A STUDY OF THE USE OF DIVERSIONARY PLANTINGS TO REDUCE PESTICIDE-RELATED HONEY BEE (*Apis mellifera*) MORTALITY.

By

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ABSTRACT

A STUDY OF THE USE OF DIVERSIONARY PLANTINGS TO REDUCE PESTICIDE-RELATED HONEY BEE (*Apis mellifera*) MORTALITY.

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A new marking system was developed to identify foragers by hive of origin. Biotic and abiotic factors influencing attractiveness of three plant species to honey bees were studied to evaluate the potential for using diversionary plants to attract honey bees away from pesticide contaminated agricultural areas. A new method was developed for estimating total nectar carbohydrate in flowers.

Daylight fluorescent pigments were determined nontoxic and used in a marking devise where exiting foragers were marked as they left and entered the hive.

Rape and borage, as diversionary plants, were always significantly more attractive than clover. Caloric reward, handling time and weather had varying effects on attraction.

Clover, rape and borage had significant differences in available sugar. Differing flower structures needed to be altered to allow for differing diffusion rates.

to the newfound

knowledge of my Higher Self

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GENERAL THESIS INTRODUCTION

Insect pollinator mortality resulting from agricultural pesticides is a serious problem. Researchers and commercial beekeepers both admit pesticides cause substantial bee kill and pose a threat to the future of agriculture systems dependent upon commercial honey bee pollination. Both groups also admit most pesticide bee kills are inadvertent and result from urban and agricultural crop management practices. These practices can often result in unintentional pesticide coverage of non-target flowering plants through direct spray or drift. Honey bees, dependent upon these flowering plants for nectar and pollen, contact this pesticide while foraging. In Michigan fruit orchards, for example, pesticide drift from cover sprays often contaminates blooming clover on the orchard floor where honey bees forage. Under these circumstances, the pesticide either kills foragers in the field or worse, is taken back to the hive. Once in the hive, it contaminates the entire colony by being introduced into the communal food supply. When this happens both adult workers and brood are poisoned thus destroying both present and future generations.

Plants on the orchard floor remain in bloom and attract bees for most of the summer, and thus hinder any attempts to develop spray programs intended to favor foraging bees. Alternatively, elimination of these blooming plants is impractical and expensive for the orchardist.

Plant species and their densities found in orchards vary considerably, therefore relative attractiveness also varies. These understory plants can become highly attractive in the absence of competing forage outside the orchard. With current urban and agricultural weed management strategies, competing forage has been greatly reduced often resulting in inadvertent bee kills in the orchard.

Integrated Pest Management (IPM) recommendations utilize several methods to alleviate inadvertent bee kills. These include callibration and timing of sprays to reduce the total amount of pesticides in the environment, night or early morning spray applications, management practices to reduce flowering plants on the orchard floor, and even bee repellents. These recommendations have not solved the problem of pesticide related bee kills. Current estimates report about 10% of U.S. colonies are killed and another 10% are weakened by insecticides (Ambrose 1983). This is probably a conservative estimate because many bee kills are not reported.

Because current solutions to the pesticide/honey bee conflict leave the problem unresolved, a design has been proposed using diversionary plantings as the key tool to eliminate pesticide related bee mortality (Ayers et al. 1984).

In Michigan, the orchard situation consists of a patchwork of small farms. Between orchards there is considerable space that is not productively managed except perhaps to reduce its nuisance value. These spaces include rights-of-way, fence rows, marshes, weedy strips along lanes, edges of woodlots and miscellaneous uncultivated areas. If these areas were to be planted with attractive bee forage, it seems reasonable that bees might be lured from potentially hazardous orchard systems to an outside "neutral" area.

The effectiveness of diversionary plants depends upon several factors. Since diversionary plants should occupy less space than the orchard floor flora, they must be more attractive than the flora within the orchard. Flowers should have a high quality and quantity of nectar per unit area to compensate for the small planting. The bloom period of the diversionary plants must coincide with the bloom period of the flora on the orchard floor, but not with the bloom period of the orchard itself. Diversionary plant species should be selected such that a continuum of bloom exists throughout the growing season. The plants should be maintenance free. It would, therefore, be advantageous if they were hardy, stress tolerant, possibly allelopathic, and either perennial or able to reseed themselves to dense stands. Finally it is imperative that they do not become problem weeds to the orchardist or society in general.

There would be many benefits to diversionary plantings.

1. Ultimately, diversionary plantings may provide a solution for reducing pesticide-related bee mortality in various agricultural situations.
2. For the beekeeper, diversionary plants would supply the extra high quality forage that current agricultural management practices have eliminated and thus, would provide

additional income for the beekeeper.

3. Diversionary plants could also serve as food and refuge for biological control organisms.
4. Diversionary plantings might reduce the orchardist's cost of managing of the waste spaces in which the diversionary planting was established. At the same time this would create pockets of color and thus increase the aesthetic value of his land.
5. In theory, the concept could be expanded to include the establishment of preferred habitats for agricultural pests.

The goals of the research described in this thesis are to develop methods to quantify and predict the comparative attractiveness of potential diversionary plant species and certain orchard flora.

The first chapter describes a new method of mass marking honey bee populations for field observation that distinguishes foragers by hive of origin. The goal of this research was to create a way of quickly identifying the foraging response of individual hives to plant attractiveness. This is important since identifiable hives can be treated as replicates for statistical purposes.

The second chapter investigates the factors influencing competitive attractiveness between two different diversionary plant species and Dutch white clover. It also discusses the potential future use of diversionary planting as an IPM tool to decrease honey bee pesticide mortality.

The third chapter describes a new method for estimating total available nectar carbohydrate per floret. The goal of this research was to create a way to predict the total available nectar carbohydrate for any size or shape of flower with only a single nectar extraction.

While this research deals with specific diversionary plant species and examines only limited aspects of diversionary plantings, the writer feels it represents a part of the basic studies needed to form the groundwork for future investigations into the use of diversionary plantings as an IPM tool.

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LITERATURE REVIEW

Pesticide Impacts on Honey Bees

Little has changed since the early 1940's concerning the problem of pesticide related bee kills. Insecticides such as paris green, sulfur, calcium arsenate, malathion, micro-encapsulated and EC formulations of methyl parathion, methomyl, carbaryl, carbofuran and permethrin have all been carried back to the hive with pollen (Erickson 1983). In the past calcium arsenate and DDT were given the most attention as causal agents for bee kills (Berg 1983). Recently, carbaryl and methyl parathion (particularly the micro-encapsulated formulation) have received the most attention (Berg 1983).

Micro-encapsulated methyl parathion (Penncap-M®) has drawn the attention of researchers and the Environmental Protection Agency (EPA) over the last 10 years after significant bee kills were found associated with this chemical in several states. Research has shown that encapsulated pesticides can be highly toxic to honey bees, are indiscriminately collected as pollen and returned to the hive, are found in nearly 10% of all pollen storage cells of contaminated hives, and can cause colony death 14 months after the initial spray application (Burgett and Fisher 1977; Johansen and Kious 1978; Sonnett et al. 1978; Stoner et al. 1978). In 1976 the EPA amended the Penncap-M label which now states that the product is highly toxic to bees exposed to direct treatment or to residues on crops and weeds. The label further states that Penncap-M should not be applied directly to or allowed to drift onto floral sources including weeds (Lowell 1978).

There is evidence of systemic insecticides being ingested and transferred to the hive in the nectar of birdsfoot trefoil, field beans, rape, citrus, cotton, onion, alfalfa, mustard, fuschia, and nasturium (Petukhov 1967; Jaycox 1976).

Residues of pesticides in hives constitute a stress factor to bee colonies just as do temperature and humidity extremes, disease, and the lack of adequate forage. It is the combination of these stress factors that determines the severity of damage a particular pesticide will cause (Erickson 1983).

Although there has been appropriate focus on these stress factors, little attention has been paid to the lack of adequate forage and its relationship to pesticide related bee kills. One of the major production problems a beekeeper faces today is a decrease in bee forage resulting from the agricultural practice of clear culture. The growth of urban and agricultural weed-management strategies over the last 30 years has effectively reduced the amount of bee forage available (Ayers et al. 1984a).

Diversionary Plantings

Ayers et al. (1984a) suggest the use of diversionary plantings as an Integrated Pest Management (IPM) tool to help eliminate pesticide related bee mortality. They define diversionary plantings as plantings of attractive floral species that have been established for the purpose of diverting bees from some area in which their presence is undesirable. In their initial observations of diversionary plantings in association with Michigan orchards, they found that the diversionary plants always attracted more bees than the orchard even through the orchard had relatively high densities of forage (clover) (Ayers et al. 1984b). It was also observed that while diversionary plants were in bloom there was no problem due to insecticide-related bee mortality. Late in August, however, when the diversionary planting had ceased blooming, pesticide related mortality began to occur forcing the researchers to move their hives.

Reports of competitive attractiveness between different plant species are common. Butler (1945) reports honey bees visiting one species of "bee-plant" and neglecting a nearby species that was found to be attractive in other situations. He also noted that it is frequently observed that when bees are moved to an agricultural system for pollination they will often ignore the crop for nearby or understory weeds. Indeed, the flowers of no two neighboring species will be equally attractive, one can always be perceived as "best". This choice of a "best" forage species is related to the relative magnitude of reward that the bee receives from competing flora (Waddington and Heinrich 1981).

Although the honey bee has been found to out-compete natural pollinators for forage "patches" of highest productivity (Schaffer et al. 1978), Hocking's (1968) studies of insect-flower associations found there was competition among plants for pollinators rather than among pollinating insects for nectar. Levin and Anderson (1970) demonstrated that two species of simultaneously flowering plants competing for pollinator service cannot co-exist. Levin (1970) discussed how plants eliminate competition. Species may diverge and specialize for a subset of pollinators. Plants may evolve to self-pollination or they may diverge temporarily in their flowering period. In species that bloom during the day, variation in the time of day at which bee visitation occurs has been observed, suggesting a variation in the timing of nectar secretion (Meeuse 1961).

Honey Bee Nutrition

The basic dietary constituents of adult honey bees are nectar or honey and pollen (Dietz 1975). Honey, to which nectar is transformed, is a carbohydrate material in which 95 to 99.9 percent of the solids are sugars (White 1975). Thus, honey is basically a source of energy on which a honey bee can live for long periods of time. Pollen provides a natural source of protein, fat, vitamins, and minerals for bees (Dietz 1975). Brood rearing is not possible unless pollen or an appropriate source of protein and vitamins is available (Haydak and Dietz 1965).

Honey bees are unable to detect differences in the nutritional value of pollen. If bees are given the choice of collecting an attractive pollen, a nutritionally worthless substance or a pollen substitute, they will not neglect collecting the worthless substance any faster than the pollen substitute (Wahl 1966).

Von Frisch (1934) tested 34 carbohydrates and related compounds and found that honey bees considered only seven of them to be attractive even though they can utilize all of them in their metabolism. Five of these sugars are found in nectar (glucose, fructose and sucrose) or in honeydew (melezitose and maltose) (Dietz 1975).

Foraging Dynamics of a Hive

A colony relies upon a small number of scout bees to find a new source of forage. These scouts justify the reward upon the amount of energy expended to obtain the nectar as opposed to the quality and quantity of nectar available. Communication concerning the level of reward is expressed through the level of excitement that is demonstrated during a food dance that is performed by the returning forager. The higher the level of reward, the more excited the dance and the greater the number of recruits. These returning recruits will also do a food dance recruiting additional foragers (von Frisch 1967). In the field, the recruit will orient to the sun to locate the general area in which the forage species is to be found, and then searches in a random manner until nectar is found. It then ceases its random searching activity and forages from subsequent blossoms in a stereotypic manner (Weaver 1956).

Singh (1950) found the average foraging bee made 3-10 flights per day depending on the type of crop. Butler (1945) estimated the total time for a forager to collect a stomachful of nectar and return to the hive to be 30-60 minutes.

Plant Fidelity

Honey bee fidelity to plant type has been well documented. Minderhoud (1931) observed that bees return time after time, day after day, to an area of forage not more than 10 yards square. Buzzard (1936) showed with marked bees that the same bees returned to the individual plant upon which they were first observed. Each plant appeared to have its own population of bees which trespassed on to a neighboring bush of the same species only when the branches of the two bushes actually interlaced. Butler et al. (1943) observed that honey bees would fly 360 yards on a route that took them over or near a number of identical dishes of syrup to get to their preferred dish. Honey bees will, however, extend their field of activity when the end of a flowers' nectar-secreting period approaches. Bees may shift to another crop when the first crop is no longer profitable (Singh 1950).

Forager Response to Flowering

Honey bee foraging activity has been shown to be closely associated with the progression of flowering throughout the season. As the number of florets increases so does foraging activity (DeGrandi-Hoffman and Collison 1982). Free et al. (1960) observed more bee visits to a particular species if bees were introduced after the plants were in bloom as opposed to before.

Factors Influencing Foraging Behavior

Foraging by honey bees is a behavioral response stimulated by a number of factors (von Frisch 1967). Among these are the sugar type and content of the nectar, the amount of nectar available, the ease of collection, the shortness in flight distance (or energy expenditure), the floral fragrance, the nutritional requirements of the hive, the weather conditions and the time of day. There seems to be no quantitative selection for pollen, rather it seems to be simply a function of its availability (Gontarski 1954).

Nectar Sugar Composition Effects on Foraging

Wykes (1952b) surmised that the sugar composition of nectar may be a major factor influencing forager visits. It was found that equal volumes and concentrations of the different sugars found in nectar were not equally attractive to bees. Instead, consistent preferences were shown in descending order for: sucrose, glucose, maltose, fructose. Bees introduced to different sugar combinations showed high preference for sucrose-glucose-fructose solutions.

Nectar Sugar Concentration Effects on Foraging

Sugar concentration also influences the preference of the forager. Indeed, Butler et al. (1943) observed that many more bees were attracted to dishes containing syrup of higher concentrations than to those containing low concentrations. Von Frisch (1967) states that bees assess quality of reward on the basis of a combination of nectar volume and concentration. This relationship of attractiveness to nectar volume (or plant density) has been seen in controlled experiments as well as field observations (Pedersen 1953; DeGrandi-Hoffman and Collison 1982). The product of numbers of flowers and nectar per flower should be the principle determinant of attractiveness since it translates into energy profit for foragers (Robacker and Erickson 1984). Butler (1945) found that honey bees respond in greatest numbers to those flowers with the highest nectar concentration. He concluded that nectar concentration determines which plant will be preferred by the foraging population and that nectar abundance determines the proportion of the foraging population of a given colony that will work the plant. He also suggested that when two or more species of potential bee forage are in flower simultaneously the one with the most concentrated nectar will attract the greatest numbers of honey bees.

Foraging Energetics

Energy expenditure through flight is a measure of cost against which the benefits of nectar are weighted. Animals are viewed as having evolved behavioral patterns which maximize net energy intake (Pyke et al. 1977). Eckert (1933) observed that bees will fly up to 13.7 km to the nearest food source, and that they have a tendency to fly in only one or two major lines of flight, neglecting similar food sources in other directions. However, honey bees usually fly to nearby or neighboring flowers thus decreasing flight time (Waddington 1980). Earlier studies (Levin 1959) indicate that, in general, foragers work close to their hives. When dishes of sugar syrup of equal concentrations were offered at distances from 160 to 400 yards, Butler et al. (1943) observed that dishes closest to the hive always received more visitors than those farther away. Lee (1961) also found that honey bees forage in greater numbers close to the hive when food sources make it possible. Honey bees can communicate quantity and quality of food sources in relations to distance. Boch (1956) has shown that when food sources are equal, more foragers are recruited to a near source than to a distant one. Levin and Kerster (1969) suggest that flight costs are largely determined by plant spacing or density. However, at higher rewards the area within which a bee is willing to forage expands as the perceived distance to the nearest neighbor decreases (Waddington and Heinrich 1981).

Flower Characteristic Effects on Foraging Behavior

Sight and smell are of importance to the foraging bee in flower recognition and discrimination. Honey bees learn floral scents quickly and can discriminate between a great variety of them (von Frisch 1967). Furthermore, they can determine the precise location of the scent in the flowers, using it as a guide to the nectar (Lex 1954). When bees have been trained to a feeding station scented with a distinctive odor, such as peppermint, the liberation of this odor in the hive by any means is sufficient to stimulate many of the experienced foragers to visit the familiar source immediately (Wenner 1971). Bees also recognize and discriminate between flower-like forms (Kamil and Sargent 1981). Visual markers on flowers serve as "nectar guides" on many flowers (Waddington and Heinrich 1981). Robacker and Erickson (1984) suggest flower colour, intensity, size and openness may be involved in: 1)attracting bees initially to flowers, 2)energy costs affecting bees working flowers and 3)serving as indicators, to experienced bees, of the flower's potential reward value.

Hive Influences on Foraging Behavior

A honey bee colony's development depends primarily on the starting population, the queen's fecundity and the nutritional status of the hive. Population, brood and flight activity are positively correlated with each other and are assumed to be related to pollination efficacy (Waller et al.1984). Barker (1971) found that the depletion of pollen and honey reserves in the hive caused an increase in foraging activity.

Meterological Influences on Foraging Behavior

It is well known that wet or stormy weather often reduces the number of foragers in the field. A large cloud passing over the sun is sufficient to cause large numbers to return to the hive (Butler et al. 1943). Boch (1956) showed that unfavorable weather will stop distant foraging while the foraging of near nectar sources may continue. Those foragers that have become established on a more distant plant will not, in unfavorable weather, switch to plants closer to the hive. Instead, they will wait in the hive until the weather clears and then resume their distant foraging (Butler et al. 1943).

Collison (1976) demonstrated that the size of the foraging force was a function of temperature, solar radiation, relative humidity, and wind speed. Wratt (1968) correlated temperature with the number of foraging honey bees. Bee flight and nectar concentration are lowest when temperature and solar radiation are low and relative humidity is high (Vansell 1934; Collison 1976). Bees do not continue to work in a wind blowing much over 24 km/hr (Gary 1975).

Daily Foraging Behavior

Honey bees extend their foraging activity from the early morning to late afternoon (Schaffer et al. 1978). Collison (1976) found a normal distribution of bee visits from 9 am - 2 pm. Vansell (1934) found that sugar concentration and numbers of bees foraging were lowest in the morning. DeGrandi-Hoffman (1983) found that when morning and afternoon temperatures were equal morning bee counts regularly gave higher numbers than afternoon counts.

Plant Nectaries

Nectaries are glandular tissue that is specialized for the export of sugars (Shuel 1967). Wykes (1952a) found sucrose, glucose and fructose in the nectar of most of the species she examined. Percival (1961) defines three general patterns of sugar composition: a) dominant sucrose nectar (Sfg), b) nectar with equal amounts of sucrose, glucose, and fructose (SFG), and c) nectar with dominant fructose and glucose (sFG). Ribbands (1953) estimated that the flowers of most nectar plants secrete between 0.02 and 7.6 mg of sugar per day.

Factors Influencing Nectar Production

Heterogeneity in nectar production within a plant species population has been demonstrated (Carpenter 1976, DeGrandi-Hoffman and Collison 1982). In general, factors that affect plant growth also indirectly affect nectar production. These factors often are distributed as gradients across a field and can usually be attributed to weather and soil (Lee 1961). Weather interactions that affect nectar production include solar radiation through its effects upon photosynthesis, air temperature and rainfall which reduces stress with a concomitant increase in nectar production. Soil moisture, temperature, aeration and fertility all affect the plants' ability to produce nectar (Schuel 1967). Butler (1945) found nectar concentration changed by the hour and was dependant on relative humidity. Vansell (1934) suggested blossom structure also affected nectar concentration with open structures leading to a dilution by rain or dew.

Dutch white Clover

Dutch white clover (*Trifolium repens*) is one of the most common pasture plants in the eastern half of the United States (Robinson and Oertel 1975). Many orchards that are in mowed sod have high densities of white clover (Ayers et al. 1984b). The plant is highly attractive to bees, and bees are likely to forage the flowers to some extent wherever they are found (Atwood 1941). White clover heads consist of 50 to 250 (average of 100) florets. The calyx is only 3 mm long so the nectar is easily reached by most nectar-collecting bees (McGregor 1976). Percival (1961) states it has an SFG type of nectar. Zauralov and Yakovleva (1973) found it to contain 54.8% sucrose, 24.0% fructose, and 21.2% glucose. Clover produces a relatively constant nectar concentration from 7 A.M. to 5 P.M. (Butler 1945).

Rape

Rape (*Brassica napus*) is highly attractive to honey bees, providing both nectar and pollen (McGregor 1976). Rape produces enough nectar to be considered a better honey plant than white or red clover (Hammer 1966). Belozerova (1960) found that rape had 2.326 mg nectar per flower at the beginning of bloom, 1.950 mg during the peak, and 1.350 mg toward the end of the blooming period. He also noted that honey bees were the primary pollinator of rape. Although rape receives foragers all day long, Ayers et al. (1984b) found it to be most attractive in the afternoon. Percival (1961) states it has a sFG type of nectar. Zauralov and Yakovleva (1973) found it to contain 9.5% sucrose, 43.5% fructose, and 47.0% glucose.

Borage

Borage (*Borago officialis*) can be an important honey plant during the dry summer months from July through August (Hodges 1958). Borage has a long flat flowering curve and shows little difference in attractiveness over the course of the day. It always attracted more bees than clover (Ayers et al. 1984b). Percival (1961) states that it has a sFG type of nectar. Zauralov and Yakovleva (1973), however, found it to contain 46.7% sucrose, 18.0% fructose, and 35.4% glucose.

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I. A SIMPLE COLOR MARKING TECHNIQUE USING DAYLIGHT FLUORESCENT PIGMENTS FOR IDENTIFYING HONEY BEE FORAGERS BY HIVE OF ORIGIN IN THE FIELD.

ABSTRACT

A new marking system was developed for field identification of foragers by hive of origin. Three fluorescent pigments were tested for acute and chronic toxicity to adult honey bees. There were no significant differences in bee mortality between the colors and the control in either acute or chronic experiments. Three additional colors were tested for acute toxicity to adult honey bees. There were no significant differences in bee mortality between these colors and the control. A simple marking devise was designed and placed on hives such that exiting bees were automatically marked as they left the hive. Field observations showed all exiting foragers were marked and remained visible for the duration of a foraging trip. Pigments created no visible changes in bee behavior nor did it accumulate in the hive. Opportunities for research using this marking method are discussed.

INTRODUCTION

Marking of honey bees *(Apis mellifera)* for tracing foraging force movement and distribution is a prerequisite for many agricultural or ecological bee behavior studies. In particular, it may be necessary to mark all the bees from a single hive or hive group for studying flight or foraging behavior. Southwood (1978) and Smith (1972) have reviewed many of the mass marking techniques available.

Genetically marked bees have been used extensively (Taber 1954; Peer 1956; Levin 1959, 1960; Lee 1961), when large populations, as from one apiary need to be identified in the midst of other populations in the field.

Radio-isotopes have been successfully fed to a colony in syrup or honey resulting in quick and positive labeling of all members of the hive (Nixon and Ribbands 1952; Levin 1960; Lee 1961).

Departing foragers can be forced to pick up a fluorescent powder that is detectable by UV light as they leave the hive (Musgrove 1949; Johanssen 1959; Free et al.1960; Dhaliwal and Sharma 1972; Frankie 1973) or in the field.

Honey bees can be permanently marked through application of a lacquer based paint (McDonald and Levin 1965; Harris 1969).

Because these methods are time-consuming in their application and/or detection they are incapable of quickly providing easily distinguishable foraging forces coded for hive of origin for between-hive behavioral studies.

This procedure modifies the method of Dhaliwal and Sharma (1972) with the use of daylight fluorescent pigments allowing individual colonies to be quickly and easily marked, creating a number of distinctly colored foraging forces.

MATERIALS AND METHODS

Chronic and Acute Toxicity Testing

DayGlo A® fluorescent pigments were selected to mark bees for their visual characteristics and because each colour uses the same organic resin as its carrier. Red (Rocket Red®), blue (Horizon Blue®) and green (Signal Green®) were tested for acute and chronic toxicity to adult honey bees. Yellow (Saturn Yellow®), orange (Blaze Orange®) and magenta (Corona Magenta®) were used in a seperate acute toxicity study. Approximately 2,500 adult honey bees were shaken from a single colony for these studies. The bees were anesthetized with carbon dioxide and were evenly divided by volume among 16 one quart cubical plastic freezer containers with lids fitted with a 5.08 cm. circular screen. The 16 containers were divided into 4 treatments (1 control and 3 colours) of 4 replications. While anesthetized, bees in all colour treatments were thoroughly dusted with a mixture of 2 gm of pigment and 2 gm of soybean flour (acting as a carrier). This was done by simply adding the powders to the container and gently shaking to assure coverage of all bees. Controls were similarly dusted with 2 gm of soybean flour. Lids were attached and sugar water feeders were placed on the screens of the container tops.

Acute toxicity data were collected over 72 hours. Numbers of dead bees were recorded every 4 hours for the first

24 hours, and every 8 hours for the remaining 48 hours. After 72 hours, the total number of dead and living bees in each container was recorded. Mortality was recorded daily for an additional 12 days as an indication of chronic toxicity.

Field Experiments

The bee marking device used in this study was modified after a similar devise described by Dhaliwal and Sharma (1972). The color marking chamber was lengthened to eight inches to facilitate mass marking of larger, high traffic hives. It was fitted with a lid to exclude wind and moisture. A nylon screen (100 mesh per 2.54 cm) was substituted for the muslin gauze as the base of the color marking chamber. This prevented moisture from interfering with the distribution of pigment onto the bodies of exiting and entering bees.

In the field pigments were stored in a desiccator and markers were kept in plastic bags to prevent absorption of moisture. Hives were set off the ground on empty hive bodies to prevent early morning dew from interfering with the markers efficiency .

With the hive marker in place a six inch horizontal landing board was added to the hive alleviating crowding at the hive entrance due to the addition of the marking device.

Markers were kept on the hives during daylight hours. Pigment levels were checked and the pigment stirred four times daily to ensure proper operation. Markers were placed on the hive one hour prior to the initiation of field observations to allow foragers already in the field time to return and be marked.

In the spring of 1985, markers were placed on 4 hives with red, green, blue and yellow pigments being used. Twenty random 5 minute observations were taken among the 4 hives noting any unmarked exiting foragers. These same 4 colours were used later in the summer, marking 4 hives during a research study where observations of any unmarked exiting foragers were again noted.

RESULTS AND DISCUSSION

As determined by average daily mortality, there were no significant acute or chronic effects of the red ,blue or green pigments (Table 1). Similarly, yellow, orange and magenta showed no significant acute toxicity effects (Table 2). These tests suggested that toxicity from any of the Dayglo® series A pigments tested was negligable.

Neither during twenty, random five-minute observations nor during any the ensuing three months of research using the markers were any unmarked bees observed exiting from the marker.

This method provided an efficient and easy to use marking system that had the following desirable attributes:

1) The system eliminated space, time and labor restrictions found in other marking systems.
2) This system created an effective, non-permanent method of marking bees that was adaptable to field use.
3) With the pigments tested it was possible to visually distinguish bees from six different hive or apiary populations in the field. Bees from more than six hives could be differentiated if genetic markers were also used.
4) All exiting foragers were automatically marked.
5) With a minumum amount of equipment, at any time of the day,

Table 1: Acute and chronic toxicity of red, blue and green fluorescent pigments to honey bees.

	Mean % Dead Bees Per Observation*	
Treatment	Acute	Chronic
Control	7.9 ± 1.8 a	5.7 ± 1.2 a
Red**	8.0 ± 2.4 a	6.6 ± 1.5 a
Blue**	8.0 ± 2.6 a	5.8 ± 1.6 a
Green**	7.8 ± 2.1 a	6.0 ± 1.4 a

* Means (± standard error) followed by the same letter within columns are not significantly different at the $P \leq 0.05$ level using Student-Newman-Keuls multiple comparison test (SAS Institute Inc. ed. 1985).

** Colors were Rocket Red®, Horizon Blue®, and Signal Green®.

Table 2: Mortality data for acute toxicity in adult honey bees from yellow, green and magenta fluorescent pigments.

	Mean % Dead Bees Per Observation*
Treatment	Acute Toxicity
Control	7.2 ± 0.7 a
Yellow**	6.2 ± 1.2 a
Orange**	6.0 ± 2.0 a
Magenta**	5.4 ± 1.0 a

* Means (± standard error) followed by the same letter within columns are not significantly different at the $P \leq 0.05$ level using Student-Newman-Keuls multiple comparison test (SAS Institute Inc. ed. 1985).

** Colors were Saturn Yellow®, Blaze Orange® and Corona Magenta®.

foraging populations were marked for field observation within a single hour.

6) Marked bees were easily distinguished without fluorescent lights and remained marked for the duration of at least one foraging trip.
7) The method is adaptable to different sized hives.
8) Marking of bees produced no visible change in hive or flight behavior.
9) Marking pigment did not accumulate in the hive or replace pollen in the food stores.

This marking method offers unique opportunities in pollination ecology studies. Inter-hive competition within or between apiaries can be easily examined. Dynamics of hive wandering and hive robbing within an apiary could be observed. Foragers marked this way could be used to study the dynamics of forager preference and constancy to plant species in the field. Differences in honey bee foraging and competition relating to hive placement could be easily observed in the field.

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II. THE ATTRACTIVENESS TO HONEY BEES OF RAPE (*Brassica napus*) AND BORAGE (*Borago officialis*), SIMULATING DIVERSIONARY PLANTS, COMPARED TO THAT OF CLOVER (*Trifolium repens*).

ABSTRACT

A method was created to compare the attractiveness to honey bees of rape (*Brassica napus*) and borage (*Borago officialis*), simulating diversionary plants, to that of clover (*Trifolium repens*). During July of 1985, attraction of honey bees to clover and rape was monitored. Rape was found to attract significantly more bees than clover at all times. Regression analysis showed that caloric reward was the most important factor in determining attractiveness. Handling time, relative humidity, and air temperature each also had a significant but smaller influence on foraging response. In August of 1985, attraction of honey bees to clover and borage was monitored. Borage also attracted significantly more bees than clover at all times. Caloric reward was shown through regression analysis to have the most significant influence on forager response. Handling time was also significant, but of the abiotic factors, only wind speed was significant. The role of these and similarly attractive plants serving as diversionary plantings in reducing honey bee mortality related to pesticides is discussed.

INTRODUCTION

Utilization of a plant species' attractiveness to directly influence honey bee foraging behavior is of special interest in agricultural research. It has been widely shown that honey bee mortality resulting from agricultural pesticides is a serious problem (Burgett and Fischer 1977, Johansen and Kious 1978, Sonnet et al. 1978, Stoner et al. 1978, and Berg 1983). Recently Ayers et al. (1984a) discussed the physical and social factors responsible for pollinator mortality in Michigan fruit orchards. In response they proposed the establishment of highly attractive diversionary plantings to be used to lure bees away from contaminated flora on the orchard floor. They found that diversionary plants always attracted more bees than an orchard floor with reasonably high levels of clover (*Trifolium repens*) (Ayers et al. 1984b). Whereas these observations were satisfactory for the conditions in which they were described, they cannot be used to quantitatively determine the effects of various physical factors on attractiveness to honey bees.

Different plant factors, such as volume of nectar per flower, flower density, color, smell, shape and size have been shown to affect plant attractiveness to honey bees (Butler 1945; Pedersen 1953; von Frisch 1967; DeGrandi-Hoffman and Collison 1982; Robacker and Erickson 1984). Collison (1976) found that foraging activity was a function of temperature, solar radiation, relative humidity and wind speed.

The purpose of this paper is to examine the effects of these various biotic and abiotic factors as they influence the differential attractiveness to honey bees of two species simulating diversionary plantings (rape - *Brassica napus*, and borage - *Borago officialis*) and clover, simulating an orchard floor.

MATERIALS AND METHODS

All studies were done during the summer of 1985, near the Michigan State University Inland Lakes Research Ponds. This area is relatively isolated and free from high densities of natural stands of attractive plant species.

The research area consisted of two plantings. In the fall of 1984, approximately 1 acre of Dutch white clover was densely planted in 6 foot-wide rows (alternated with 8 foot-wide strips of non-floral vegetation) to simulate a Michigan fruit orchard floor.

The second planting, 300 meters away, consisted of two plant species, rape and borage, of approximately 1/2 acre each. These two species, simulating diversionary plantings, were chosen on the basis of their attractiveness to honey bees over the length of the day (Ayers et al. 1984b), their sequential and limited overlapping blooming periods (Hodges 1958) and their ability to bloom during their first year of growth (McGregor 1976).

During the spring of 1985, rape and borage were planted in alternating 4 foot-wide rows (with 2 foot-wide strips of non-floral vegetation between each row) such that rape would finish blooming before borage started. Interspersed strips of non-floral vegetation in all three plantings consisted largely of rye grass and other plants unattractive to bees.

Plant types were treated as treatments. Clover was compared to rape over Julian Dates (JD) 190-200 (7/9-7/19) after which the rape was mowed. Clover was compared to the newly emerging borage over the span of JD 221-248 (8/9-8/28). Within each treatment 20 sampling units were randomly selected in which observations were made throughout the study. Each observational unit in clover was five square meters. Observational units in the other two species were three square meters.

Within all observational units a stake was randomly placed representing the center of a one square meter subsampling unit. This area was used for censusing density of blooms daily. Since clover is a multi-floreted flower, the number of influorescences per square meter were recorded once per day. The average number of open mature florets per influorescence was measured from 30 random influorescences once per week. The product of these two yielded average florets per square meter on a daily basis. Rape and borage were tallied as florets per square meter per day.

Four Caucasion honey bee colonies were treated as replicates and were placed equidistant between plantings (approximately 150 meters from either planting area). Besides being genetically marked, each colony was individually marked using a new marking method (see Chapter I). Daylight fluorescent pigments red, green, blue and yellow were used to mark the hives. Exiting foragers were marked as they left the hive creating four distinct foraging forces that were distinguishable in the field.

Sampling was always carried out simultaneously on the two plant species being compared. Time of day was treated as a block effect for all types of observations. To adjust for photoperiodism one minute was added daily to the starting time of sampling (Nautical Almanac 1985).

To determine the comparative attractiveness between two treatments, timed samples were taken. One minute samples were taken on all 20 units at 900, 1100, 1300, and 1500 EDST (Eastern Daylight Savings Time) noting color and number of bees.

To determine the state of bloom throughout the day for each treatment one randomly chosen plant per sample unit was monitored at 1000 and 1400 EDST. Each plant was protected from nectar foragers by a muslin gauze cage set in place the evening before sampling. A single day-old floret was taken from each of the 20 units and deposited in a plastic vial with distilled water. The flowers were rinsed for 60 minutes after which they were shaken into the vial to remove excess nectar and rinsate. The rinsate was frozen for later analysis.

The percentage sugar compositions for the nectar of each species was determined using the high pressure liquid chromotography (HPLC) method of McLaughlin (1985). Five determinations per species were used to compute overall composition.

Total nectar sugar from the remainder of the samples was determined using Roberts (1979) spectrophotometric analysis based on standards with appropriate percent compositions as determined by the HPLC analyses.

To determine the handling time of each plant species, one honey bee from each unit (or surrounding area) was monitored at 1000 and 1400 EDST. Handling time was defined as the time during which the proboscis penetrated the floret.

Honey bee populations in each hive were estimated at the end of each study (JD 200 and JD 248) using the method of Burgett (1985).

Temperature and solar radiation were recorded on a Cambell Instrument CR-21 Micrologger®. Wind speed and relative humidity were obtained from the U.S. Weather Service located at Lansing City Airport.

RESULTS AND CONCLUSIONS

Laboratory analysis of nectar sugar content for each plant species was comparable to earlier reports (Table 3).

There was no significant difference between hives for foragers found per square meter regardless of population size. This was observed in clover and rape and in clover and borage (Table 4). It was interesting that in both studies that average number of foragers per hive did not necessarily correspond to the size of the hive population. This pattern could indicate that some foragers might have been in search of food outside the system. This could also explain the distribution of foragers found in the study between clover and borage. This study was conducted in the early fall when foragers, reacting to both the need to build their winter stores and to the scarcity of flowering plants, would have expanded their field of search for food sources. It is possible, therefore, that the two weaker hives, having fewer foraging scouts competed strongly for the resources closest to the hive. The two stronger hives having more foraging scouts, took advantage of their size in response to that competition, and recruited more foragers to areas farther away and outside the system.

Honey bee response by hive to rape (Table 5) or borage (Table 6) showed there was a pattern of foraging common to all hives as well as to bees coming from outside the system. The

Table 3: Percentage sugar compositions for clover, rape and borage.

Flower	Percival(1961)*			Zauralov & Yakovleva(1973)			1985 Data		
	amt(%)**			amt(%)**			amt(%)**		
	S	F	G	S	F	G	S	F	G
Clover	50	25	25	54.8	24.0	21.2	50.2	27.2	22.6
Rape	10	45	45	9.5	43.5	47.0	7.1	55.2	37.7
Borage	10	45	45	46.7	18.0	35.4	11.9	40.5	47.6

* Values for Percival's (1961) nectar amounts are my estimates based on her published nectar types.
** S = sucrose, F = fructose, G = glucose.

Table 4: Differences between hive populations and number of hive foragers found.

Hive	Field Study			
	Clover and Rape		Clover and Borage	
	Est. of Pop.*	Ave.no.bees /sq.m.**	Est. of Pop.*	Ave.no.bees /sq.m.**
Red	36,402	0.103 ± 0.058 a	49,572	0.037 ± 0.027 a
Green	24,551	0.037 ± 0.046 a	22,130	0.060 ± 0.032 a
Blue	13,175	0.027 ± 0.044 a	35,002	0.038 ± 0.026 a
Yellow	17,824	0.085 ± 0.050 a	15,268	0.050 ± 0.030 a

* Burgett (1985) method of estimating honey bee colony populations.
** Means (± standard error)followed by the same letter within columns are not significantly different at the $P \leq 0.05$ level using Duncan's multiple range test (SAS Institute Inc. ed. 1985).

Table 5: Percentages of bees found foraging by source and day and corresponding F value showing the relationship between plant type and bee response to clover and rape.

Bees	Plant*	Percentage of Bees							F value
		Day							
		190	191	193	197	198	200	mean±SE	
Red	C	5	9	11	13	18	4	10 ±0.93	34.38***
	R	95	91	89	87	82	96	90 ±0.93	
Green	C	21	30	27	26	29	5	23 ±1.25	10.27***
	R	79	70	73	74	71	95	77 ±1.25	
Blue	C	38	44	33	38	44	25	37 ±1.10	4.28***
	R	62	56	67	62	56	75	63 ±1.10	
Yellow	C	19	27	28	53	52	38	36 ±1.53	16.19***
	R	81	73	72	47	48	62	64 ±1.53	
Other	C	19	18	18	25	32	17	21 ±0.99	59.03***
	R	81	82	82	75	68	83	79 ±0.99	
Total	C	17	20	19	26	33	17	22 ±1.03	85.74***
	R	83	80	81	74	67	83	78 ±1.03	

* C = clover, R = rape.
*** Highly significant ($P \leq .0001$) with 1 degree of freedom.

Table 6: Percentages of bees found foraging by source and day and corresponding F value showing the relationship between plant type and bee response to clover and borage.

Bees	Plant*	220	221	224	226	228	231	233	235	240	Mean**	F value
		Percentage of Bees — Day										
Red	C	0	0	13	4	2	0	2	3	1	3±0.67	21.61***
	B	100	100	87	96	98	100	98	97	99	97±0.67	
Green	C	20	0	3	1	2	0	0	1	0	3±0.85	46.87***
	B	80	100	97	99	98	100	100	99	100	97±0.85	
Blue	C	0	100	56	58	30	16	17	19	4	33±1.89	7.88***
	B	0	0	44	42	70	84	83	81	96	67±1.89	
Yellow	C	29	50	26	16	15	5	10	5	2	17±1.30	20.78***
	B	71	50	74	90	85	95	90	95	98	83±1.30	
Other	C	66	70	57	43	46	14	27	13	4	38±1.64	8.94***
	B	34	30	43	57	54	86	73	87	96	62±1.64	
Total	C	57	64	35	27	23	7	13	7	2	26±1.57	77.37***
	B	43	36	65	73	77	93	87	93	98	74±1.57	

* C = clover, B = Borage.
** Mean ± standard error.
*** Highly significant ($P \leq .0001$) with 1 degree of freedom.

majority of bees were found on the diversionary plants in almost all cases, indicating this was a case of choice on the part of the bees.

Plant type yielded a significant differance in bee response in every comparison. In all cases, whether by hive (as distinguished by color), bees from outside the system, or by the total bees present there was a significantly greater response to each diversionary species than to clover. There appeared to be no direct relationship between mean number of foragers (Figures 1 and 2) and the mean number of flowers per square meter. Clover always had more flowers, but significantly fewer bees than rape or borage. However, after multiplying the mean number of flowers available, by the average sugar per flower, the resulting caloric reward per unit area showed a stronger relationship to foraging distribution (Figures 3 and 4). Borage and clover showed a distinct forager distribution in relation to available caloric reward that was not as obvious as in rape and clover. This could have been a result of the lack of influence of other factors, such as weather, on the ability of caloric reward to influence bee response in borage and clover. In rape and clover these same factors could have altered the influence of caloric reward to affect forager distribution in the pattern seen.

Table 7 shows the mean nectar handling time for both rape and borage was greater than that for clover. This, combined

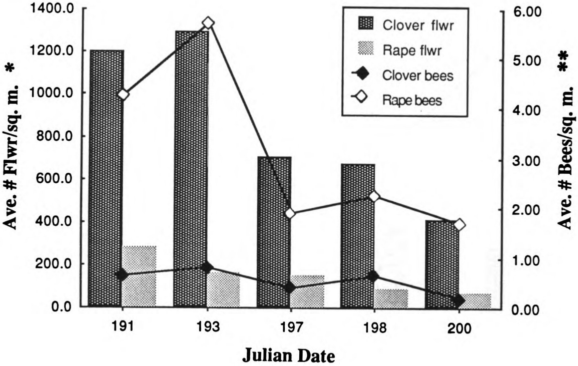

Figure 1. Total honey bee response to available florets in clover and rape. (see Table 5)

*Standard errors are less than 5% of means.
**Standard errors are less than 10% of means.

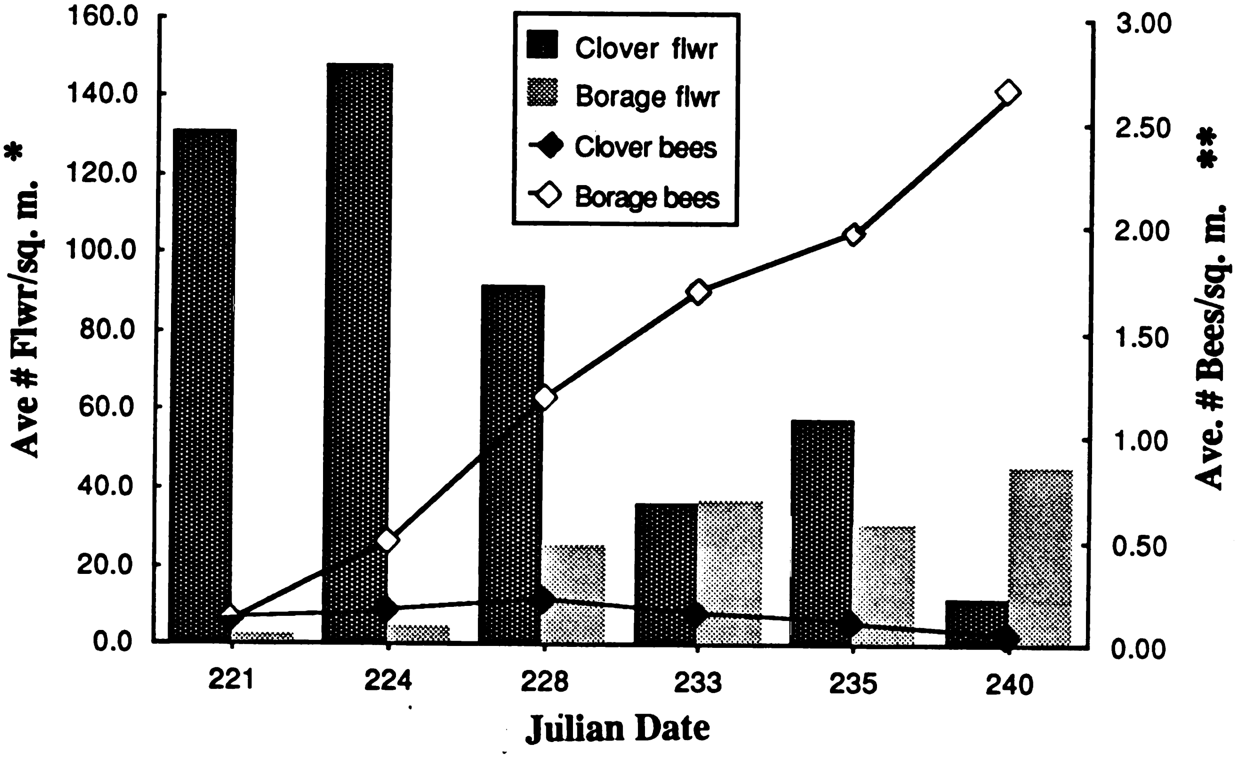

Figure 2. Total honey bee response to available florets in clover and borage. (see Table 6)

*Standard errors are less than 5% of means.
**Standard errors are less than 10% of means.

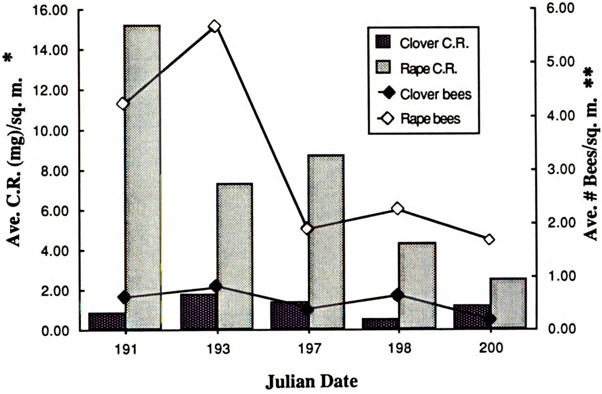

Figure 3. Total honey bee response to available caloric reward (C.R.) in clover and rape. (see Table 5)

*Standard errors are less than 5% of means.
**Standard errors are less than 10% of means.

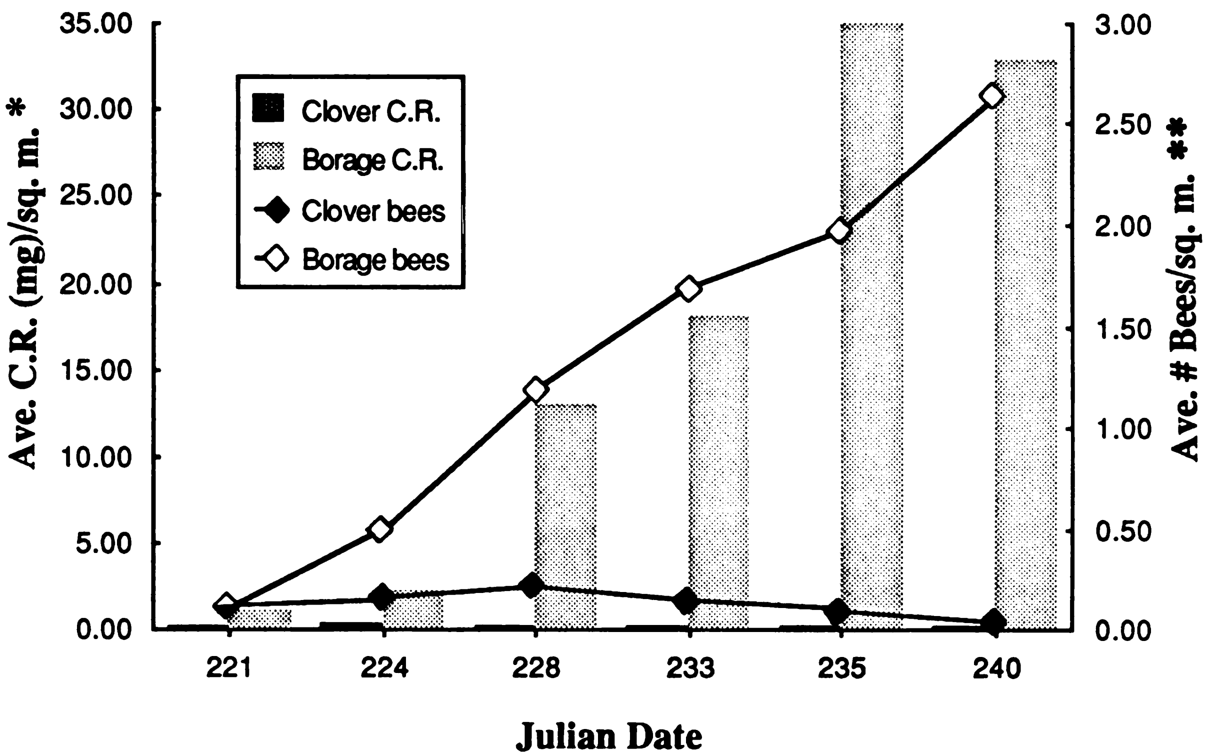

Figure 4. Total honey bee response to available caloric reward (C.R.) in clover and borage. (see Table 6)

*Standard errors are less than 5% of means.
**Standard errors are less than 10% of means.

Table 7: Average handling time for different plant species.

Flower	Day 190	191	193	197	198	200	mean*
clover	1.71	1.93	1.30	1.40	1.12	1.74	1.53 ± 0.23 a
rape	2.70	2.70	2.08	2.84	2.66	2.80	2.63 ± 0.22 b

Mean Handling Time (seconds)

Flower	Day 220	221	224	226	228	231	233	235	240	mean*
clover	1.58	1.44	1.36	1.04	0.88	1.32	1.13	1.28	0.93	1.22 ± 0.16a
borage	9.98	9.66	7.56	7.28	6.92	6.65	6.61	5.66	5.59	7.32 ± 0.42b

* Means (± standard error) followed by the same letter within columns are not significantly different at the P≤0.05 level using Duncan's multiple range test (SAS Institute Inc. ed. 1985).

with the data of honey bee foraging response, probably indicates that rape and borage flowers are not harder to work than clover, but rather offered more reward than clover.

It is interesting that borage showed a steady decrease in handling time, when both caloric content and foraging response were increasing (Figure 4). This is probably not the result of a decrease in nectar but rather of an increase in foraging competition.

Review of some of the independent factors involved in bee response to plant attractiveness revealed a complex picture. In all species there was an observable difference in nectar sugar content between morning and afternoon samples. (Figures 5-8). Morning nectar sugar contents were almost always less than or equal to afternoon contents. The amount of sugar in clover was always much smaller than in rape or borage so that the relative change was always smaller. The pattern, however, almost always matched that of rape or borage.

Some of the factors involved in partitioning of bees in response to clover and rape are demonstrated by a General Multivariate Linear Model Analysis of Variance (SAS Institute Inc 1985) (Table 8) and expressed in the resulting regression prediction equation (Table 9). Main plant factors - plant type, time of day, and plots nested within plant type, along with interaction plant factors - time of day, caloric reward, handling time, relative humidity and air temperature, all showed significant relationships to honey bee partitioning.

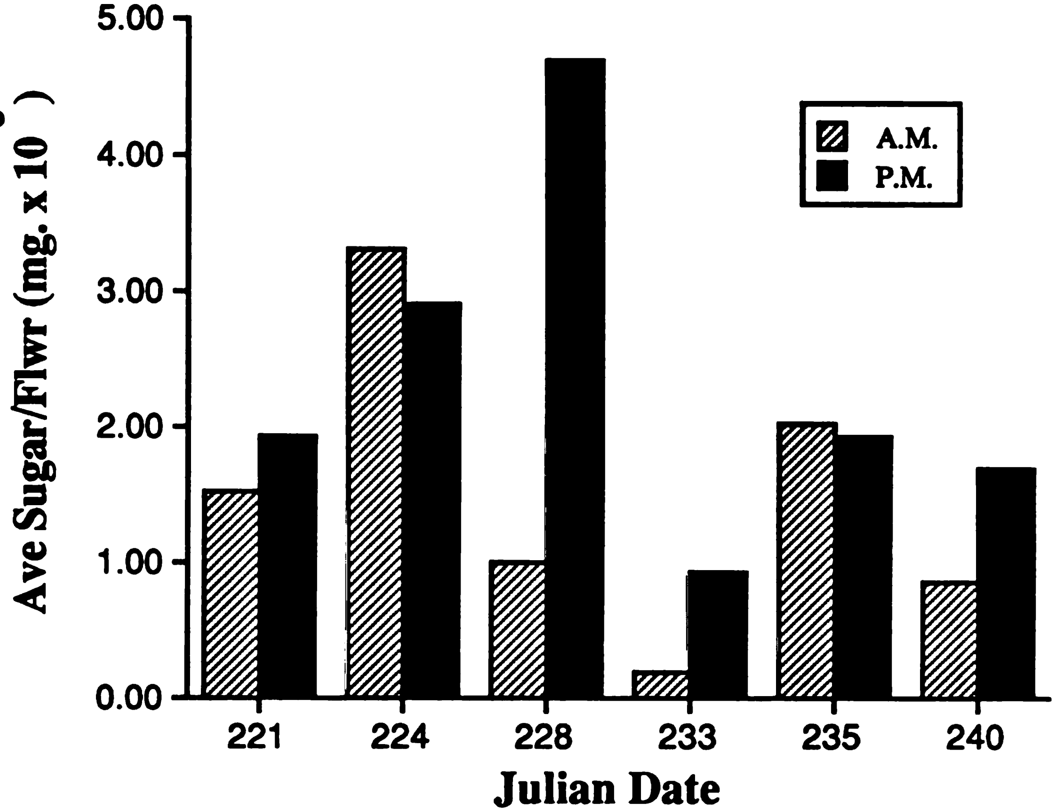

Figure 5. Differences between morning and afternoon nectar sugar contents per flower in clover during rape study.

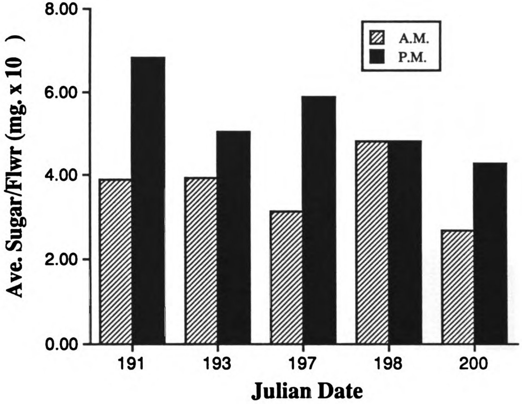

Figure 6. Differences between morning and afternoon nectar sugar contents per flower in rape.

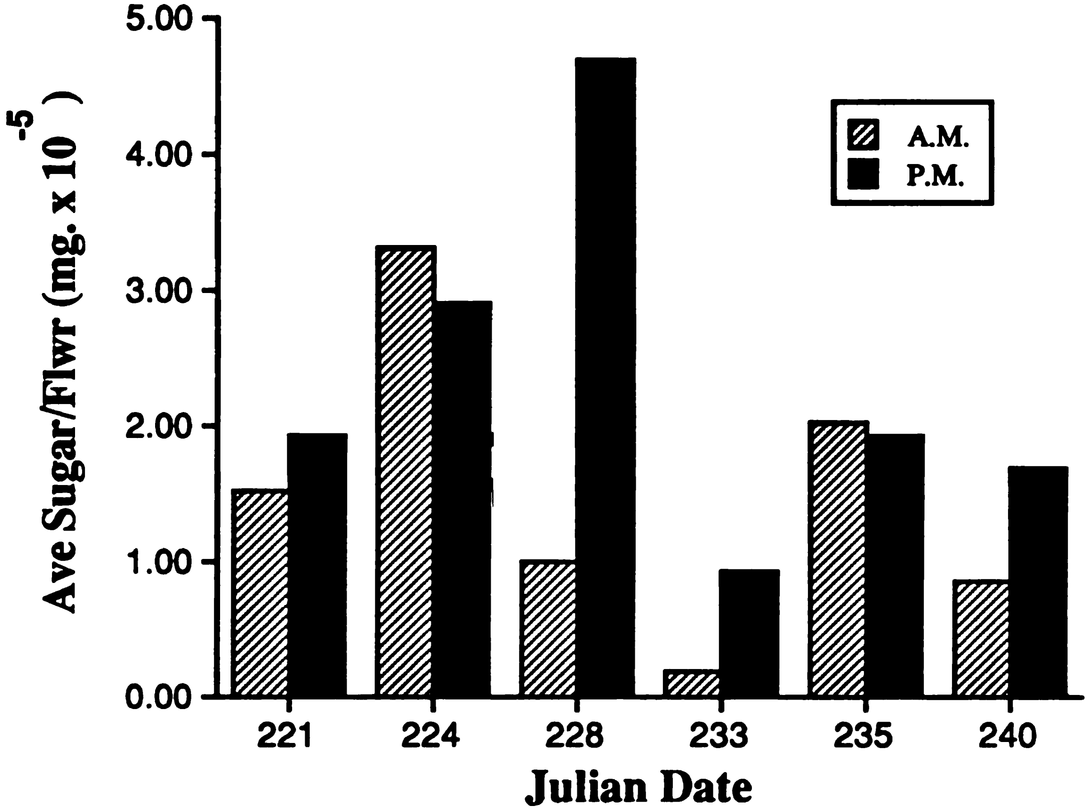

Figure 7. Differences between morning and afternoon nectar sugar contents per flower in clover during borage study.

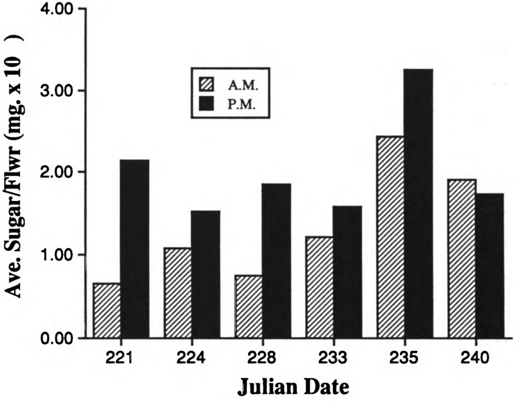

Figure 8. Differences between morning and afternoon nectar sugar contents per flower in borage.

Table 8: F values showing the regression relationships between various factors of plant attractiveness and honey bee partitioning in clover and rape.

Source of Variation	df	F value
<u>Main Plant Factors</u>		
Plant Type	1	1195.44***
Time of Day	3	32.66***
Plots within Plant Type	2	8.25***
<u>Interaction Plant Factors</u>		
Time of Day	3	10.17***
Caloric Reward	1	95.49***
Handling Time	1	72.56***
Solar Radiation	1	0.09
Relative Humidity	1	19.22***
Temperature	1	40.22***
Wind Speed	1	2.47

***Highly significant ($P \leq .0001$)

Table 9: The regression prediction equations by time of day for honey bee partitioning in clover and rape.*

B=Potential Bees A=Main Plant Factors C=Caloric Reward H=Handling Time S=Solar Radiation RH=Relative Humidity T=Temperature W=Wind Speed

The regression prediction equation for clover and rape is:

$$\mathrm{Log10}(B+1) = A_0 + A_1(\mathrm{Log10}(C+1)) + A_2(\mathrm{Log10}(H+1)) + A_3(\mathrm{Log10}(S+1)) + A_4(\mathrm{Log10}(RH+1)) + A_5(\mathrm{Log10}(T+1)) + A_6(\mathrm{Log10}(W+1))$$

where R=0.79:

Time of Day	A_0	
	Clover	Rape
900	-1.04	-8.50
1100	-1.04	-9.08
1300	-1.13	-9.37
1500	-1.12	-9.52

and where the estimates for coefficients are:

Interaction With Plant	Clover		Rape	
	A_n	±SE	A_n	±SE
C	57.31	23.62	-0.60	6.70
H	0.09	0.03	0.40	0.05
S	-0.02	0.01	0.03	0.02
RH	0.43	0.32	3.45	0.57
T	0.46	0.18	2.21	0.38
W	-0.16	0.11	0.42	0.23

*** Based on a single multivariate analysis.**

Only solar radiation and wind speed showed no significant relationship.

Table 9 gives the regression prediction equation for bee response to clover and rape by hour. The factors involved with bee response were multiplicative, therefore, to create a model, a log transformation was used to linearize the data. Since the assumption of homogeneity of variences under the General Linear Model was violated, a weighted analysis was performed. To avoid taking the log of 0.0, 1.0 was added to each of the variables in the equation.

Caloric reward by far had the highest regression coefficient, while relative humidity and temperature had the next highest regression coefficients in either plant species.

Some of the factors involved in honey bee response to clover and borage are demonstrated by a General Multivariate Linear Model Analysis of Variance (SAS Institute Inc 1985) (Table 10) and the resulting regression prediction equation (Table 11). Main plant factors - plant type, time of day, and plots nested within plant type, along with the interaction plant factors - time of day, caloric reward and handling time all showed a significant relationship to honey bee response. Of the abiotic (weather) factors measured, only wind speed showed a significant relationship.

Table 11 gives the regression prediction equation for bee response to clover and borage by hour. The factors involved with bee response were multiplicative, therefore, to create a

Table 10: F values showing the regression relationships between various factors of plant attractiveness and honey bee partitioning in clover and borage.

Source of Variation	df	F value
<u>Main Plant Factors</u>		
Plant Type	1	1164.90***
Time of Day	3	17.49***
Plots within Plant Type	2	5.44*
<u>Interaction Plant Factors</u>		
Time of Day	3	2.68**
Caloric Reward	1	184.09***
Handling Time	1	4.32*
Solar Radiation	1	1.44
Relative Humidity	1	0.18
Temperature	1	0.61
Wind Speed	1	3.60*

*** Highly significant ($P \leq .0001$)
** Highly significant ($P \leq .005$)
* Highly significant ($P \leq .05$)

Table 11: The regression prediction equations by time of day for honey bee partitioning in clover and borage.*

B=Potential Bees A=Main Plant Factors C=Caloric Reward H=Handling Time S=Solar Radiation RH=Relative Humidity T=Temperature W=Wind Speed

The regression prediction equation for clover and borage is:

$$\mathrm{Log10}(B+1) = A_0 + A_1(\mathrm{Log10}(C+1)) + A_2(\mathrm{Log10}(H+1)) + A_3(\mathrm{Log10}(S+1)) + A_4(\mathrm{Log10}(RH+1)) + A_5(\mathrm{Log10}(T+1)) + A_6(\mathrm{Log10}(W+1))$$

where R=0.74:

Time of Day	A_0	
	Clover	Borage
900	8.18	-0.60
1100	8.20	-0.53
1300	8.20	-0.54
1500	8.16	-0.51

and where the estimates for coefficients are:

Interaction With Plant	Clover		Borage	
	A_n	±SE	A_n	±SE
C	52.47	48.68	15.62	1.47
H	-0.001	0.008	0.03	0.02
S	0.004	0.006	-0.006	0.013
RH	-0.15	0.09	0.06	0.23
T	0.05	0.04	0.02	0.10
W	-0.08	0.07	0.36	0.15

* Based on a single multivariate analysis.

model, a log transformation was again used to linearize the data. Since the assumption of homogeneity of variences under the General Linear Model was violated, a weighted analysis was also performed. To avoid taking the log of 0.0, 1.0 was again added to each of the variables in the equation.

Caloric reward again demonstrated the highest regression coefficient in both plant species. Wind speed had the next highest regression coefficient in borage, but not in clover.

DISCUSSION

Rape and borage are highly effective diversionary plants in attracting honey bees away from clover. They always attracted more bees than clover. This agrees with observations of Ayers et al. (1984b). Close examination of independent variables responsible for honey bee foraging response gave some insight into criteria to use in choosing future diversionary species.

Von Frisch (1967) points out that foraging by honey bees is a behavior response stimulated by a number of factors. Among them are the sugar type and content of the nectar, the amount of nectar available, the ease of collection, the foraging distance (or energy expenditure), the floral fragrance which helps in locating the nectar source and in communicating this to other foragers, the nutritional requirements of the hive, the weather conditions and the time of day.

The fact that the nectars of rape and borage are high in fructose and glucose may have contributed to these species attracting greater numbers of bees than clover whose nectar is a balance of sucrose, fructose and glucose. This would agree with Wykes' (1952b) findings. She found that given equal volumes and concentrations of nectar, sugar composition was the major contributing factor influencing forager visits. She pointed out, however, that for a given nectar type, concentration plays the most important role in foraging behavior.

For each hive (color), as well as for bees arriving from outside the experimental area, there were always more bees on rape and borage than clover. Sugar analysis showed that the average nectar sugar content per flower was always greater in rape and borage than in clover. This agrees with Butler's (1945) findings. He observed that honey bees respond in greatest numbers on those flowers of plants with the highest nectar carbohydrate content.

Although attempts to study nectar volume were unsuccessful, DeGrandi-Hoffman (1981) essentially equated nectar volume with plant density. Since plant density, as well as the average nectar sugar contents per flower, were monitored throughout both studies, the total sugar or caloric reward available per given area was easily calculated as the product of the two.

In the analysis of honey bee response to clover and rape and to clover and borage, caloric reward had the highest significant F value among plant interaction variables. The regression coefficient for caloric reward in either of the prediction equations was also higher than those of any other independent variable. This demonstrates that caloric reward had the most influence on forager response. It appeared in each regression prediction equation that handling time and weather had little influence on attractiveness to foragers.

During the study of bee response to clover and rape, weather had little significant influence on attractiveness. This may have been a result of the small variability in weather factors experienced during this study.

During the study of forager response to clover and borage, weather again had little significant influence on attractiveness. Inspection of the data again revealed little variability in any meterological factors over the time the study was conducted. Only wind speed in borage demonstrated any significant influence and may have been a result of the vulnerability of these taller plants to wind.

A second reason that there was little weather influence on bee response may have been a result of energetics. Late in the summer when this study was conducted, there were fewer plants suitable for foraging at a time when the bees were storing reserves for winter. The energy costs of foraging in slightly adverse weather conditions may have been outweighed in the face of hive needs and the benefits of available borage and clover nectar. This could have effectively reduced any minor effects weather might have had under other circumstances.

The time to handle a flower was always greater in the diversionary species than in clover. This could simply be a response by foragers to the amount of nectar available. In a comparison of average sugar per floret to the corresponding handling times, both were found to be lowest in clover, higher in rape and highest in borage.

Both nectar sugar and numbers of foraging bees were generally lowest in the mornings and increased throughout the day. In the regression predictive equation for clover and borage the main plant factors were lowest in the morning and rose through the day. Here main plant factors directly reflect numbers of bees.

In the regression predictive equation for clover and rape, main plant factors were highest in the morning and decreased through the day. This would imply that numbers of bees decreased during the day. Observations, however, showed increasing numbers of bees through the day. It is possible, therefore, that the weather factors that were found to have a significant influence on bee response in the regression analysis were responsible for this.

It is easy to see from these studies that the predictive models for attractiveness would not be appropriate for predicting attractiveness at any other time and place. The window of variability for weather factors was too small to reflect the rapidly fluctuating weather patterns found in a state like Michigan.

However, under these types of conditions a pattern does exist. Caloric reward by far has the greatest effect on attracting foragers. Therefore to compete against natural flora, it will be important to develop diversionary plants that not only have a high density of flowers per unit area, but also a nectar with high sugar concentration.

Handling time might not be as much a function of plant structure as of total available reward. However, handling time offers a way to observe and enter the complex cost/benefit interactions of plant structure on attractiveness.

The lack of weather effect was surprising. Our expectations were that these abiotic factors would greatly influence bee behavior. They did not seem, however, to vary enough to affect the cost/benefit ratio influence on attractiveness.

It would not be prudent at this time to declare rape and borage the best diversionary plants possible. There are far too many good species of bee forage from which to choose to make that claim based on this study. As annuals their ability to competitively reseed and bloom in natural setting is highly suspect. Competitive perennials would seem to be more suitable for establishing permanent diversionary plantings.

These studies, however, have demonstrated some of the factors that need to be measured to predict plants' comparative attractiveness.

In light of the results of this study, future studies involving other factors would be interesting and appropriate and include:

1) Examination of plants with higher caloric rewards under different weather conditions.

2) Examination of the effects of distance of the plantings from the hives on attractiveness.

3) Examination of nectar type effects in plants with high caloric reward on attracting foragers.

4) Examination of bee flight energetics involved when the above factors fluctuate.

5) Examination of honey bee mortality in response to presence or absence of diversionary plants.

Not all of these are necessary to begin practical implementation of diversionary plantings to reduce pesticide related pollinator mortality. However, they would supply a wealth of input into formulating a long term IPM model to relieve this problem.

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III. A METHOD FOR APPROXIMATING TOTAL NECTAR SUGAR IN FLOWERS.

ABSTRACT

A new method was designed for determining the total quantity of sugar available from flowers. Nectar was washed from the flowers of three plant species and analyzed for total sugar content. After one hour there were significant differences in the amounts of sugar extracted from clover (*Trifolium repens*), rape (*Brassica napus*) and borage (*Borago officialus*). After the keel petal of clover, the fused petals of borage and the unfused petals of rape were physically removed from the flower, 4 hourly extractions of nectar sugar were again made and total sugar per species after 5 extractions was again significant. Clover, however, demonstrated a lower significant percentage of total nectar extracted in the first hour, than in the second hour after the keel petal was removed. Regression prediction equations for each species were developed to predict the total sugar extractable after 5 hourly extractions. Significance of the continued presence of sugar in all species over the 5 hour extraction period is discussed.

INTRODUCTION

Determining the total quantity of sugar available in the nectar of blossoms is a problem that is encountered in both agricultural and ecological research.. Foraging by honey bees is a behavioral response stimulated by a number of factors, but is primarily determined by quality of reward (a combination of nectar volume and concentration) (Butler 1944; Pedersen 1953; von Frisch 1967; DeGrandi-Hoffman and Collison 1982). Therefore, quantification of available sugar is a key to determining insect-flower interactions (Heinrich and Raven 1972).

Several methods have been developed for assaying the quantity of available nectar sugar. The most direct method is removing nectar by micropipette. Research has shown that this method is difficult and unreliable (Butler 1945). Other methods have since been developed: squeezing the flower (Nunez 1977), squeezing the gut of a returning forager (Gary and Lorenzen 1976), and simple rinsing of the floret (Dubois et al. 1956; Kapla 1978). None produce an accurate estimate of the total sugar available to a forager.

Roberts (1979) introduced a method that relied on extended rinsing of a flower that he reports gave a consistently accurate estimate of the available sugar in the nectar of American linden, *Tilia americana* (Roberts and Rajotte 1978), blueberry (Rajotte and Roberts 1979) and cranberry (Roberts 1979). Whereas this method was reported as very excellent for the studies in which it was used, it does not take into account the effect differing blossom structures have on the nectar extraction.

In our observations of flowers with narrow corollas, such as Dutch white clover (*Trifolium repens*), it appeared that sugar still remained in the flower after a 1 hour rinsing. However, there appeared to be no additional sugar present after a 1 hour rinsing in cup-like or relatively open flower species, such as rape (*Brassica napus*) or borage (*Borago officialis*).

The method described here investigates these observations and extends Roberts'(1979) method, taking into account the effect that differing blossom sizes and shapes have on rinsing nectar from them. This produced a dependable method of estimating the total available sugar in the floral nectaries of the three species used in these studies.

MATERIALS AND METHODS

Three plant species, Dutch white clover, rape and borage, were located on the campus of Michigan State University. These three species had been previously examined for comparative attractiveness (see Chapter II) and conveniently offered simultaneously blooming flowers of differing structure.

Optimum sampling size was determined with previous analytical data (see Chapter II) by the method of Karandinos (1976). Plant types were treated as treatments and replicated 5 times. Sampling occurred once per day (1000-1030 EDST) over a 3 day period. Eighteen hours prior to each sampling period, premature flowers from each species were covered with a guaze cage preventing early morning foragers from depleting nectar from the newly opened flowers.

A single one-day-old floret was placed in a polypropylene vial with a measured amount of distilled water. After soaking in the rinsate for one hour as described by Roberts (1979), the flower was removed and the rinsate frozen for later analysis. Since each species had a different flower structure, the keel petal of clover, the fused petals of borage and the unfused petals of rape were physically separated from each flower using forceps, thus exposing the nectaries while keeping structural changes relatively equal. The flower was then deposited in a second vial for 60 minutes with a measured amount of distilled water. At the end of the second hour the flower was transferred to a third vial. This procedure was continued for 2 additional hours with all vials of rinsate being frozen for later analysis. At the conclusion of each sampling period for each flower there were 5 vials where the first hour's vial had been extracted using the method of Roberts (1979).

All samples were analyzed for total carbohydrate spectrophotometrically (Roberts 1979). Total sugar for 5 hours, for each species, was determined with standards corresponding to the carbohydrate types of that species (see Chapter II).

Regression prediction equations were generated using a single multivariate General Linear Model to determine the total sugar for 5 hours of extraction from the first hour's extraction.

RESULTS

Mean sugar content of the first hour's sample was significant for all three species (Table 12). Mean total sugar (the sum of the 5 extractions) was also significant for all species.

Of the total sugar from 5 hours of extraction, rape and borage flowers respectively diffused 47.1 and 44.4 percent during the first hour's rinse (Table 12). Clover was significantly lower, diffusing only 18.0 percent during the first hour's rinse.

Comparative sugar ratios between species were substantially higher in the first hour's extraction, when compared to the end of five hours' extraction, after each species' nectaries had been exposed (Table 13).

The importance of blossom structure to the amount of sugar extracted using Roberts' (1979) method is evident from the data from the three species. Rape and borage, having relatively open floral structures, exhibited only slight changes in sugar content when their flowers were opened prior to the second hour of extraction (Figures 9 and 10). Clover, having a relatively closed floral structure, exhibited a dramatic increase in sugar content when its nectaries were exposed to the rinsate during the second hour's washing (Figure 11).

Figures 12a-12c show the regression prediction equations

Table 12: Apparent average sugar content in three plant species as affected by single and multiple extractions.

Plant Type	Sugar (mg) Extracted*		
	Mean Total		Mean %
	After 1 Hr	After 5 Hr**	After 1 Hr
Borage	48.41 ±1.56 a	102.78 ±8.38 a	47.10 ±2.64 a
Rape	14.67 ±1.72 b	33.01 ±3.79 b	44.43 ±4.02 a
Clover	1.23 ±0.50 c	7.01 ±0.56 c	18.02 ±1.44 b

*Means (± standard error) followed by the same letter within columns are not significantly different at the P=0.05 level using Duncan's multiple range test (SAS Institute Inc. ed. 1985).
** Petal structures were removed after the 1st hour's extraction to expose the nectaries.

Table 13: The effect of single and multiple extractions on comparative nectar sugar ratios between species.

Plant Comparison	Nectar Sugar Ratio	
	After 1 Hour	After 5 Hours
Clover vs Borage	1:38	1:15
Clover vs Rape	1:12	1:5
Rape vs Borage	1:3	1:3

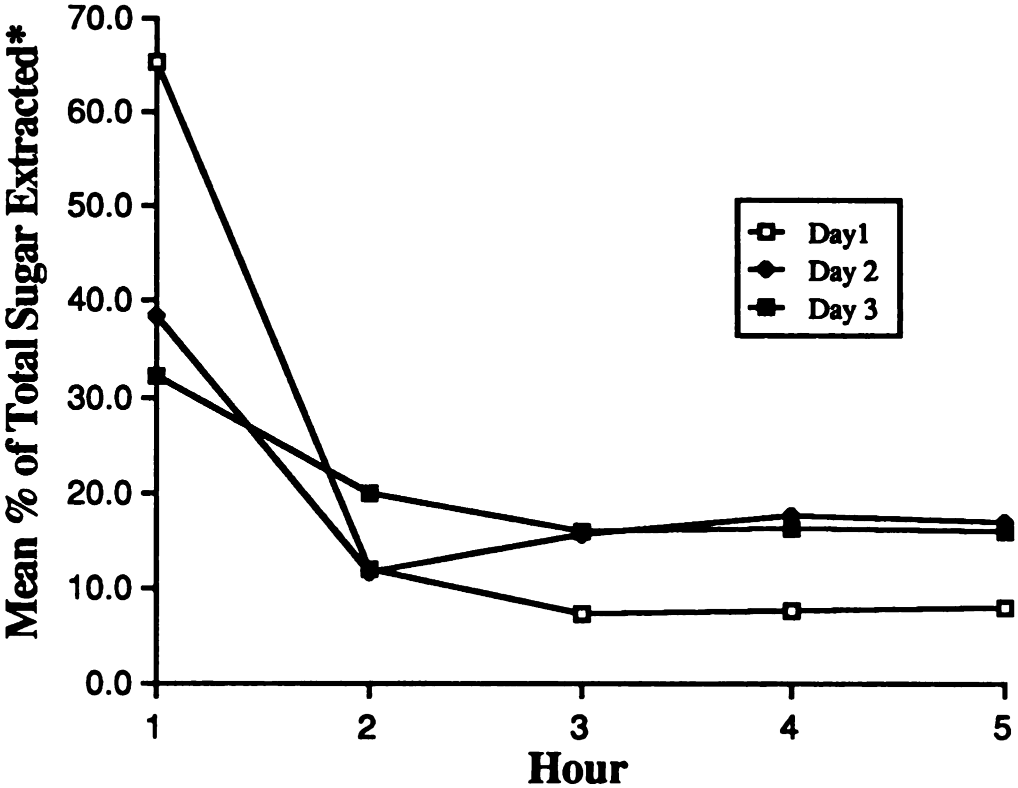

Figure 9: Hourly extraction percentages of total extracted sugar found in rape in 3 consecutive days. *Standard errors were less than 5% of the means.

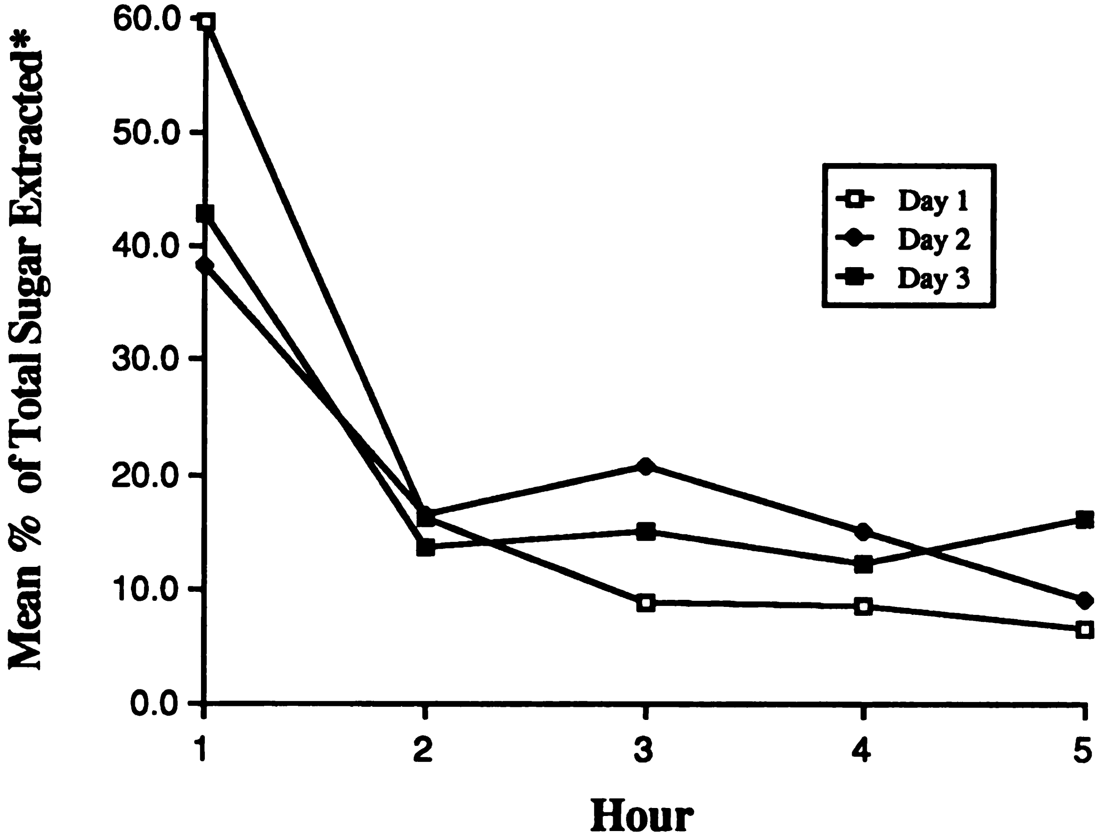

Figure 10: Hourly extraction percentages of total extracted sugar found in borage in 3 consecutive days. *Standard errors were less than 5% of the means.

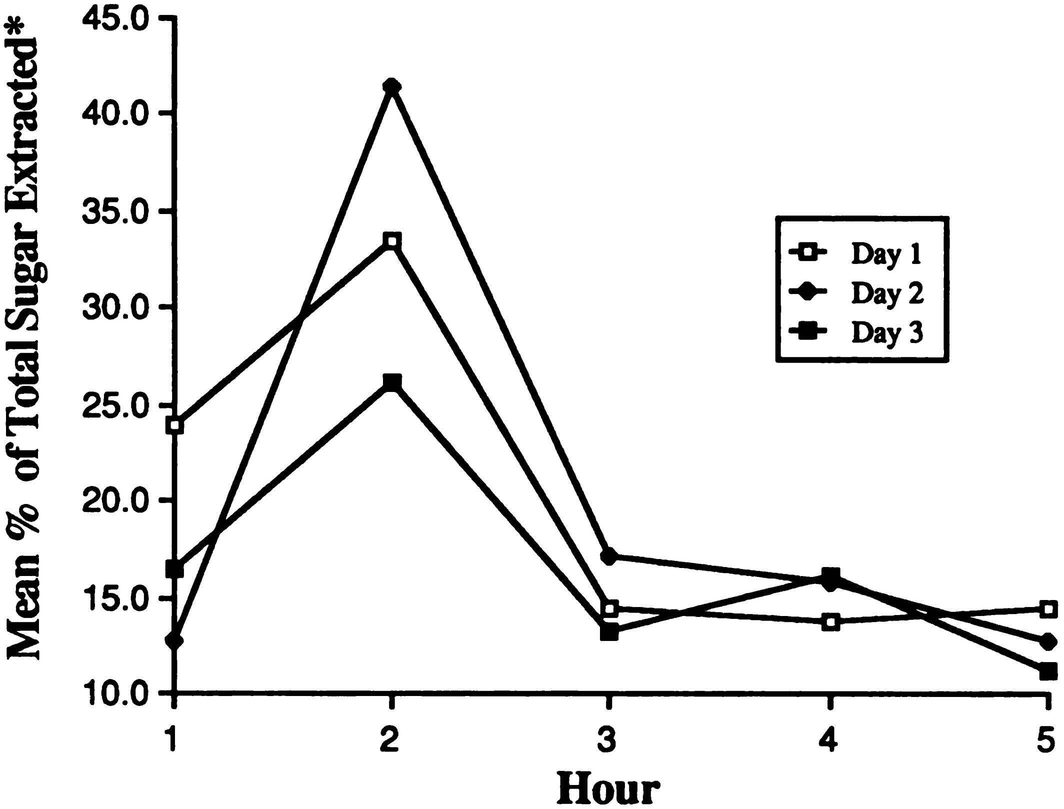

Figure 11: Hourly extraction percentages of total extracted sugar found in clover in 3 consecutive days.

*Standard errors were less than 5% of the means.

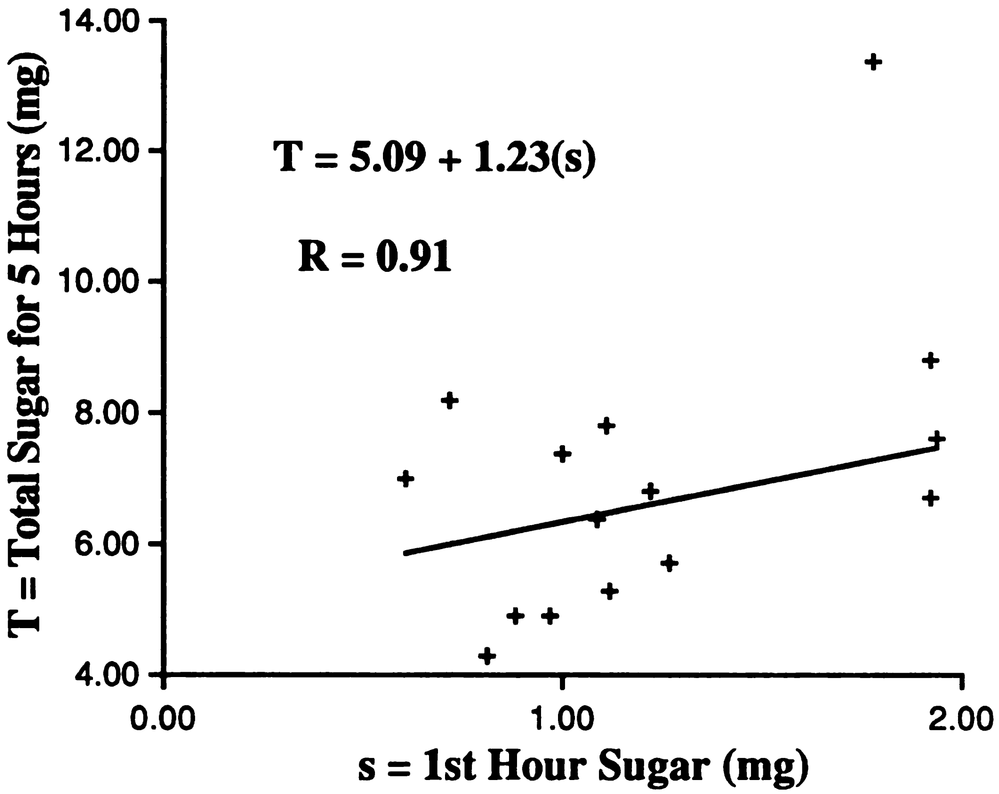

Figure 12a: Prediction equation* for total sugar for 5 hours in clover based on 1st hour sugar.
*Based on a single multivariate analysis.

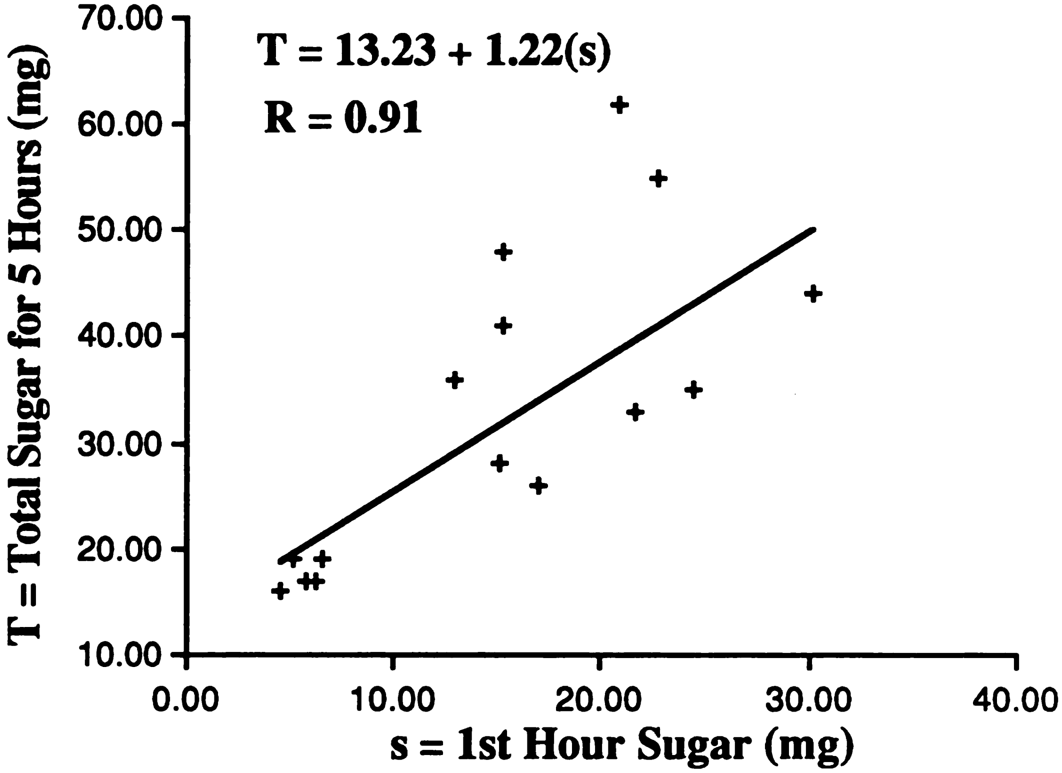

Figure 12b: Prediction equation* for total sugar for 5 hours in rape based on 1st hour sugar.
*Based on a single multivariate analysis.

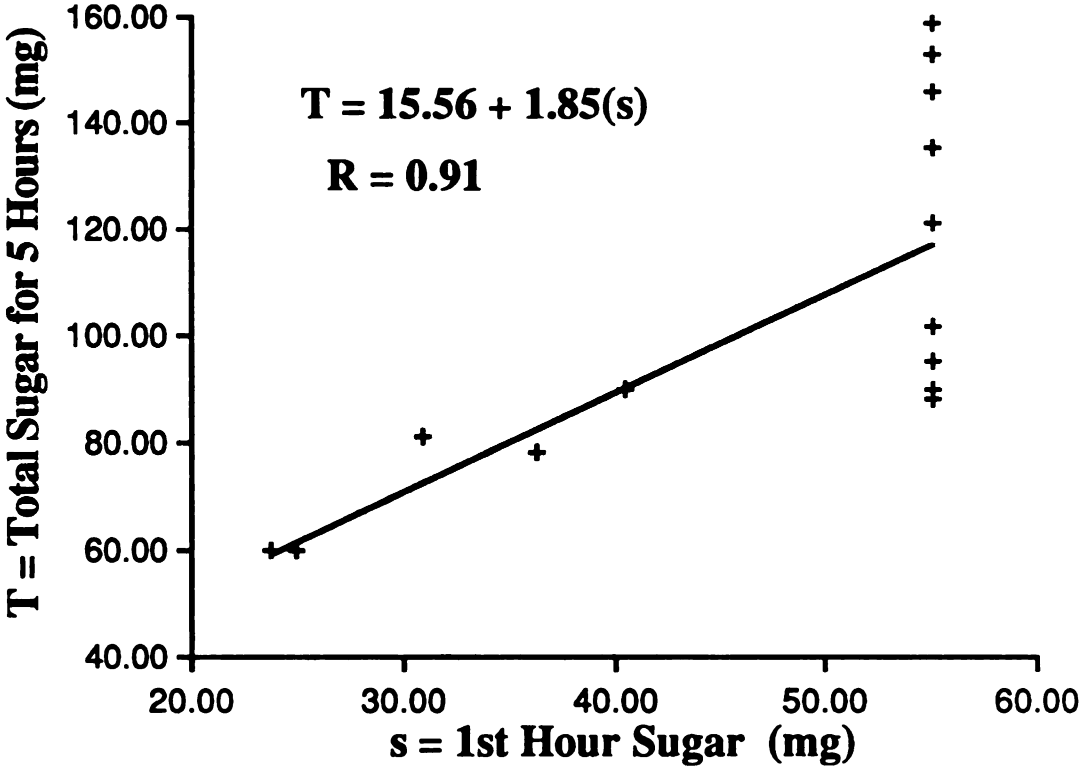

Figure 12c: Prediction equation* for total sugar for 5 hours in borage based on 1st hour sugar.
*Based on a single multivariate analysis.

for total diffused sugar after 5 hours of extraction based on the amount of sugar found in the 1st hour extraction for each plant species. Although these equations are based on a single multivariate analysis, because of differential sugar content, representation in a single figure would have distorted the data.

DISCUSSION

The fact that extracted sugar in clover was highest in the second hourly extraction indicates that physically exposing nectaries of relatively closed flowers is necessary if a large percentage of the nectar is to be removed during a single 1 hour extraction. Although rape and borage flowers underwent the same physical process, neither exhibited any increase in corresponding second hour carbohydrate levels. Instead they both showed a substantial decrease in the amount of sugar extracted from them. This suggests that leaching of plant liquids containing carbohydrates, other than nectar, has little significant effect on total carbohydrate levels in the rinsate. This agrees with studies by Crunden and Hermann (1983). They found with nectar volumes being equal, that nectar sugar concentrations of cut flowers placed in water and rinsed for more than 12 hours were significantly lower than those extracted from rinsed flowers left intact on the plant.

Flower structure, therefore, seems the primary factor influencing the effectiveness of carbohydrate extraction by rinsing. Flower species with narrow corollas should have their nectaries exposed to extract most of the nectar sugar present if a single extraction is to be used.

In all three species, after the major extraction of sugar during the first or second hourly extractions, there remains a relatively consistent presence of low levels of sugar in the remaining 3 hourly extractions. This may be explained by the nectaries' ability to continue secreting nectar after picking. Cruden and Hermann (1983) found bagged or protected flowers produced less carbohydrate than flowers visited several times during the secretion cycle. They concluded the nectaries responded during the secretion cycle to any depletion of carbohydrate with the secretion of more carbohydrate. Therefore, exposing the flower to repeated rinses might stimulate the nectaries to continue sugar secretion. This results in a low, but consistent reading of sugar in the rinsate of each extraction.

It has been observed that clover (Butler 1945), rape and borage (Ayers et al. 1984) are attractive to foraging bees throughout the day. In these studies it was assumed that nectar secretion occurred throughout this period. Cruden and Hermann (1983), however, suggest that the presence of nectar is not a true indicator of the duration of nectar secretion. They observed in some plants that although they ceased nectar production by noon, there was an actual increase in nectar sugar concentration as a result of evaporation of water from the nectar throughout the day. The data from this study seems to indicate that nectar secretion is active in all three species for at least 5 hours (from 1000 to 1500 EDST).

The significant difference between species for first hour extractions of sugar agrees with our previous study (see

Chapter II). Total sugar for 5 hourly extractions remained significantly different between plant species. The sugar extraction ratios between clover and the other two species, however, is reduced by half after 5 hours. This indicates that comparisons of total carbohydrate of first hour samples alone greatly exaggerate differences between species. It seems imperative, therefore, to determine the total carbohydrate that will be made available by the plant to make accurate comparisons between plant species' attractiveness values. Using the method developed in this study, it might be possible to generate a predictive model to be used with Roberts' (1979) method to estimate total sugar that would be available to bees if they continued to deplete it.

Genetic heterogeneity of nectar production potential among plants (or populations) has been demonstrated (Carpenter 1976; DeGrandi-Hoffman and Collison 1982), but factors such as weather and soil will also affect nectar production (Schuel 1979). For these reasons, this method would need to be developed de novo for each system encountered.

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CONCLUSION

Study of the use of diversionary plantings to reduce pesticide related pollinator mortality is an intriguing practical approach to a serious problem. Elimination of pesticides or restrictions in application, removal of apiaries to noncontaminated areas, or just doing nothing are costly and ineffective solutions.

Although there are many known bee plants and there are many studies investigating honey bee foraging behavior, there is little quantitative data that simultaneously bears on the various factors influencing attractiveness. The role of diversionary plants as an IPM tool will remain theoretical until more of the questions concerning competitive attractiveness are answered.

The objectives of this thesis were: 1) to add new methodologies for investigating some of the factors involved in making diversionary plantings attractive and 2) to use these methods to study factors affecting attractiveness.

To create a method for studying honey bee forager response to plants of varying attractiveness, a new marking system was developed. This marking system created a method of identifying foragers from a given hive or apiary that is both inexpensive and easy to use. It can be employed anywhere and at anytime. Although temporary in duration it continuously and automatically marks foragers with a color identifying the hive of origin. This allows the researcher more time to attend to other duties involved with collection of data. It allows for statistical analysis where hives of origin, now clearly identifiable, may be treated as replicates.

This study revealed that the two diversionary plants rape and borage always attracted more bees than clover. Caloric reward, the product of plant density and nectar concentration, is the most significant factor effecting foraging response. Handling time and weather had varying minor effects influencing attractiveness, but could potentially become more important in other situations. While it would be incorrect to conclude that rape and borage could fullfill all the requirements of diversionary plantings, examination of the factors affecting their attractiveness created a building block in the design of such a system. A methodology has been developed that can be used to examine such important factors as: 1) the effect of distance from the apiary to the diversionary planting and/or orchard, 2) the relative sizes of bee foraging populations from different hives, or 3) the effect of pesticides on the efficiency of a diversionary planting.

As caloric reward had the most significant effect on attractiveness, a method was created to estimate the total nectar sugar available to a group of foragers. As a result of this work, it was concluded that other methods of sugar extraction do not correctly access the total sugar available in flowers with restrictive corollas. With this method it was also seen that rape, borage and clover all apparently continued to produce sugar after the initial extraction. Further research should be done to reveal if this were indeed a product of continuous nectar secretion. If so, this would place the concept of total attractiveness of a plant over time and through repeated visits in a new light. The technique started in this work may have created a new and more accurate method of predicting total sugar available to a foraging population over a period of time. Such a technique might prove useful in more accurately predicting plant attractiveness.