returns that causes them to intensify their efforts. Finally, the resource is depleted. A "fixes that fails" archetype is another pattern that explains a quick fix, but often creates unforeseen long-term consequences that require even more fixing later. A "growth and underinvestment" archetype is used when growth approaches a limit that could be eliminated if individual or organizational investments were to add capacity. However, investment must be aggressive and rapid to forestall reduced growth. Most of the time, the key goal is to lower the performance standard to justify underinvestment. When it happens, a self-fulfilling prophecy leads to lower expectations, which causes more underinvestment.

The systems archetypes have been used successfully in business. For example, Royal Dutch Shell applied "limits to growth" to improve sales productivity (Kim, 1994). Identifying behavior or growth over time helped A to Z Corp to improve market share rather than achieve better profits (Kim, 1994). Goodman and Kim (1993) applied "limits to growth" to gain insight into declining sales for the EnviroCo company.

CHAPTER III

RECOGNITION OF PATTERN-THINKING BY AGRICULTURAL CONSULTANTS AND EARLY MANAGEMENT OF WEED RESISTANCE

1. ABSTRACT

Pattern-thinking enables people to recognize phenomena that recur over time. Processor field representatives from two processing plants in the Willamette Valley of Oregon recognized weed population growth patterns and described early detection of weed resistance. Weed population growth patterns were described from a "limits to growth" archetype (Senge, 1990). Individuals defined indicators (numbers of weeds per .093 square meters) at different stages of the weed population growth pattern. Pattern-thinking encouraged individuals to predict and manage the potential problem of weed resistance at an early stage.

2. INTRODUCTION

Repetition of a weed control practice leads to weed resistance. Persistent cultivation results in development of a population of deep-rooted perennial weeds, while continuous mowing of a lawn causes a shift from upright weeds to prostrate types. Similarly, repeated use of herbicides with similar modes of action has contributed to selection for increased resistance within formerly susceptible species. Therefore, weed species or biotypes tolerant to any practice will rapidly develop resistance.

Detection of herbicide resistance in weeds began in 1969 when simazine resistant common groundsel (*Senicio vulgaris*) was described (Ryan, 1970). Simazine is a member of the s-triazine herbicide family. It kills plants by inhibiting electron transfer within the plant photosystem. During the 1970's, triazine resistant biotypes of common groundsel were found in several locations in the Pacific northwest. In North America, pigweed (*Amaranthus* spp.) and lambsquarters (*Chenopodium* spp.) with the same mechanism of resistance to triazine also were reported (Bandeen et al, 1982).

In the early 1980's, growers in the midwest recognized weed shifts including appearance of triazine resistant pigweed in crops such as field corn (William, 1980). By the early 1990's, Oregon growers recognized weed resistance in sweet corn. It took more than ten years for growers to recognize weed resistance in Oregon, despite several attempts by weed scientists to contribute to the knowledge of weed resistance as well as its management (William, 1980).

Because similar patterns have developed among several commodities, weed scientists have questioned whether pattern-thinking as described by Senge (1990) would provide insight into early detection and management of weed resistance. Senge developed pattern-thinking from a standard growth curve (Figure 3.1). Initially growth is slow, becomes exponential, and levels off as resources become limiting. Weed infestations follow a similar pattern. Senge defines this pattern as a "limits to growth" archetype.

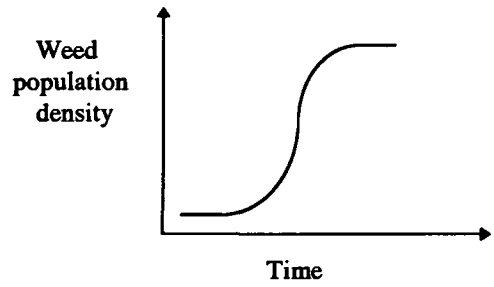


Figure 3.1. Standard growth curve representing weed population growth patterns

Another explanation of "limits to growth" archetype involves two interconnected circles. The first circle is a reinforcing or amplifying process (Figure 3.2; left side). This reinforcing loop creates a cycle of success that also creates weed abundance. However, resources such as light, water, or nutrients become limiting, thereby, causing eventual slowing of the infestation process (Figure 3.2; right side). The two circles connect at the point of inflection on the graph (Figure 3.1) when the slope of the curve changes. To describe weed population dynamics, words can be inserted within the circles as a way of clarifying the recurring patterns (Figure 3.2). Another aspect of the archetype involves time or the delays inherent in the system. As weed control practices are repeated, time delays contribute to explosive weed infestations. Only when substantial delay has occurred do farmers begin to recognize resistant weed infestations in their fields. Weed populations increase rapidly (delay) because the weed control practice no longer provides control (Figure 3.2; right side).

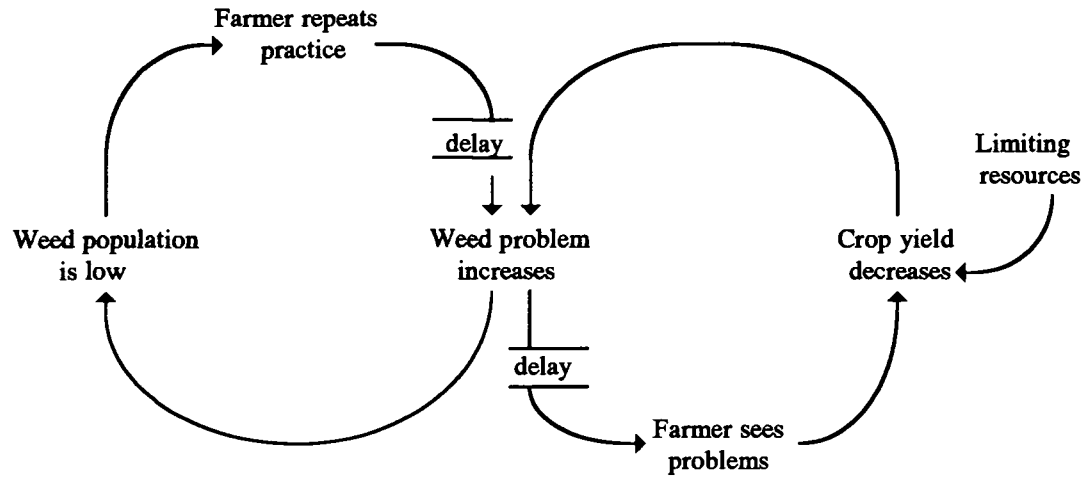


Figure 3.2. A "limits to growth" archetype or story describing weed population growth patterns resulting from repeated use of a weed control practice

It has been hypothesized that pattern-thinking might contribute to principles of early detection by growers or processor field representatives. It was reasoned that growers might initiate preventative measures before the resistant population becomes abundant.

Objectives of this study were to: 1) quantify pattern recognition among field representatives in relation to weed resistance using a modified "limits to growth" structure or archetype; and 2) determine how individuals relate their pattern-thinking of weed control experience to recognition of weed resistance at an early stage of detection.

3. METHODOLOGY

Agricultural consultants are frequently involved in growers' weed control strategies. Since they influence growers' points of view, field representatives were asked to participate in this study. Varied experience among the participants was a selection criterion because it represented a broad perspective. Since this study attempted to describe individual's pattern-thinking, all opinions expressed by each respondent were included.

This study involved six interviews of eight field representatives from two processing companies in the Willamette Valley. Interviews began on 3 March 1994 and were completed on 4 April 1994. Each interview was conducted with a single field representative, except one which involved three persons with individual perspectives. All field representatives had experience working with the companies from 3 to 23 years and all had worked with growers for at least 10 years.

The study used open-ended questions[1]. All questions related to weed control in sweet corn. Respondents were asked a series of questions to assess pattern-thinking relative to early detection of weed resistance. At the beginning, each field representative described his weed control experience in sweet corn. This part of the questionnaire included experience of respondents as well as the possibility of knowing the existence of weed resistance in the region where they work.

[1]See Appendix A.

Each person selected one of his most common weeds. Individuals described when the weed appears within each corn planting. Also, management practices were described. The standard growth curve or weed population growth pattern (Figure 3.1) was then introduced to each respondent. Each person was asked to divide this growth curve into meaningful segments to represent their experience with developing weed populations or infestations. Each stage was described with their perception of weed population density. Using the same growth curve, each person was asked to identify the most appropriate time to intervene with weed control practices. Weed resistance was introduced only if respondents lacked experience or failed to respond to this subject.

While, exploring the same standard growth curve, respondents were asked to describe patterns of weed resistance at each stage they identified. This step led individuals to indicate when weed resistance was first recognized. Using the same growth pattern, field representatives identified the point at which weed resistance management should be initiated.

4. RESULTS AND DISCUSSION

All field representatives (Table 3.1) recognized the standard population growth curve as a way to describe the development of weed infestations (Figure 3.3). Seven of eight field representatives divided the growth curve into three stages (Table 3.1 and Figure 3.3). One field representative refused to believe that weed infestations should progress to Stage 2 and 3. Thus, only Stage 1 was identified (Table 3.1).

Table 3.1. The results of interviewing eight field representatives from two Western Oregon processing companies.

Results of the interviews		Number of response
Part I Experience of respondents		
1.1. Specific weed problems familiarity		
Pigweed		5
Lambsquarters		5
Proso millet		3
Part II Recognition of pattern-thinking		
2.1. Recognizing weed population growth pattern		8
2.2. Identifying Stage 1 as slow growth stage and low weed density		8
2.3. Identifying Stage 2 as rapid growth stage; one said that weed infestations should never enter Stage 2		7
2.4. Identifying Stage 3 as overwhelming stage		7
2.5. Identifying the best time to intervene with weed control strategies during Stage 1 or at the beginning of Stage 2		8
Part III. Link experiential weed problem to existing weed resistance problem		
3.1. Previous experience of weed resistance	Yes	5
	No	3
3.2. Recognition of weed resistance by using pattern-thinking archetype		7
Part IV Combining pattern-thinking with rapid microcalorimetric assay		
4.1. Believed that combining pattern-thinking and rapid microcalorimetric assay would enhance weed control strategies		8

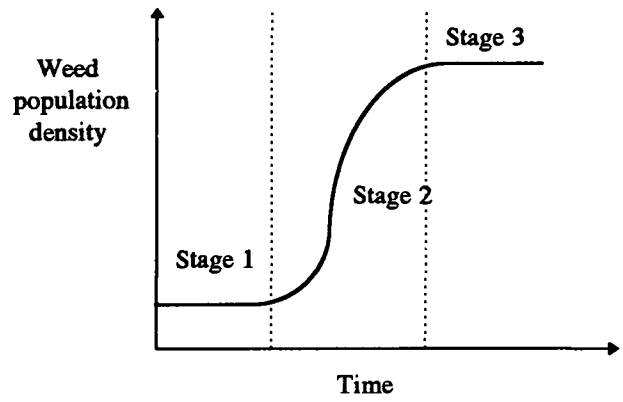


Figure 3.3. Weed population growth pattern, as divided by seven of eight field representatives

Three major weeds were identified by all field representatives; pigweed, lambsquarters and proso millet (Table 3.1). Proso millet was commonly identified as the most serious weed pest because it reproduces rapidly and competes significantly with corn. Pigweed and lambsquarters were familiar to all field representatives. One respondent suggested that sweet corn was not a high value crop, which explained why growers failed to pay attention to weed problems until they became serious.

Field representatives described Stage 1 (Figure 3.3) as a slow growth stage in which weeds were under control (Table 3.1). Weed density in this stage was not harmful to the crop. The population density associated with Stage 1 varied depending on the weed species. Four field representatives who chose proso millet identified Stage 1 density with zero or very few plants in entire fields. The other three persons selected either pigweed or lambsquarters, and identified weed densities in Stage 1 as 3 to 4

plants per .093 square meters.

Stage 2 (Figure 3.3) was described as the rapid growth phase (Table 3.1) which began when someone noticed a proso millet or a small patch of pigweed that survived control practices. Soon weed populations began to compete with the crop. The population density associated with Stage 2 for proso millet was identified by four field representatives as 3 to 5 plants at the early stage, rapidly increasing to 1,000 plants per .093 square meters in the late stage.

Stage 3 (Figure 3.3) was described as an overwhelming stage by seven field representatives (Table 3.1). Stage 3 densities for all selected weeds were more than 1,000 plants per .093 square meters. Weed control managers could not allow weed populations to reach this stage. Crop yield or quality would be completely destroyed.

Atrazine resistant pigweed was the only weed resistance problem referred by five field representatives. There was no evidence of resistance in proso millet. Even though there was some evidence for lambsquarters resistance, it was not considered a problem. Therefore, pigweed was used as an example of weed resistance by respondents in this study. All field representatives described pigweed resistance in sweet corn after atrazine failed to control the weed population. Growers repeatedly used this herbicide because it was inexpensive. This repeated use of atrazine caused a weed shift. Once atrazine resistant pigweed developed, the population rapidly increased (Figure 3.3). When resources became limiting, intraspecific competition became a major cause of weed populations to level off.

The next step involved converting the verbal results from field representatives into pattern-thinking archetypes (Figure 3.4). This step focuses attention on "leverage" or where the greatest improvement can be made within the system. Most field representatives recommended that weed control practices should be considered somewhere between late Stage 1 and early Stage 2 when weed populations begin to increase (Figure 3.4). Populations beyond that level resulted in weed problems that are very expensive to control, or if not controlled, result in serious yield loss. In other

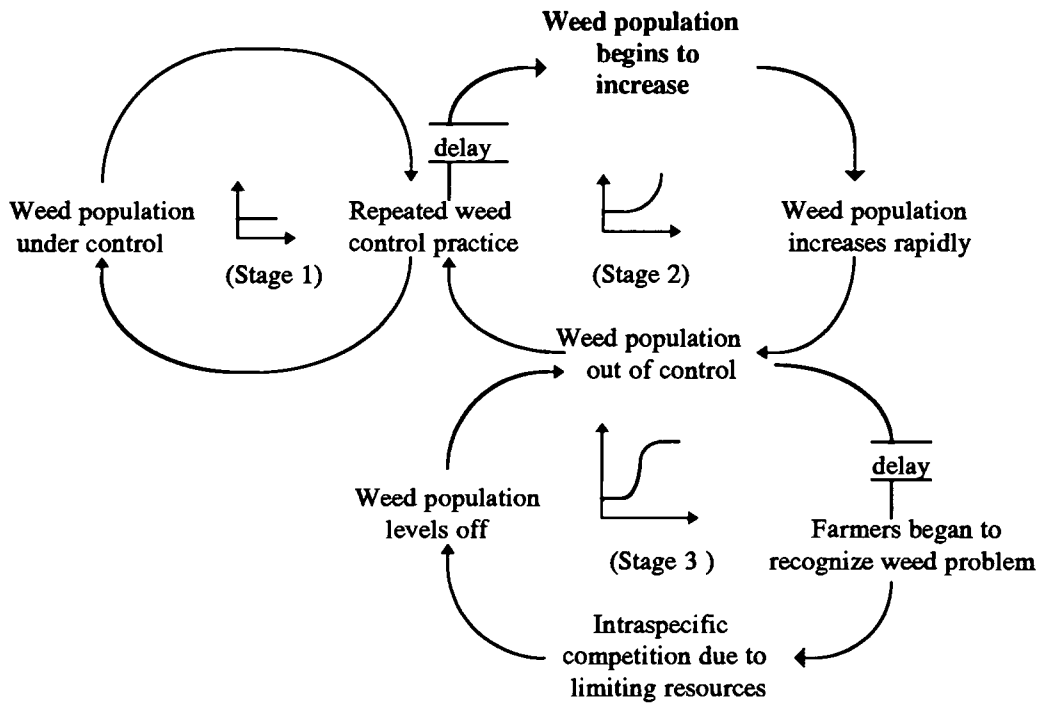


Figure 3.4. Pattern-thinking of field representatives, describing development of weed population from their experience

words, weed populations at Stage 2 should be suppressed as soon as they are recognized. The growth curve must level off at a low threshold instead of entering the exponential growth phase.

Early recognition of weed populations is an important link in controlling weed resistance. This fact applies to all weed populations since patterns develop similarly. Thus, weed resistance can be substituted for weed population throughout Figure 3.4. Again, leverage or fundamental improvement occurs when weed resistance begins to increase. Indicators or numbers of weeds per .093 square meters were similar for general weed populations. It is essential that individuals understand weed infestations in their fields so that they can initiate timely weed control strategies. A crucial step in this process is recognizing and doing something about the delays in Figure 3.4.

The greatest leverage occurs in either recognizing or reducing delays in the system. All field representatives responded affirmatively to the question about a rapid microcalorimetric assay being developed to distinguish a herbicide resistant weed from susceptible plants (Chapter 4). The results of this test would validate resistance or susceptibility within 24 hours. By reducing the validation time, field representatives believed that early detection could be improved significantly compared to reconstructing possible treatment problems during application or waiting one or two years when atrazine might be applied again. They valued this rapid assay by considering a dollar value to analyze a sample. They thought growers would be willing to pay a similar fee as their soil analyses.

In conclusion, pattern-thinking of weed resistance development encouraged field representatives to predict weed resistance at an early stage by relating the existing problem to their experience with weed infestations. Lack of time is a major reason that weed control managers fail to recognize a resistant weed problem before the population explodes. A rapid microcalorimetric assay for potential herbicide resistance and use of pattern-thinking to make decision makers aware of the urgency for action will help field representatives take appropriate steps in implementing an effective management plan.

Appendix A.

Interview Structure to Study Pattern-Thinking of Field Representatives

Part I. Background of Respondent

1. Goal: To understand respondents experience with weed control and weed resistance

2. Questions:

    1. How many years have you been working with corn growers?

    2. What crops do corn growers usually rotate with corn?

    3. What crop rotation was used for the past 10 years?

    4. What weed control practices do corn growers use in corn?

    5. Have weed control practices changed in the past 10 years?

    6. Do you have any specific weed problem?

    7. When did your growers recognize this weed as a problem?

    8. How do your grower manage this weed in sweet corn?

Part II. Research Strategies, Recognition of Pattern-Thinking

1. Goal: Field representatives relate an existing weed problem to weed resistance by using the "limits to growth" pattern

2. Questions:

    1. Could you list weeds in sweet corn that concerned you the most?

        1 .....

        2. .....

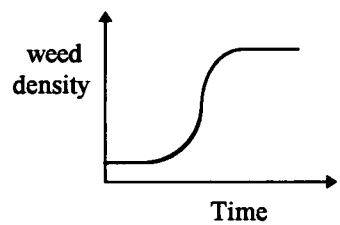
3. .....

4. .....

5 .....

2. Please select one weed with which you are most familiar.....

3. If I draw this graph having X axis as time and Y axis as weed density (as shown in Figure 1)



4. Could you divide this curve the way you understand?

5. Could you describe what is happening during Stage 1 (2 and 3)?

6. What would be the most distinguishing characteristic in this stage (1, 2 and 3)?

7. How would you recognize this stage (1, 2 and 3) in your field?

Part III. Link Experiential Weed Problem to Existing Weed Resistance Problem as well as Weed Control Strategies

1. Goal: Field representatives link existing weed problem to weed resistance problem

2. Questions:

Our experience suggests that producers practice weed management some where along this curve, could you show where on the curve (weed population growth curve on question part I) you would use your various weed management strategies

1. Where on the curve would you use your weed management strategies?
2. What are you striving for in weed management?
3. What indicators/ measures do you base your decision on for this weed control management?
4. There always are some weeds that seem to escape our weed control practice, what is allowing these weeds to survival?

Note:

If the respondents do not include weed resistance in the answer on question 4, introduce this topic to them: After the same weed control practice has been applied for several years, some weeds may develop resistance to a particular practice and that practice will no longer be effective.

If the answers include "weed resistance" go to question 5.

5. Knowing that you (will) have resistant weeds in your corn field, how would you recognize them (explain by using the same growth curve)?

Part IV. Combining Pattern-Thinking with Rapid Microcalorimetric Assay

1 Goal: Combining pattern-thinking of a development of weed resistance with rapid detection method by microcalorimetry.

2. Explain overall research hypothesis, both pattern-thinking and rapid microcalorimetric assay to field representative:

I am working on a simple test by measuring heat evolution from plant respiration of resistant and susceptible biotypes. The results showed that this rapid

assay can distinguish between the two biotypes within 1 or 2 days. However, you may have to spend some money for the test.

3. Questions:

1. Would you mind bringing the suspected weeds to OSU for running a test?

2. What do you think would be a fair price for this test?

Part V. Closing Statement

Explain Research Hypothesis:

Linking pattern-thinking (social study) to rapid microcalorimetric assay should lead to early detection of weed resistance. The "limits to growth" archetype (Standard growth curve) can be used to relate experiential weed problem to existing weed resistance problem.

Ask if respondent has any questions or suggestions concerning this interview as well as this research.

I hope this research will be helpful to you. Thank you very much for your cooperation and your valuable time.

CHAPTER IV

MICROCALORIMETRY: A RAPID METHOD FOR DETECTING HERBICIDE RESISTANCE

1. ABSTRACT

Microcalorimetry was tested as a promising method to detect herbicide resistance in three weed species including triazine resistant *pigweed (Amaranthus powellii* S. Wats), diclofop-methyl resistant wild oat (*Avena fatua* L.) and metsulfuron-methyl resistant prickly lettuce (*Lactuta serriola* L.). Heat evolution rate as a measure of plant respiration was determined for meristematic tissue from treated plants. This rapid method can be completed within 24 hours after the plant is sprayed with herbicide. Removing the plant sample from the plant soon after spraying did not diminish accuracy of the method. Therefore, this microcalorimetric method is very applicable for rapid detection and improves preventative weed management practices for growers as well as extension persons.

2. INTRODUCTION

Several methods have been used to discriminate between herbicide resistant 'R' and susceptible 'S' weed biotypes. Each method was developed depending on the particular herbicidal mode of action. A fluorescent test was used to diagnose atrazine resistant weeds. This method is based on the difference in photosynthetic efficiency between 'R' and 'S' biotypes. Atrazine inhibits electron transfer on the reducing side of

photosystem II (PS II) by binding on a D1 polypeptide, the apoprotein of the secondary quinone acceptor of PS II (Trebst 1987). Replacement of serine with glycine in position 264 on D1 protein confers resistance to the triazine in weeds (Hirshberg and McIntosh, 1983). The alteration of the 32 KD protein of PS II changes the plants' ability to oxidize the PS II primary quinone acceptor and is the principle basis for distinguishing an atrazine resistant biotype from a susceptible one. A study by Fuks et al. (1992) on photosynthetic performance of *Solanum nigrum* L. and *Chenopodium album* L. treated with atrazine used photoacoustic spectroscopy as a tool for monitoring the 'R' and 'S' biotypes. Changes in oxygen and photothermal components to photochemical fluorescence quenching were compared by fluorimetry.

The sulfonylurea (ALS/AHAS) resistance mechanism is attributed to an altered form of acetolactate synthase (ALS) that no longer binds the herbicides (Saari et al., 1990 and Thill et al., 1991). Rapid diagnosis of ALS/AHAS resistant weeds by Gervick et al. (1993) was based on the differential accumulation of acetoin in the presence and absence of an ALS/AHAS inhibitor herbicide. Accumulation of acetoin in quantities sufficient to distinguish between 'R' from 'S' biotypes took between 2 to 8 hours. HPLC analysis also was used to quantify the levels of branched chain amino acids in 'R' and 'S' *Kochia scoparia.* Levels of valine and isoleucine were higher in 'R' than in the 'S' biotype (Dyer et al. 1993).

Microcalorimetry is a measurement of energy in the form of heat. This technique was first developed by Joseph Black in 1730 (Beezer 1980). Since then

microcalorimetry has undergone numerous modifications to increase sensitivity and extend versatility. This technique has been applied to various fields including chemistry, biochemistry, physics, microbiology, and plant physiology (Hansen and Criddle 1990). The output from microcalorimetric measurement on living plant tissue is the rate of heat production as a function of time and temperature. The values of heat evolution rate over tissue mass are proportional to metabolic rates and can be comparable for different plants, tissues, growth stages and environmental conditions. Microcalorimetry can be used with cells in liquid culture, callus cultures, root tips and other tissue cuttings such as an excised leaf. Isothermal calorimetry can be used to examine total metabolic rates of plant samples and to study the effects of a wide variety of naturally or artificially imposed factors on those rates. It also can be used to measure a tissue's response to stress from salt, pathogen infection, herbicides or other conditions (Criddle et al., 1990).

The metabolic heat evolution rate of plant tissue is an accurate and sensitive measure of stress response in the whole plant, with increasing stress indicated by a decreasing metabolic heat evolution rate. Isothermal microcalorimetry was used to measure metabolic heat evolution rate of barley (*Hordeum vulgarae* L.) root tips exhibiting differential tolerance to salinity. Criddle et al. (1989) concluded that isothermal microcalorimetry could detect different levels of salinity stress.

Numerous instances of weed resistance were developed from herbicides with various modes of action. However, current techniques used to discriminate between 'R'

and 'S' biotypes are usually specific for a herbicide. The study presented here was an attempt to develop a broad based method to distinguish between herbicide resistant and susceptible weed biotypes. Heat evolution rate from microcalorimetry was a measure of a secondary symptom rather than a primary effect at the target site of a particular herbicide. Objectives of this study were to: 1) compare the heat evolution rate between resistant and susceptible biotypes of three herbicide resistant weeds, each herbicide represented a different mode of action; 2) determine the best time to sample and measure heat evolution rates in herbicide resistant and susceptible biotypes so that the method has good discrimination and meets the criteria of a rapid detection method; 3) validate the use of microcalorimetry as a rapid detection method in a real field situation by comparing this method with a conventional bioassay.

3. MATERIALS AND METHODS

Greenhouse studies. The three weed species used in this study included atrazine resistant *Amaranthus powellii* S.Wats[1], diclofop-methyl resistant *Avena fatua* L.[2], and metsulfuron-methyl resistant *Lactuca serriola* L[3]. Each species was represented by both a resistant biotype (R) and a susceptible biotype (S).

Greenhouse studies consisted of two experiments. The first was conducted during August to October 1994; the second during December 1994 and January 1995. All greenhouse studies were conducted in a growth chamber at 20/15C with a 16/8 h,

day/night regime and irradidiance of 480 μE $m^{-2}s^{-1}$ photosynthetic photon flux density.

Experiment 1. All three weed species, both 'R' and 'S' biotypes, were planted in the greenhouse on 15 August 1994. After 2 weeks, *A. fatua* seedlings were treated with topical spray of diclofop-methyl (354.5 g ai L^{-1}) at the rate of 1136.4 g ai ha^{-1}(13 liters of water). At the 3 week-stage, *L. serriola* seedlings were sprayed with metsulfuron-methyl (60% dry flowable) at the rate of 4.2 g ai ha^{-1} (13 Liters of water). At the 4 week-stage, *A. powellii* seedlings were sprayed with atrazine (454.6 g ai L^{-1}) at rate 1136.4 g ai ha^{-1} (13 liters of water).

Comparisons were made between 'R' and 'S' biotypes and the intervals between spraying and sampling (0, 1, and 2 days). Microcalorimetry was used to discriminate between the 'R' and 'S' biotypes. Metabolic heat evolution rate measurements were made in a Hart model 4207 differential scanning calorimeter (Hart Scientific, Pleasant Grove, UT 84062). Isothermal experiments measured heat evolution rate at 20C for at least 3,000 seconds; data were collected at 20 second intervals. Heat of metabolism

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[1] *A. powellii* seeds were collected from a grower's field in the Willamette Valley of Oregon and tested for resistance to atrazine in the greenhouse at Oregon State University.

[2] *A. fatua* seeds were received from Dr. Ian Heap, Courtesy Professor in the Department of Crop and Soil Science, Oregon State University.

[3] *L. serriola* seeds were provided by Dr. Daniel Ball, Assistant Professor of Weed Science, Oregon State University.

rates were expressed on a dry weight basis. Measurements of metabolic heat rate and CO_2 evolution rate were made according to Criddle et al. (1990, 1991). The NaOH was placed in a small glass vial to ensure that the sample did not contact the base.

Samples for calorimetric measurements were prepared by excising only the meristematic and associated tissue. *A. fatua* and *L. serriola* samples were collected from the base of the stem. *A. powellii* samples were collected from shoot apices. Size of samples ranged from 1 to 2 centimeter in length, leaving enough space in the respiration chamber (ampule) for the sample to continue its respiration. The microcalorimetric measurements were started immediately after the samples were excised from the plant.

Statistical analysis of this data was performed by analysis of variance using a randomized complete block design with unequal number (4-5) of replications. Means were compared by LSMEANS (SAS).

Experiment 2. All three weed species, both 'R' and 'S' biotypes, were planted in the greenhouse on 12 December 1994. Greenhouse conditions and methods including planting, spraying, and metabolic heat evolution rate measurement were the same as for the first experiment. The second experiment focused mainly on discriminating between the 'R' and 'S' biotypes since the time after spraying included only the 1 day sampling period. However, for this experiment, 7 to 10 centimeter-long plant segments were excised from the plants 1 hour after spraying. This delay allowed the herbicide to dry before immersing the base of the samples in the deionized water. All samples were kept

with their bases in water under the same greenhouse conditions for 1 day before microcalorimetric measurement. Samples of *A. powellii* consisted of only the top 10 centimeters of shoot, while *A. fatua* and *L. serriola* samples included the base of the stem from the soil level in order to include the meristematic tissue, the tissue most responsive for heat evolution rate measurement. The major reason for excising the samples from the plants in the manner described was to develop a technique more applicable to field use.

Field studies. For validation of the methodology tested in experiments 1 and 2, only atrazine resistant *Amaranthus powellii* was used. This species was chosen because it exists in the Willamette Valley and this rapid method was assumed to be broadly applicable for all herbicide modes of action. Both suspected 'R' and 'S' biotypes were randomly selected in corn fields in the Willamette Valley. Specimens of each biotype were sprayed with atrazine (454.6 g ai L^{-1}) at the rate of 1136.4 g ai ha^{-1} (13 liters of water). A shoot tip (about 10 to 15 centimeter long) from each plant was excised and the base of the sample immersed in water and kept under room conditions for 1 day before sampling for metabolic heat evolution measurement. Each sprayed (and sampled) plant was evaluated after 2 weeks in order to determine its response to the herbicide. These were designated from observation as resistant or susceptible.

4. RESULTS AND DISCUSSION

Greenhouse studies. The results from experiment 1 showed that for all three species, the heat evolution rates of treated plants were significantly different for resistant (R) and susceptible (S) biotypes (Table 4.1, 4.2 and 4.3; Figure 4.1, 4.2 and 4.3). For all weed species, these differences were not always apparent on day 0 but were consistent 1 day after spraying (Figure 4.1, 4.2 and 4.3).

There were no significant differences in CO_2 evolution rates and heat-/CO_2 evolution rates between the two biotypes for all species. CO_2 evolution, a product of respiration, showed parallel heat evolution. CO_2 evolution rate as well as heat-/CO_2 evolution rate appeared to be inconsistent in all species and biotypes. This may be because the heat evolution rate was the first calorimetric measurement while CO_2 evolution rate as well as heat-/CO_2 evolution rate were determined from the second and third measurement. Each measurement took at least one hour to obtain the complete results. Meanwhile, the sample plant tissue was taken from the most vulnerable part of the plant. The equipment could not obtain accurate respiration measurements from the dying tissue. Any technique that can prolong the longevity of plant tissue should result in more accurate measurements. However, since the objective of this study is to develop a method for rapid detection of weed resistance to herbicides, the significant and direct measurement of differences between 'R' and 'S' biotypes of heat evolution rate would be a superior method. This study strongly suggested that the best time

Table 4.1. Heat evolution rate, CO_2 evolution rate and heat-/CO_2 evolution rate of pigweed (*Amaranthus powellii*) after being sprayed with atrazine at the rate of 1136.4 g ai ha^{-1}

Treatment	Heat evolution rate			CO_2 evolution rate			Heat-/CO_2 evolution rate		
	Day			Day			Day		
	0	1	2	0	1	2	0	1	2
	μW/mg			pmole/sec/mg			KJ/mole		
R[b]Control	16.8 a[a]	16.4 b	15.5 b	44.7 a	57.8 b	53.1 c	366 b	284 a	320 a
S[c]-Control	16.1 a	18.6 b	17.5 b	47.3 ab	61.3 b	60.5 c	324 b	290 a	335 a
R-Treated	17.1 a	17.2 b	15.9 b	50.8 ab	56.1 b	44.5 b	315 b	297 a	419 b
S-Treated	14.5 a	10.0 a	8.8 a	60.8 b	34.0 a	29.0 a	229 a	300 a	340 a

[a]Means within the columns followed by the same letter are not significantly different by LSMEANS at the 0.05 level of significance.

[b]R: Resistant biotype, [c]S: susceptible biotype.

Table 4.2. Heat evolution rate, CO_2 evolution rate, heat-/CO_2 evolution rate of wild oat (*Avena fatua*) after being sprayed with diclofop-methyl at the rate of 1136.4 g ai ha^{-1}

Treatment	Heat evolution rate			CO_2 evolution rate			Heat-/CO_2 evolution rate		
	Day			Day			Day		
	0	1	2	0	1	2	0	1	2
	μW/mg			pmole/sec/mg			KJ/mole		
R[b]-Control	22.4 a[a]	28.5 c	22.6 b	46.9 a	51.5 a	38.2 a	435 b	493 b	568 b
S[c]-Control	24.5 a	22.4 b	15.1 a	66.6 a	42.1 a	27.4 a	392 a	485 b	497 b
R-Treated	24.3 a	20.6 b	21.6 b	47.7 a	31.5 a	41.9 a	470 b	603 b	541 b
S-Treated	20.1 a	12.1 a	11.6 a	58.6 a	36.8 a	35.2 a	314 a	305 a	300 a

[a]Means within the columns followed by the same letter are not significantly different by LSMEANS at the 0.05 level of significance.

[b]R: Resistant biotype, [c]S: susceptible biotype.

Table 4.3. Heat evolution rate, CO_2 evolution rate and heat-/CO_2 evolution rate of prickly lettuce (*Lactuca serriola*) after being sprayed with metsulfuron-methyl at the rate of 4.2 g ai ha^{-1}

Treatment	Heat evolution rate			CO_2 evolution rate			Heat-/CO_2 evolution rate		
	Day			Day			Day		
	0	1	2	0	1	2	0	1	2
	μW/mg			pmole/sec/mg			KJ/mole		
R[b]-Control	20.4 b[a]	19.0 bc	24.5 c	41.4 a	51.3 a	58.3 b	514 b	375 ab	389 a
S[c]-Control	15.8 b	15.6 ab	14.8 a	35.2 a	35.2 a	35.6 a	418 ac	328 ab	372 a
R-Treated	19.1 b	19.8 c	20.3 b	38.9 a	38.9 a	40.2 a	458 bc	478 b	453 a
S-Treated	12.3 a	12.7 a	13.8 a	36.2 a	36.2 a	35.9 a	293 a	269 a	337 a

[a]Means within the columns followed by the same letter are not significantly different by LSMEANS at the 0.05 level of significance.

[b]R: Resistant biotype, [c]S: Susceptible biotype.

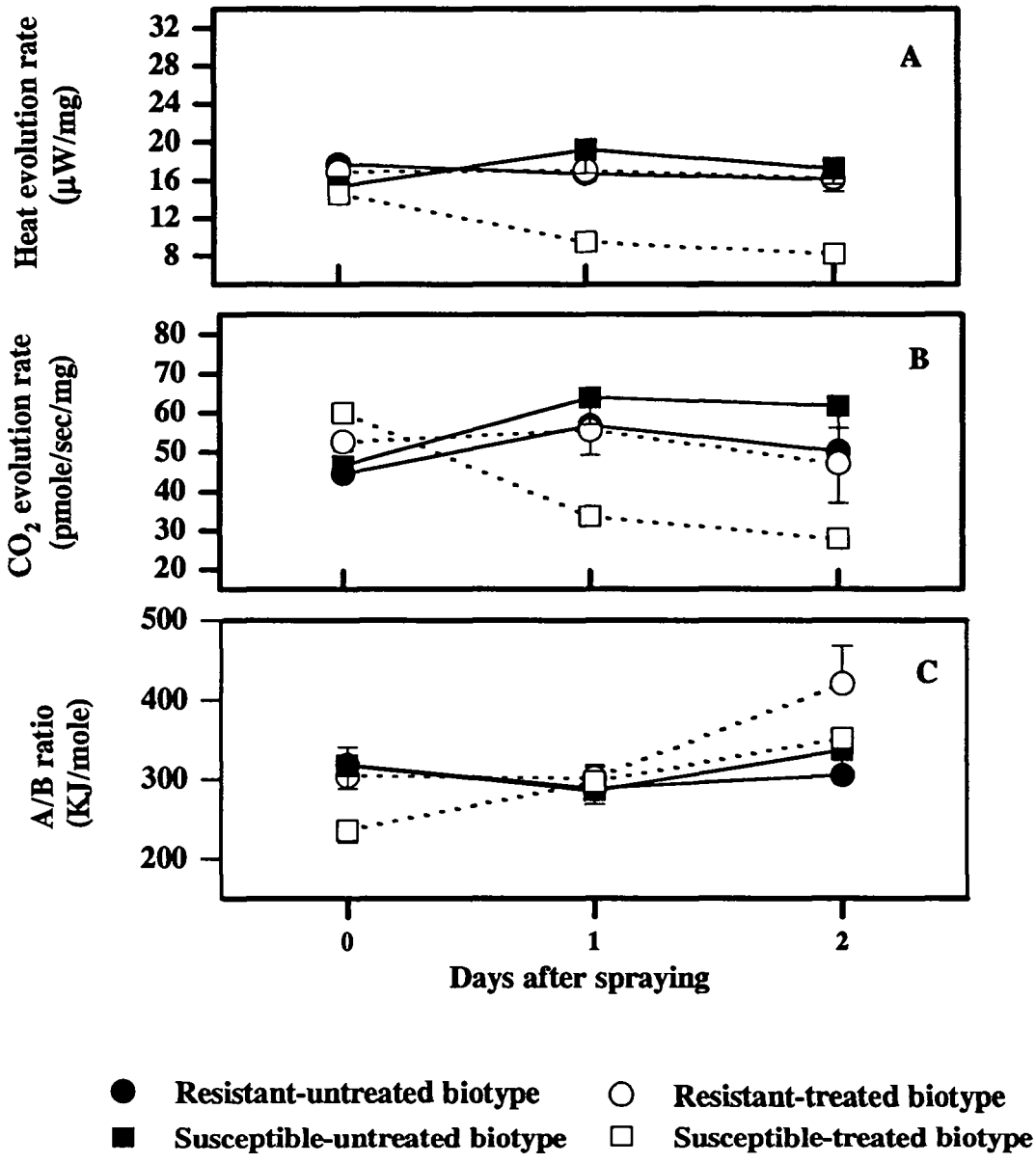

Figure 4.1. Heat evolution rate (a), CO_2 evolution rate (b) and heat-/CO_2 evolution rate (c) of *Amaranthus powellii* untreated or treated with atrazine at the rate of 1136.4 g ai ha^{-1}, vertical lines represent standard error of the mean

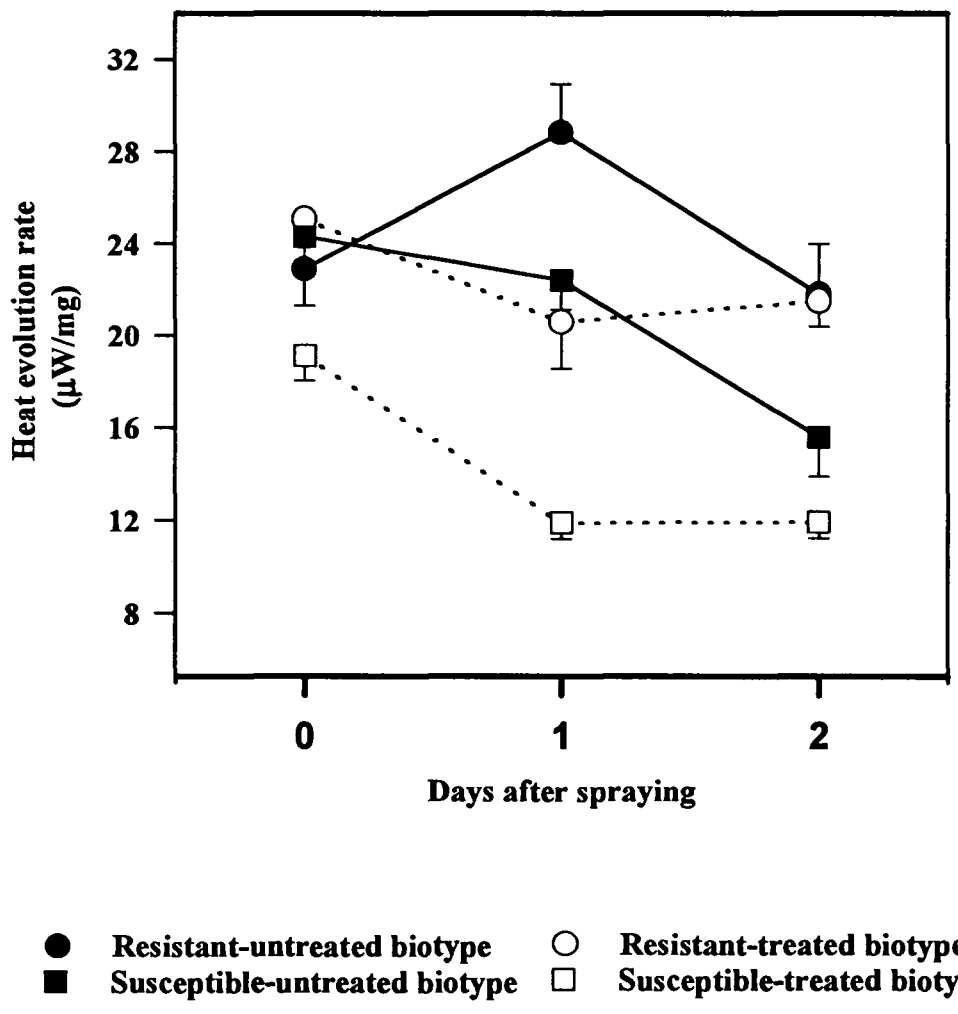

Figure 4.2. Heat evolution rate of *Avena fatua* untreated or treated with diclofop-methyl at the rate of 1136.4 g ai ha^{-1}, vertical lines represent standard error of the mean

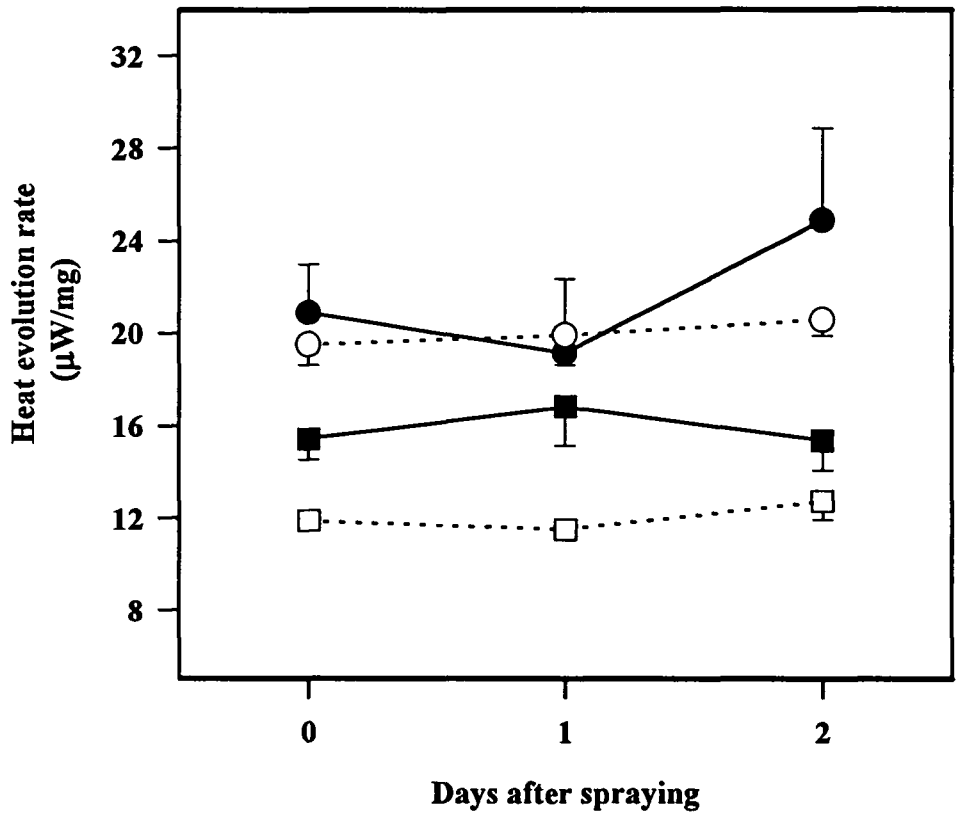

Figure 4.3. Heat evolution rate of *Lactuca serriola* untreated or treated with metsulfuron-methyl at the rate of 4.2 g ai ha^{-1}, vertical lines represent standard error of the mean

for microcalorimetric determination of herbicide resistance was the measurement taken one day after spraying the herbicide.

The results from the first experiment clearly demonstrated that microcalorimetry has a potential for rapid detection. However, in order to be more applicable in real field situations, the second experiment was carried out to improve the technique by removing only a portion of the treated plant to see whether or not the heat evolution rate from the excised sample was still consistent. The results from experiment 2 strongly suggested that heat evolution rate in all three weed species was not affected by removing the sample tissue from the treated plant one day prior to measurement (Figure 4.4 and Table 4 4).

Table 4.4. Heat evolution rate of pigweed (*Amaranthus powellii*) untreated or treated with atrazine at the rate of 1136.4 g ai ha^{-1}, wild oat (*Avena fatua*) untreated or treated with diclofop-methyl at the rate of 1136.4 g ai ha^{-1} and prickly lettuce (*Lactuca serriola*) untreated or treated with metsulfuron-methyl at the rate of 4.2 g ai ha^{-1}, 1 day after being sprayed.

Treatment	Heat evolution rate		
	A. powellii	*A. fatua*	*L. serriola*
		μW/mg	
R[b]-Control	13.6 b[a]	27.3 b	27.9 b
S[c]-Control	14.7 b	25.6 b	27.4 b
R-Treated	16.9 c	26.3 b	27.9 b
S-Treated	11.2 a	17.8 a	19.7 a

[a]Means within the columns followed by the same letter are not significantly different by LSMEANS at the 0.05 level of significance.
[b]R: Resistant biotype.
[c]S: Susceptible biotype.

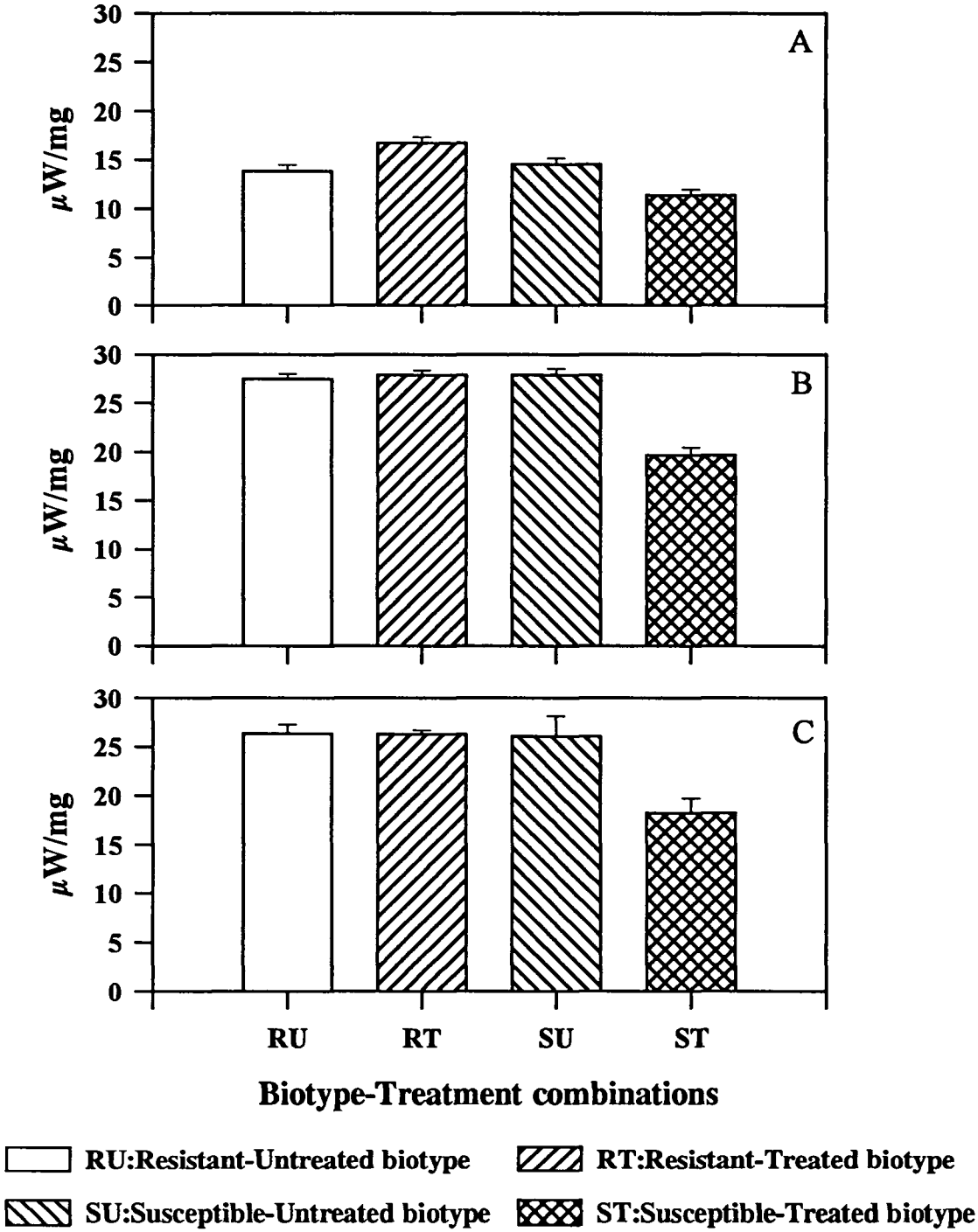

Figure 4.4. Heat evolution rate of *Amaranthus powellii* untreated or treated with atrazine at the rate of 1136.4 g ai ha^{-1} (a), *Avena fatua* untreated or treated with diclofop-methyl at the rate of 1136.4 g ai ha^{-1} (b), *Lactuca serriola* untreated or treated with metsulfuron-methyl at the rate of 4.2 g ai ha^{-1} (c), vertical lines represent standard error of the mean

Results from the first two experiments clearly suggested that microcalorimetry is feasible as a rapid detector of herbicide resistance. However, validation of the method in field situations, where weeds were naturally established, was necessary to insure that the new method is practical. Four sites of suspected resistant and susceptible pigweed (*Amaranthus powellii*) were randomly sampled for the test. All four sites with different stages of development of pigweed (*A.powellii*) showed significant differences in heat evolution between the 'R' and 'S' biotypes. In this experiment the calorimetric measurements were validated by comparing the results of heat evolution rate with the conventional method (delayed observation) as a check. The results from all four sites exhibited the significant difference of heat evolution rate between the two biotypes regardless of differences in sites or plant stages (Table 4.5).

Table 4.5. Heat evolution rate of pigweed (*Amaranthus powellii*) 1 day after being sprayed with atrazine at the rate of 1136.4 g ai ha^{-1}

Site	Biotype	Heat evolution rate	
		Treated	Untreated
		____ μW/mg ___	
1.	Susceptible	12.1 a[a]	17.7 a
2.	Susceptible	11.8 a	19.3 b
3.	Susceptible	11.2 a	16.1 a
4.	Resistant	16.2 b	16.1 a

[a]All means followed by the same letter are not significantly different by LSMEANS at the 0.05 level of significance. All mean comparisons within the table are valid.

In conclusion, microcalorimetry is a very promising method for rapid detection of herbicide resistance. It is applicable to field situations and is easy for extension persons as well as growers to collect the samples. However, the study reported here needs further development in order to be certain that it applies to all weed species and herbicide modes of action. Even though this study represented three weed species treated with herbicides with different modes of action, modifications may be needed if it to be used with other weed species and herbicides.

CHAPTER V

GENERAL CONCLUSIONS: COMBINING PATTERN-THINKING BY AGRICULTURAL CONSULTANTS AND RAPID MICROCALORIMETRIC ASSAY

The recognition of a "limits to growth" pattern encouraged field representatives to acknowledge patterns of weed resistance development at different stages. Thus, individuals could identify weed resistance before it became a problem. This pattern-thinking was based on individuals' experience and provided an understanding of the potential for the development of herbicide resistance in a weed population.

A rapid detection of weed resistance by microcalorimetry was developed (Chapter 4) as a general method to differentiate between resistant and susceptible weeds within one or two days. It was tested with three herbicides; atrazine, metsulfuron-methyl and diclofop-methyl, representing three modes of action; photosynthetic inhibitor, ALS/AHAS and ACCase inhibitor. The procedure is simple and sample preparation and collection could be handled by field representatives as well as growers. In addition, the results are obtained quickly and are accurate. However, the samples should be taken as early as possible in order to prevent a serious infestation of resistant weeds.

The rapid assay was introduced to respondents to explore the possibility of weed resistance management in the future. All field representatives indicated that it is necessary to combine the two processes, the thinking process and development of

microcalorimetric assay, to accomplish a successful weed control strategy. Hence, one provided insight, and the other provided supporting evidence.

The results from this study showed that processor field representatives recognized that detection of weed resistance in the field must occur just as the first biotypes appear. They all believed that a new rapid microcalorimetry method for detection of herbicide resistant weeds could enhance their weed management practices. They suggested that a reasonable cost for the test should be comparable to a soil test or between $50 to $200 per farm. They looked forward to the availability of the new test. They realized that recognition of the weed population growth pattern could encourage them to predict the potential weed resistance problem. Hence, results from the microcalorimetric rapid bioassay would validate this pattern-thinking.

It is important to combine pattern-thinking or the "limits to growth" pattern and the rapid microcalorimetric assay since doing so can lead to early detection of weed resistance. Economically sound and environmentally safe weed control programs depend on early detection of herbicide resistant weeds and rapid intervention measures. Taking measures early to reduce the potential development of herbicide resistant weeds will provide long-term lower production costs. These steps should also reduce farmers' practice of using greater amounts of a herbicide as it begins to fail with development of resistant weed populations. Using less herbicide results in less environmental impact.

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