

**EVALUATION OF IMAGE ANALYSIS
FOR STUDYING MITE BEHAVIOUR**

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The aim of this study was to investigate the usefulness of image analysis for studying mite behaviour. Image analysis was used to convert video recordings of mites' locomotory behaviour into a series of x,y coordinates that, when joined, closely resemble the paths of mites. The coordinates were also used to calculate walking speed, direction of travel, turning frequency, turn bias and tortuosity. Two experimental arenas were developed and used to study the movement of three mite species: 1) a leaf disc arena for two-spotted spider mite, *Tetranychus urticae* Koch and European red mite, *Panonychus ulmi* (Koch); and 2), a cover-slip/tack-trap arena for *Typhlodromus pyri* Scheuten.

Two-spotted spider mite exhibited a change in locomotory behaviour through a 48 minute period. Mites exhibited a significant decline in distance travelled, whereas the mean stationary time (per four minute interval) more than doubled, and the duration of stationary events increased steadily over the same period.

A reduction in sampling frequency of mite coordinates from one per second to one every two seconds and every four seconds produced a 5% and 12% 'loss' in path length respectively.

Sample period length was shown to greatly influence the results produced for some of the mean parameters calculated, however, a reduction in sample length from 3000 to 1500 coordinates was not considered to cause a major loss in information. The influence of the inherent mite movement could not be ignored and made it difficult to make decisions on the 'best' sample length to use.

Some strong correlations were found between parameters used to analyse mite locomotory behaviour. In particular, arithmetic mean vector length, speed, total stationary time and total distance travelled were significantly correlated with each other. Mean angular deviation and weighted mean vector length, which both measure the degree of clustering around the mean heading angle, were strongly negatively correlated.

Parameters which differentiated between 'straight' and 'tortuous' mite movement were found to be mean meander, absolute mean turn and fractal dimensions. Mean meander

was thought to be the most 'powerful', while coefficient of a straight line, a commonly used parameter for measuring tortuosity, did not significantly differentiate between the two different behaviours.

Frequency distributions of turns and standard deviations of the three mite species were very similar. All three species had a slight bias to turning right (clockwise) rather than to the left (counter-clockwise) and for each species certain angles occurred more often than would be expected in a 'perfect' normal distribution. A similar pattern also occurred with the frequency distribution of two-spotted spider mite heading angles, in that angles which were expected to occur more often, did not, and vice versa.

The potential to use saturated salt solutions to control relative humidity on the arena was demonstrated and indicated that relative humidity is likely to have an important influence on mite behaviour. Two-spotted spider mites appeared to move more quickly in an attempt to escape the unfavourable, extreme (10% and 95% R.H. at 25°C) moisture conditions.

All three mite species displayed a characteristic edge-walking behaviour around the arenas. However, when 'edge' and 'non-edge' behaviours were compared, mean meander was the only parameter (of the parameters tested) which gave a significant difference.

Behavioural responses of European red mite and *T. pyri* to sub-lethal (field rate) esfenvalerate were investigated and the results indicated that these mites did not seek the unsprayed halves of the arenas during the first 48 minutes. However, significant differences in most behavioural parameters to esfenvalerate residues were found with European red mite when whole arenas were compared.

Image analysis is an extremely useful research tool for studying mite behaviour because of its ability to measure many parameters quickly.

Careful choice of the environmental conditions, the sampling framework, and interpretation of data is essential for meaningful results.

Keywords: Image analysis, locomotory behaviour, two-spotted spider mite (*Tetranychus urticae*), European red mite (*Panonychus ulmi*), *Typhlodromus pyri*, esfenvalerate, sub-lethal effects.

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CHAPTER 1

INTRODUCTION

Movement behaviour for most insects determines how individuals encounter environmental features that vary in space (Wiens *et al.*, 1993). Following a representative sample of individuals' actual movement tracks, investigators can rapidly test their ideas about the environmental cues that affect insect movement (Turchin *et al.*, 1991). With the advances in computer and video technology, behavioural entomologists are seeking more rapid, accurate and simple methods for recording the paths of arthropods. In the past, many behavioural studies relied on hand tracings of frame-by-frame images of locomotion recorded by time-lapse photography (Hoy *et al.*, 1983). However, such methods are tedious and human error, bias, and poor resolution can be likely results (Hoy *et al.*, 1983). Studies of arthropod locomotion are suited to video technology particularly where the locomotion is on a 2-dimensional plane and can be contained in a moderate-sized arena (Varley *et al.*, 1994). By using a computer to digitize movement tracks, the tedium of measurement is removed and problems such as bias and unintentional errors are avoided in analysis (Hoy *et al.*, 1983).

Several computer-assisted tracking systems have been used to date (Hoy *et al.*, 1983; Mueller-Beilschmidt and Hoy, 1987; Berry and Holtzer, 1990), but they tend to either measure simple units such as activity in pixels, or do not use the computer system to its full potential to collect data where, in many cases, additional software may extend the use of the data collected. Because of their small size (0.54 mm long) and high mobility, the movement of mites is difficult to study.

The aim of this study was to investigate the usefulness of an image analysis tracking system as a research tool for studying locomotory behaviour in three species of mites, *Tetranychus urticae*, *Panonychus ulmi* and *Typhlodromus pyri*.

The main objectives of the study were:

1. To design and test suitable arenas for the three mite species.
2. To develop computer software appropriate to measure and analyse several facets of locomotory behaviour.
3. To characterise the behavioural responses of TSM on a leaf disc arena over a 48 minute period.

4. To determine the effects of arena edge-effects on mite behaviour.
5. To test a range of behavioural parameters to measure certain movement characteristics.
6. To apply the image analysis system to examine the effects of esfenvalerate residues on ERM and *T. pyri* behaviour.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Developments in computer technology have led to major hardware and software advances in movement tracking systems for behavioural studies over the last decade. The aim of this literature review is to outline the most relevant to the main areas covered in this thesis. The literature review has been divided into four sections, viz., behavioural studies of arthropod movement, locomotion tracking systems, application of image analysis for use in behavioural studies, and sub-lethal effects of pesticides on arthropod behaviour.

2.2 Behavioural parameters used in studies of arthropod movement

Survival and reproductive success of all animals is to a large degree determined by their mobility (Cain, 1985) and the means by which individuals search for their resource requirements. Arthropod species are thought to exhibit different types of searching movement paths depending on their recent experience or feeding status (Sabelis *et al.*, 1984; Berry and Holtzer, 1990). For example, the searching strategies of arthropods that use chemo-orientation can include a noticeable change in the amount or degree of turning and/or locomotion to a stimulus. Many arthropods exhibit turns that are $> 90^\circ$ when they encounter a change from a favourable to an unfavourable stimulus (Bell and Tobin, 1982). A common behaviour observed in invertebrate predators is the reduction in walking speed (inverse orthokinesis) and increased rate of turning (klinokinesis) directly after some host-related stimulus has been detected. Such behaviour is thought to increase the chances of finding a prey resource (Takabayashi and Takahashi, 1989). This information is useful to help understand environmental cues that affect arthropod behaviour, yet simplistic models such as those described above do not assist in the characterisation of more complex behaviour due to their non-quantitative nature.

2.2.1 Characterising movement components

Perhaps the most authoritative, comprehensive and prominent studies describing general animal movement to date is the work on classification of orientation patterns devised by Fraenkel and Gunn (1940, revised 1961). They classified animal reactions or movements on whether they were directed (taxes) or undirected (kinesis). Their classification was

based upon locomotory patterns (consisting of speed, turning rate and linearity), gradient of stimulus, position and number of receptors, and the mechanics of stimulus interpretation. Most studies of animal movement have adopted the kinesis-taxes approach (Visser, 1988), however, this classification method has been criticised for two main reasons: 1) a lack of consideration of the role memory and internal control systems contribute to orientation; and, 2) insufficient experimentation before classification of orientation occurred (Jander, 1975; van der Steen and ter Maat, 1979; Kennedy, 1986; Bell and Tobin, 1982). Visser (1988) has revised the classification of Fraenkel and Gunn (1961) to include some of these omissions. Irrespective of the criticisms of Fraenkel and Gunn's (1961) classification, these orientation categories remain useful as behavioural descriptors of movement, however, analytical measurements are required for more quantitative studies.

'Movement' can be defined as the spatial displacement of the whole animal. Any movement of an animal in a two-dimensional space can be pictorially described by segmenting a continuous track into a series of connected straight lines of equal time or distance. Kitching and Zalucki (1982) proposed a movement model based on six components thought to be sufficient to describe a two-dimensional track of an animal in time or space. These six components were: 1) mean of angles turned; 2) variance of angles turned; 3) mean speed of movement; 4) variance of movement speed; 5) initial angle of bearing; and, 6) proportion of time spent moving. Using these components, Zalucki and Kitching (1982) showed that simple but realistic tracks of the gastropod, *Polinices incei* (Phillipi) in beach sand could be modelled. The authors perceived the beach sand to be a homogeneous environment, free from any behaviour-modifying stimuli, however, such homogeneity is unlikely in field situations.

Another procedure for quantifying simple movement sequences is based on the assumption that the movement of many animals is a 'correlated random walk' (McCulloch and Cain, 1989). Kareiva and Shigesada (1983) developed a model based on move length (distance between two sample points) and turning angle (change in direction expressed in degrees) probability distributions that relate expected square displacements (distance from original position) to the number of consecutive moves. In studies on white butterflies (Kareiva and Shigesada, 1983) the random walk model closely predicted net displacements while ovipositing in a collard garden, however, when feeding on nectar in a goldenrod field, movement did not fit the derived model. These authors concluded that some behaviour may be too complex to be described by a simple correlated random walk

formula (Kareiva and Shigesada, 1983). The correlated random walk model was also used to study slug foraging behaviour in response to commercial baits (Howling, 1991). Slugs (*Deroceras reticulatum* Müller) randomly searched in the absence of any bait and with the commercial metaldehyde baits, but deviated significantly from the random walk model in the presence of commercial methiocarb baits. Howling (1991) suggested the methiocarb baits contain an attractant that resulted in the difference in behaviour.

Other workers have taken different approaches to quantifying animal movement, especially under natural conditions. For example, Turchin *et al.* (1991) quantified the movement of hilltopping butterflies (*Euphydryas editha* Boisduval) in the field using triangulation, and illustrated some of the problems of working with insects in the field. Such constraints are that many insects move too fast, are too small to be seen and their accessibility to the observers is poor. Aluja *et al.* (1989) suggested that because laboratory studies allow greater control over experimental conditions, the sophistication and precision of movement pattern analysis can be increased.

2.2.1.1 Measures of tortuosity

The path by which a single animal moves is rarely straight, and as a rule, a more or less pronounced detour occurs with left and right deviations (Batschelet, 1981). It is therefore essential to characterise tortuosity of the animal's trail when seeking factors influencing animal orientation (Dicke and Burrough, 1988). One of the simplest methods of characterising animal trails is the subjective analysis of calculating the percentage of time spent walking 'straight' or 'tortuously', as was previously done in studies involving the orientation and behavioural changes of predatory mites towards prey odours (Sabelis *et al.*, 1984; Dicke, 1986). To remove the subjectivity, some sort of relative scale that allows quantitative comparisons of different behavioural sequences is required. Such a measure is the index of straightness (Suski and Naegele, 1968).

2.2.1.1.1 Index of straightness (linearity of travel; coefficient of travel)

The index of straightness is calculated by dividing the 'beeline' (shortest distance between the start and end points) by the actual length of the path. This measure has been used in behavioural studies on twospotted spider mite, *Tetranychus urticae* Koch in response to chemical attractants (Penman and Cone, 1972) and temperature and humidity (Penman and Chapman, 1980), Colorado potato beetle *Leptinotarsa decemlineata* Say

(Thiery and Visser, 1987) and for larval searching of the aphidophagous coccinellid *Harmonia axyridis* Pallas (Ettifouri and Ferran, 1993). However, one criticism of this method is that all paths having the same total length and same net displacement have the same index of straightness, regardless of their actual 'wiggleness' (Dicke and Burrough, 1988). However, this is less of a problem when the paths are short and there is some degree of tactic orientation. For example, the type of orientated behaviour observed with parasitoid movement in odour plumes of host microhabitats (Vet and Papaj, 1992). In another odour orientation study of tsetse flies (*Glossina pallidipes* Aust.), Gibson *et al.* (1991) observed a distinct bimodal frequency distribution in the straightness index for two different flight tracks. In this study the index allowed the authors to distinguish behavioural differences between treatments. In another study, Visser (1988) used this method of measuring path straightness to assess the stimulation of behavioural responses in Colorado beetles by three modalities, light, wind and plant odour.

2.2.1.1.2 Weighted mean vector length (r)

One way to avoid the problem described above by Dicke and Burrough (1988) is to break the movement path into small sections, or vectors, that are recorded at equal time intervals. The weighted mean vector length uses the directions of all the vectors and describes their concentration around a mean direction. A value of zero indicates no clustering around the mean angle (i.e., evenly spaced around 360°) and when $r=1$, all heading angles are identical and no deviation exists (Batschelet, 1981). This parameter has been used to compare the relative searching behaviour of three caterpillar species, cabbage butterfly (*Pieris rapae* L.), diamondback moth (*Plutella maculipennis* Curt.) and the alfalfa looper (*Plusia californica* Speyer) under different environmental conditions (Jones, 1977).

2.2.1.2 Fractal dimensions

Another way to quantify insect movement paths is to calculate their fractal dimensions. The term 'fractal' was invented by Mandelbrot (1975) to describe complexity at different scales. Many natural objects have a complexity that can not be described in terms of lines or planes. In addition, the complexity also changes according to the scale of

measurement used. For example, the coastline of New Zealand has major features visible at a broad scale, such as Banks Peninsula. Someone walking the coastline would observe more detail due to the greater resolution imparted by the finer scale. An important characteristic of fractals is their self-similarity when observed at different scales, therefore, as the resolution is increased, greater detail is seen (Williamson and Lawton, 1991). Similarly, as the movement scale (step size) is changed when measuring the length of the path of an insect trail (with a pair of dividers, for example) greater or lesser amounts of detail are included. As a consequence, the length of the trail changes according to the scale of measurement used. The graph of $\log(\text{length})$ against $\log(\text{step size})$ is a straight line with a slope of $1-D$, where D is the fractal dimension. Fractal dimensions are therefore an alternative measure of pathway tortuosity (Wiens *et al.*, 1993) where a linear fractal function, the Hausdorff-Besicovitch dimension (D), may vary between 1 and 2. When $D=1$ the linear path is considered to be perfectly straight, whereas when $D=2$, paths are considered to be highly contorted (Mandelbrot, 1983). Dicke and Burrough (1988) found the index to be a good, discriminative index for twospotted spider mite trails even though such paths are not "pure" fractals due to "smoothing-out" procedures. Because of the difficulties of measuring insect trails manually, Dicke and Burrough (1988) advocate the use of automatic recording techniques, such as image analysis, to reduce such problems.

2.2.1.3 Turning characteristics (parameters)

Frequency distributions of an insect's turning responses over time can give information about its behavioural state. Altered distributions of turning angles in test individuals is known to be a common response of organisms to changing environmental stimuli and has been adopted by researchers as a standard movement parameter in recent times (Bell, 1985; Wendler and Scharstein, 1986; Heinzl and Bohm, 1989; Lönnendonker and Scharstein, 1991; Obeng-Ofori, 1991; Vet and Papaj, 1992; Collins *et al.*, 1994). This method involves the measurement of directional change at intervals of a subjectively chosen length (Dicke and Burrough, 1988). Despite that, the turning angles calculated are scale dependent, such that, an increase in step size will result in a decrease in the amount of directional change. This method does provide information on the direction of turning which allows distinction between left and right (anti-clockwise and clockwise) turns.

McEwen *et al.* (1993) showed in their study of the searching behaviour of the green

lacewing *Chrysoperla carnea* (Stephens) that the turning frequency changed more significantly than turning angle in response to honeydew produced by the black scale *Saissetia oleae* (Olivier), showing it could be a useful measure in behavioural studies. The shape of search paths can be affected by the tendency of an animal to turn predominately in one direction or another (Collins *et al.*, 1994). Such turn biases generate circling paths that may increase searching efficiency (Bornbusch and Conner, 1986). Casas (1988) used the succession of turn angles (i.e., to the left or right) to analyse searching movements of leafminer parasitoids (*Prigalio soemius* Walker) when encountering leaf mines. Successive angles were analysed to determine whether the leafminer parasitoid movements were random (non-stimulus searching) or non-random (altered by a stimulus).

Meander (or turning per unit distance) is a convenient measure that combines the effects of both changes in speed and turning rate (Bond, 1980). Collins *et al.* (1994) in a study of local search behaviour by the house fly (*Musca domestica* L.) found the turning parameters locomotory rate, turning rate, meander and turn bias to be highly correlated with environment.

2.3 Tracking systems for quantification of locomotor behaviour

There are currently two broad types of systems used for analysis of locomotor behaviour: 1) the locomotion compensator apparatus; and, 2) various levels of sophistication in video-computing technology as outlined in Young *et al.* (1994) and Varley *et al.* (1994).

2.3.1 Locomotion compensator

The spherical treadmill, servosphere or locomotion compensator (Berg, 1971; Kramer, 1976) is an indirect tracking system that compensates for transitional movements of the insect, allowing free rotation. Motion of the sphere is translated into incremental x , y coordinates both for analysis and display on a computer (Bell *et al.*, 1989). Locomotion compensators have been used to measure the orientation response of granary weevils *Sitophilus granarius* L. to gravitational stimuli (Wendler and Scharstein, 1986), walking phonotaxis (a directed response to sound) in field crickets *Gryllus bimaculatus* (De Geer) (Doherty and Pires, 1987), anemotaxis (directed response to wind) in larder beetles *Dermestes ater* De Geer (Bell *et al.*, 1989), and optomotor and odour-conditioned anemotaxis response of Colorado beetles (Lönnendonker and Scharstein, 1991; Visser,

1988). The main disadvantage of the locomotion compensator compared to image analysis techniques is that it can not be used to observe behavioural responses of arthropods to different treatments or stimuli on a natural surface such as a leaf.

2.3.2 Image analysis techniques

Image analysis is a process whereby images of interest are digitised by a computer and then quantified (Anon., 1985). Image analysis can identify and quantify parameters such as size, number, shape, position and optical density of identifiable parts of an image. Where a large number of repetitive measurements are required image analysis can give deliver greater accuracy in quick time.

Image analysis has been used for a wide range of applications covering many disciplines (Anon., 1985). Entomologists have also shown image analysis to be useful for insecticide droplet analysis (Jepson *et al.*, 1987; Franz, 1993), for assessing insect defoliation (Notting and Edwards, 1985; Hagerup *et al.*, 1990; Escoubas *et al.*, 1993; Kokko, *et al.*, 1995), quantification of mandibular wear (Kokko *et al.*, 1993) and for insect systematics (Yu *et al.*, 1992). The application of image analysis to the analysis of animal movement is relatively recent and has been investigated by entomologists as a powerful tool in insect behavioural research.

2.3.3 Applying image analysis to behavioural studies

For the purposes of this thesis "image analysis" will be used in reference to all computer-automated video tracking systems. Image analysis systems have been used to study locomotory behaviour of a wide range of subjects including: stallion sperm (Jasko *et al.*, 1988), leukocytes (Tumanov *et al.*, 1990), fish (Royce-Malmgren and Watson, 1987), spiders (Baatrup and Bayley, 1993), insects and mites (Hoy, *et al.*, 1983; Hoy, 1994; Hoy and Dahlsten, 1984).

Before advances in technology allowed the interface of video cameras with computers, movement behaviour of arthropods was recorded on video tape and the paths were traced from a video monitor onto a transparent film such as acetate (Varley *et al.*, 1994; van Vorhis Key and Barker, 1982). Parameters such as path length, heading angles and turns had to be painstakingly calculated. Because of the financial constraints of acquiring more sophisticated technology (Jouen and Lepecq, 1989), such methods are

still used. However, they are likely to introduce errors through operator mistakes, bias, and have poorer resolution (Hoy *et al.*, 1983). Technology is now available that allows computer-assisted tracking that provides a rapid and sensitive method of analysing animal behaviour (Dunsenbery, 1985). Since the pioneering work of Davenport *et al.* (1961) a series of improved electronic tracking systems that use different methods, have been reported (Aluja *et al.*, 1989).

One method is to digitise the trace of the walking patterns using a computer bit pad and use the x,y coordinates generated to reconstruct a stylised path for subsequent analysis (Young *et al.*, 1994). Two types of ambulatory dispersal of the predatory mite *Neoseiulus fallacis* (Garman) were studied using this method (Berry and Holtzer, 1990), however, problems can arise when tracks run on top of each other particularly on arena edges. Another limitation of this method is that the process can be very slow; one minute of recorded behaviour may take a several of hours to digitise (Young *et al.*, 1994).

A video-computer interface that can track and record movement paths in 'real time' eliminates the laborious manual digitising component. Two types, in current usage, i.e., teletracker and image analysis, are described in detail by Young *et al.* (1994). Briefly, the teletracker uses standard video signals which it compares with a brightness threshold set by the operator to isolate the object (e.g., mite) to be tracked. The coordinates of the scanned objects reaching the brightness threshold for each sequential video image sampled are joined to produce a series of points representing the track. In image analysis, successive pictures are loaded into the computer memory using a frame-grabber to compare sequential 512 x 512 arrays with grey level (brightness) values for each given point or 'pixel'. Comparing digital images of successive array sets, coordinates of moving objects are recorded and joined to produce a track. The image analysis method has advantages over the teletracker system as the latter requires good contrast between the object or arthropod of interest, and the background. An additional advantage over the teletracker system is the ability to track more than one subject, in some image analysis systems (Young *et al.*, 1994).

Simple video-computer interfaces such as that used by Mueller-Beilschmidt and Hoy (1987) measure only an activity level (in pixels/second), however, given the x,y coordinates, many other useful parameters can be calculated. One recent innovation uses robotics to allow camera displacement and can measure locomotor activity of up to 60 individuals and monitor many more. Such a system was used to monitor more than

400 individuals in a study using parasitoids *Asobara tabida* (Nees), *Leptopilina heterotoma* (Thomson), and *Trichogramma brassica* (Bezdenko)(Allemand *et al.*, 1994). They found the existence of adult parasitoid emergence rhythms (from host) and circadian activity rhythms by measuring the percent activity in real time using an automatic image analysis system. Commercial tracking products with a potential for many uses are also becoming available (Anon., 1994). Examples include studies in olfactometry, time patch allocation and boundary responses, foraging behaviour on natural substrates, and wind tunnel activity.

2.4 Sub-lethal effects of pesticides on arthropod behaviour

In a review of the sub-lethal effects of insecticides on insect behaviour Haynes (1988) stated that "certain insecticides stimulate or depress general locomotory behaviour such as walking or flight." Pesticide-altering behaviour in pest or beneficial arthropods is an important consideration when pesticides are used as a control strategy (Margolies and Kennedy, 1988). For example, spiders have shown responses to sub-lethal concentrations of pesticides in their web building behaviour, particularly with pyrethroids (Samu and Vollrath, 1992; Lengwiler and Benz, 1994). Hoyt and Dahlsten (1984) studied the behavioural effects of malathion-bait-treated surfaces on the parasitic wasp *Encyrtus saliens* Prinsloo and Annecke and found walking speeds to be significantly faster on treated surfaces. Because of their pest status, spider mites are probably one of the most studied arthropods in relation to their responses to sub-lethal doses of pesticides. Pyrethroids are known to have a major effect on the natural regulation of spider mite populations (Penman and Chapman, 1988). Of particular interest has been the pyrethroid-induced dispersal behaviour of spider mites (Penman *et al.*, 1981; Iftner and Hall, 1983; Penman and Chapman, 1983; Penman *et al.*, 1986; Margolies and Kennedy, 1988; Holland and Chapman, 1990, 1991, 1994).

Pyrethroid-induced mite outbreaks have been a recognised problem since early work (Hoyt *et al.*, 1978) and outbreaks have now been associated with most pyrethroids and at least nine spider mite species (Gerson and Cohen, 1989). The main response to pyrethroids is irritability and dispersion which has been shown to occur at sub-lethal concentrations (Penman *et al.*, 1981; Iftner and Hall, 1983; Penman and Chapman, 1983; Penman *et al.*, 1986; Holland and Chapman, 1990 and 1994; Holland *et al.*, 1994a and 1994b). These studies indicated that spider mites disperse rapidly from treated to

untreated surfaces by either running off or spinning down on silk threads. Margolies and Kennedy (1988) have identified aerial dispersion to be another significant behavioural response to fenvalerate-treated leaves with a wind speed of 1.5m/s. Anti-feedant responses are also induced by pyrethroids (Iftner and Hall, 1983; Hurkova, 1984; Iftner *et al.*, 1986; Margolies and Kennedy, 1988; Holland *et al.*, 1994a).

Even though there has been considerable interest in spider mite responses to pyrethroids, little work has been conducted on trying to understand the behaviour in detail by analysis of locomotory responses.

CHAPTER 3
MATERIALS AND METHODS

3.1 Introduction

Mites were chosen to test the use of image analysis system for behavioural studies because ample numbers of mites were readily available and because they were very mobile arthropods that would quickly generate sufficient data allowing parameters to be tested.

3.2 Mite colonies

Three mite species were used in this study (Plates 1-3). Two-spotted spider mite *Tetranychus urticae* Koch (TSM) and European red mite *Panonychus ulmi* Koch (ERM) are phytophagous mites, and *Typhlodromus pyri* Scheuten is a predator mite of the ERM and TSM.

3.2.1 Two-spotted spider mite

The TSM strain (LN-R) used in this study was established in the laboratory in 1985 by combining several strains to ensure genotypic variation. The colony was reared on dwarf French beans *Phaseolus vulgaris* L. at $25\pm 1^\circ\text{C}$, $50\pm 5\%$ RH under a mercury-vapour growth lamp on a timer to produce a 16L:8D photoperiod. Fresh dwarf bean plants were added to the colony when required.

3.2.2 European red mite

ERM were collected from nectarine leaves, *Prunus persica* (var. 'Fantasia') in the Horticultural Research Area at Lincoln University. The nectarine trees had not been sprayed with insecticides, acaricides or fungicides since 1986, except for one application of dicofol (Kelthane® 35) in 1988 (Keith McIntosh, pers. comm.). Because this species is not easy to rear in the laboratory, mites were collected from the field when they were needed for experiments.

3.2.3 SP-resistant *Typhlodromus pyri* Scheuten

The colony was started using *T. pyri* collected from apple trees (cv. 'Gala') from the

Plate 1. Adult female twospotted spider mite and egg on bean leaf.



Plate 2. Adult female European red mite in midrib of a bean leaf.



Plate 3. Adult female predator mite *Typhlodromus pyri* on bean leaf.



Appleby Research Orchard, Nelson. The predators were reared on 12x7 cm, 6 mm thick black acrylic plates. A 2 mm deep groove 5 mm in from the edge of the plates was syringed with Tack-trap (a sticky polybutene-based substance) to confine the populations. The plates were placed on to water-saturated damp paper towels in plastic food trays with clear lids. The lids were raised 5 mm to allow air movement to reduce fungal growth. The trays were kept in a Contherm controlled environment cabinet at $27\pm 0.5^{\circ}\text{C}$, $75\pm 5\%$ RH and a 16L:8D photoperiod.

Each plate was started with 20-40 adult predators and after 2-3 generations the progeny were transferred to fresh plates. The predators were fed TSM of all life stages by flicking them off leaves through a clean funnel on to each plate every second or third day. Fresh pollen collected from local plants was provided as a food supplement. Two or three small sections of bean leaf were placed on each plate as refuge sites and oviposition substrates for adult females.

The resistance status of the colony was confirmed when mites survived for 24 h on Petri dishes treated with esfenvalerate at 15x field rate using a Potter precision spray tower (Burkard Manufacturing Co. Ltd, U.K.). SP-resistance was maintained by regularly spraying with esfenvalerate (Hallmark[®] 5EC, Shell Chemicals Limited) at the recommended field concentration of 0.015 g a.i. l⁻¹.

3.3 Behavioural arenas

The design of the behavioural arenas had to be appropriate for containing the mites within the arena without being optically obtrusive to enable image analysis to identify the mites from the background (i.e., the materials making up the arena). The poor resolution of the translucent body of the predator *T. pyri* compared to the highly pigmented bodies of the phytophagous mites, ERM and TSM, meant that two arenas were needed for image analysis. For both types of arena (described below), care had to be taken to avoid trapping air bubbles beneath them. The dark appearance of the bubbles made it difficult for the computer to differentiate them from mites. In addition, static buildup on the Petri dishes on which the arenas were placed was found to cause mites to unnaturally 'jump' from arenas in early preliminary experiments. This problem was easily eliminated by earthing the Petri dishes.

3.3.1 Arena 1: Agar/leaf disc method for TSM and ERM

The leaf disc arena (Plate 4) provided a natural surface for movement and feeding for the phytophagous mites TSM and ERM. Care was taken to avoid large midribs, 'unbalanced', uneven or highly pigmented leaves to allow the image processor to distinguish the mites from the darker areas of the leaf.

Using a cork borer, 24 mm diameter leaf discs were cut out of flat and smooth sections of freshly picked broad bean (*Vicia faba* L.) and nectarine (*Prunus persica*, var. 'Fantasia') leaves for TSM and ERM respectively. Young healthy mated female mites were placed on to a leaf disc that were placed bottom side up on semi-soft agar (0.1% w/v) in a small (50 x 9 mm) Petri dish base (Falcon 1006, Dickenson and Co., Cockysville, U.S.A.). Reasons for using semi-soft agar were to create a barrier to contain the mites, to keep the leaf disc fresh, and to allow light from below to silhouette the mite to create an image with good contrast.

3.3.2 Arena 2: Tack-trap/cover slip method for *T. pyri*

Glass cover slips were washed of 'production residues' (that appeared to irritate the mites) and dust to provide a smooth, inert and symmetrical surface on which to apply the insecticide treatments.

Each 16 mm diameter microscope slide coverslip was washed in distilled water, dried, and placed on a thin layer of Tack-trap in a Falcon 1006 (50 x 9 mm) Petri dish base. The Petri dish base was glued inside a standard sized Petri dish and sealed with a lid. The use of a saturated salt solution (Winston and Bates, 1960) as a surrounding 'moat', created a humid micro-environment during experiments (Plate 5). For each experiment using *T. pyri* described in this thesis the lid was removed and the arena allowed to equilibrate for five minutes to the constant relative humidity (50±5%) and a temperature (25°C) of the controlled temperature room.

3.4 Lighting

Even lighting was produced by directing a light source through an opalised sheet of acrylic (5 mm thick). The light source used was a Schott KL1500 fibre optic cold light

with a 70 mm diameter Zeiss terminal ring illuminator. The light source was set at 1 x 0.5 for *T. pyri* and, depending on leaf thickness, 1 x (1 to 3) for ERM and TSM. An earthed aluminium spacer was used between the Petri dish and the acrylic sheet to avoid static charges that could influence mite movement. The apparatus was enclosed in an acrylic blackout box (400 x 400 x 400 mm) to prevent air currents and directional light influencing mite behaviour (Fleschner *et al.*, 1956; Mori, 1962; Smitley and Kennedy, 1985).

3.5 Image Capture

A black and white Burle (TC304EX) low light, high resolution (2/3 inch format CCD) video camera fitted with a 55 mm Micro-Nikkor lens was mounted over the blackout box with the lens protruding through a hole. The aperture of the lens was set at *f*5.6 for *T. pyri* and *f*2.8 for ERM and TSM. The mite images were recorded using a Hitachi DA4 video cassette recorder (VCR) and viewed using an AWA Colortrack television monitor.

3.6 Hardware

Mite behaviour was recorded onto a TEAC E-180HX cassette tape which was then played-back into the image analysis system. Images were sent to the image analysis system through an I.DEN IVT-9SP digital time-base corrector (TBC) which electronically corrected the improper tuning relationships of the synchronization signals created by mechanical and electronic errors in the VCR. The signal was then fed via an RGB transcoder into the Magiscan II full colour image analysis system (Joyce Loebel, Gateshead, Tyne and Wear, England). The system was mouse-driven (Logitech) through menus by a '386' computer with MS-DOS version 4.0 and a specialised software package, Genias version 3.5. The image screen used was a 512 x 512 pixel format which was manipulated using a light pen. Figure 1 illustrates the linkages between hardware components.

Plate 4. Agar/broad bean leaf-disc arena contained in a Petri dish.

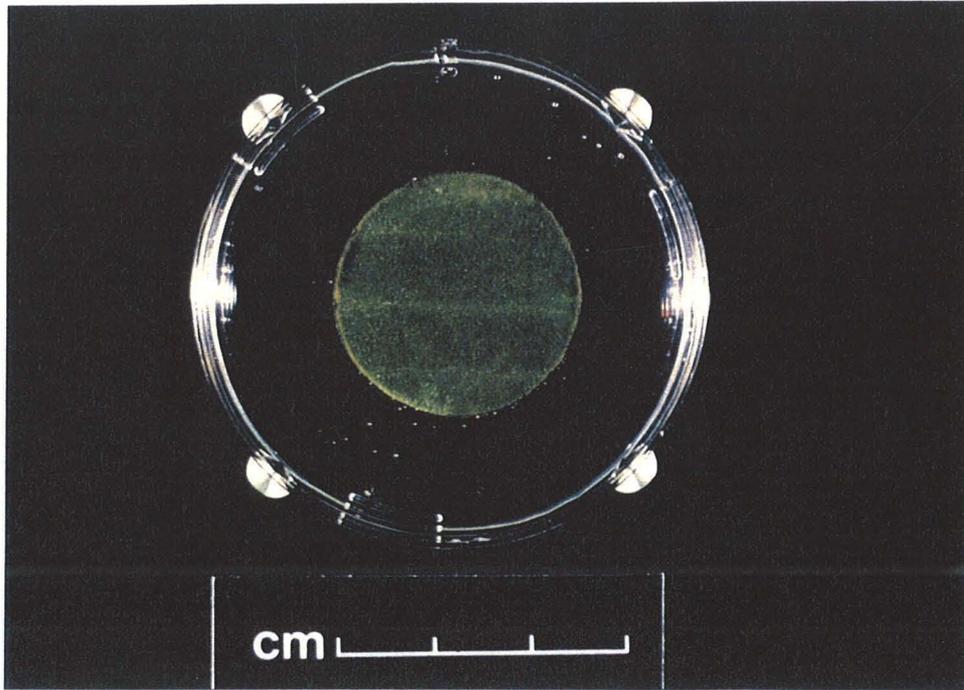


Plate 5. Tack-trap/coverslip arena surrounded by saturated salt solution.

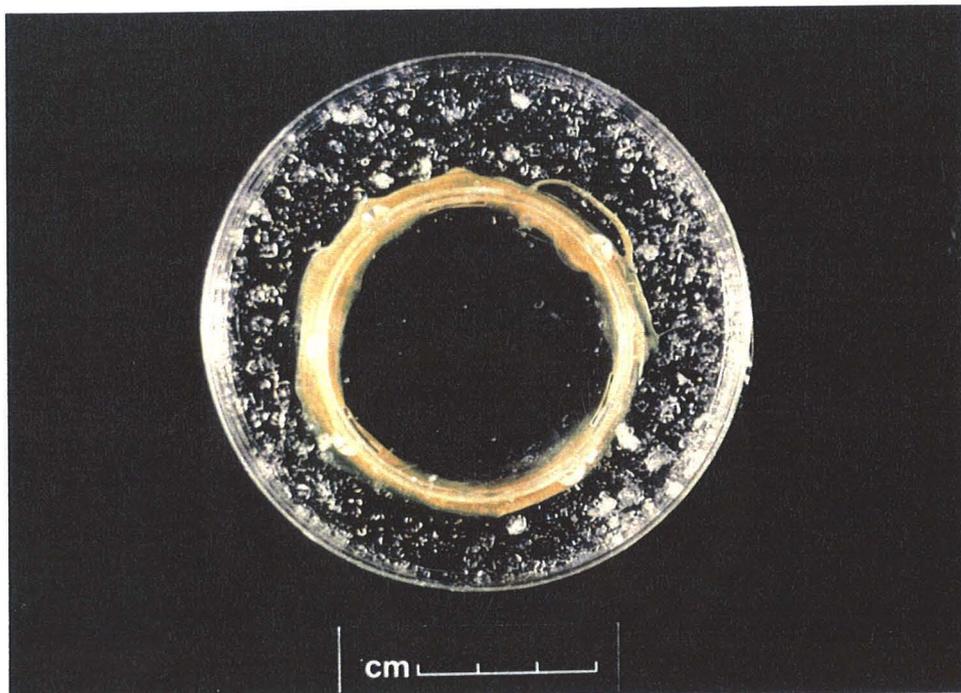
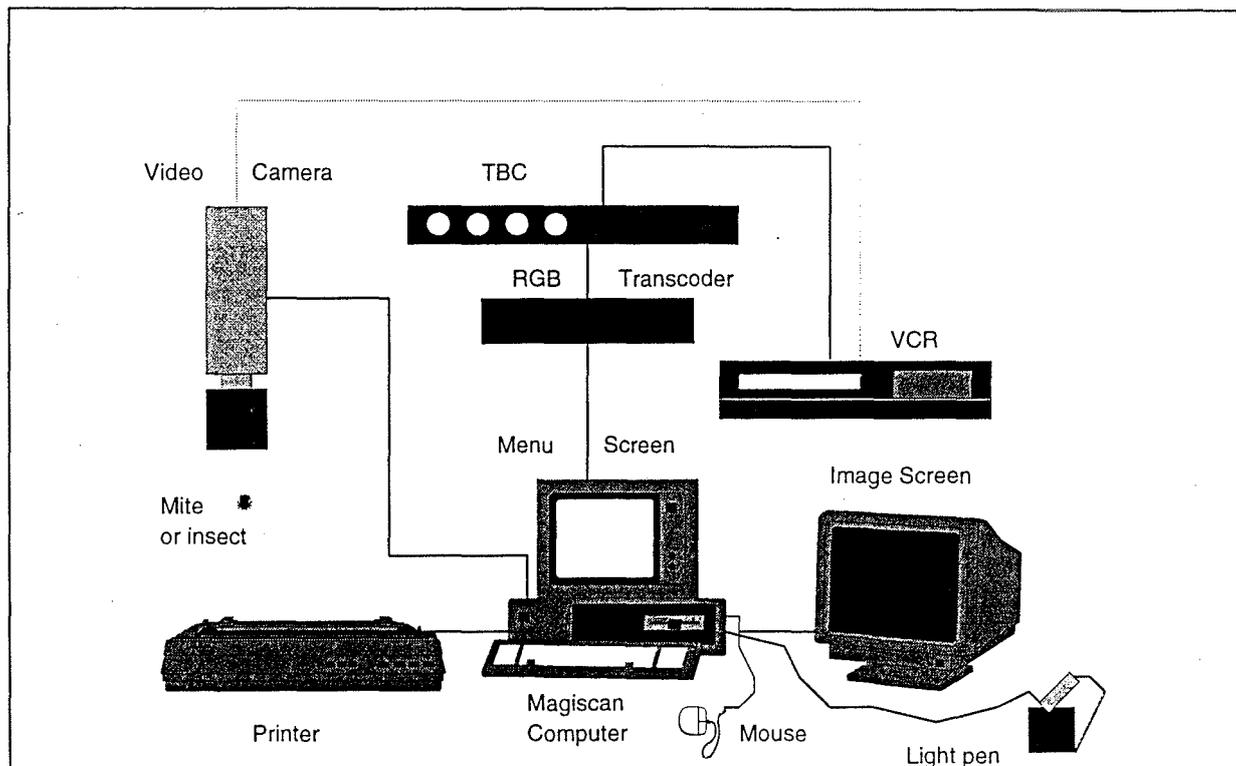


Figure 1. Diagrammatic representation of the equipment used image analysis experiments.



3.7 Image Processing

The set up used was teletracker-type system described by Young *et al.*, (1994), but for the purpose of this thesis it will be referred to as image analysis. A Magiscan computer (Joyce Loeb) digitised the images using the software program BUGSY (Appendix I, Joyce Loeb). Using a mouse, the brightness threshold level was set to separate the mite image from the background. In addition, adjustment of the video level and black level on the TBC optimised the image quality. In order to convert pixels to a common unit of measure (mm), calibration of a known distance (a ten mm micrometer) was videoed using the experimental set-up, and was entered using the light pen. The scale factor calculated from the input value was then used in the following analysis. The frequency of the sampling interval was then entered (1/second).

Once an analysis was initiated, the mite's x and y coordinates were automatically recorded and joined in real time to produce a track that modelled its movement. Sequential x,y data generated during each run was viewed at the end of each run up to

a maximum of 3000 coordinates (approximately 48 minutes). Data sets and binary images of tracks were edited to obtain standard sample lengths. Both binary images and data were saved on disc for future reference or for additional analysis using statistical packages.

3.7.1 Software

A separate FORTRAN program called MIKE (Appendix II) was written to analyse x,y coordinates and compute the required parameters (see analysis section below). The MIKE program produced three output files: a report file consisting of columns of sequential movement data (Table 1); a table file of movement statistics that summarised the data (Table 2); and, a movement file which recorded the number and duration of events (in chronological order) where there was activity and inactivity. Single pixel jitter, a problem found by other authors (Mueller-Beilschmidt and Hoy, 1987), was compensated for in the MIKE program by ignoring single pixel movement so that the mite being tracked had to move at least a distance of two pixels before movement was recognised.

A simple FORTRAN program called DFILE (Appendix III) was used to create files of equal length for additional analysis. An additional FORTRAN program called CFILE (Appendix IV) was used to select specific parameters of the statistical output from MIKE for input into the statistical packages MINITAB (Minitab Inc.) and STATISTIX (Analytical Software). Another program, BUG2EPS (Appendix V), was developed mainly to print laser-quality images of mite paths, however, this program also allowed visual analysis of tracks on a step-by-step basis.

To examine the potential use of fractal dimensions as a measure of tortuosity of mite paths, an additional FORTRAN program, FRACTAL (Appendix VI), was developed to analyse mite movements in relation to step length (sampling interval).

3.7.2 Analysis of parameters

Movement parameters calculated by the MIKE program are divided up into measurement between coordinates and those that describe an entire track. The brackets contain the abbreviated form used in the thesis.

3.7.2.1 Between coordinates (Table 1):

(a) *Distance travelled* (DIST) - distance moved per specified time interval (mm).

(b) *Angle turned* (TURN)- angular deviation from previous direction. A clockwise turn is positive and a counter-clockwise is negative (-180 to 180°). Calculation for this parameter is based on the smallest angular distance ($\leq 180^\circ$) of two directions: $\arccos[\cos(\phi-\psi)]$, where ϕ and ψ are the two angles (Batschelet, 1981).

(c) *Heading angle* (ANGLE) - gives direction on a 360° scale determined by a line joining the last two coordinates.

(d) *Speed* (SPEED) - distance/time (mm/second).

3.7.2.2 Over entire track (Table 2):

(a) *Mean speed* (SPEED) - average rate of movement (mm/second).

(b) *Stationary time* (STIME) - period of inactivity (measured in seconds).

(c) *Mean angle* (ANGLE) - mean heading angle, indicates the preferred direction (360° scale). The mean angle of the sample was calculated based on the method of Batschelet (1981).

(d) *Weighted mean vector length* (WMVL) - describes concentration of sample points around mean direction. A value of zero corresponds to no cluster, and a value of one corresponds to no deviation.

(e) *Arithmetic mean vector length* (AMVL) - mean distance between coordinates (mm).

(f) *Mean angular deviation* (MAD) - measures clustering or dispersion (s) of heading angle values around the mean angle (expressed in degrees), where $s = [2(1-r)]^{1/2}$ (Batschelet, 1981).

(g) *Absolute mean turn (AMT)* - average of the absolute turn values expressed in degrees.

(h) *Mean meander (MM)* - mean of turn/distance at each sampling point (degrees turned/mm moved) and was based on the calculation described by White *et al.* (1984) where mean meander = $\Sigma \text{ABS}(\text{turn angle}/\text{dist})/n-1$.

(i) *Coefficient of a straight line (CSL)* - also known as linearity of travel or index of straightness. This was calculated by dividing the 'beeline' (shortest distance between the start and end points) by the actual length of the pathway, where one is a straight line and values approaching zero are tortuous.

(j) *Total distance travelled (TDIST)* - a running total of distance travelled (mm).

3.8 Mechanics, processes and data analysis

The behavioural parameters (above) were tested for potential problems or factors influencing the results.

3.8.1 TSM time-course/settling time

To determine whether the mites should be given time to equilibrate to their new surroundings (behavioural arena) before behaviour is monitored, the following experiment was conducted. TSM were placed onto leaf discs (arena 1) and their behaviour was recorded onto video tape for 48 minutes (3000 *x,y* coordinates). Twenty-seven replicates were used and each replicate consisted of a fresh leaf disc and new mite. Each segment of 48 minutes was divided up into 12 chronological segments of four minutes (250 *x,y* coordinates each). Means and the standard errors for each four minute segment over the 27 mites were calculated for seven parameters: absolute mean turn, total distance travelled, time stationary, mean meander, tortuosity, arithmetic mean vector length and angular deviation. Regression equations and r^2 values were calculated for each parameter to determine whether there were any significant linear (or non-linear) relationships over time.

Figure 2. Sample of digitised mite track with ten data points. The associated image analysis output is given below (Table 1 and 2).

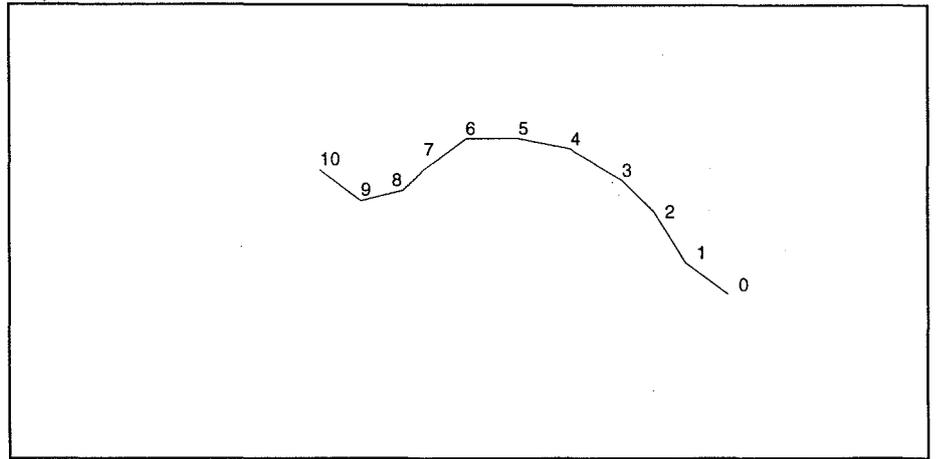


Table 1. An example of a report file produced by MIKE program of x and y coordinates of the above mite track (Figure 2).

THIS FILE IS: 200792.DAT
 SCALE FACTOR: 3.000E-02
 TIME IS EXPRESSED IN : SECONDS

INDEX	X	Y	DIST	ANGLE	TIME	TURN	SPEED	TDIST	DIGITIME
0	435	311			13:52:41:54				
1	408	344	1.28	129.29	13:52:42:48	-129.29	1.361	1.28	.94
2	396	388	1.37	105.26	13:52:43:46	24.03	1.396	2.65	.98
3	381	420	1.06	115.11	13:52:44:40	-9.86	1.128	3.71	.94
4	344	445	1.34	145.95	13:52:45:39	-30.84	1.353	5.05	.99
5	304	454	1.23	167.32	13:52:46:32	-21.37	1.323	6.28	.93
6	261	452	1.29	182.66	13:52:47:31	-15.34	1.304	7.57	.99
7	230	430	1.14	215.36	13:52:48:24	-32.70	1.226	8.71	.93
8	208	412	0.85	219.29	13:52:49:23	-3.93	0.861	9.56	.99
9	168	405	1.22	189.93	13:52:50:17	29.36	1.296	10.78	.94
10	137	435	1.29	135.94	13:52:51:15	53.99	1.321	12.07	.98

Table 2. Example of table file produced by the MIKE program with summary statistics of behaviour above (Figure 2 and Table 1).

TABLE FILE IS:	200792.DAT
MEAN SPEED PER SECOND	= 1.26
STATIONARY TIME (SECS)	= .00
MEAN X (XBAR)	= -.08
MEAN Y (YBAR)	= .08
MEAN ANGLE	= 136.88
WEIGHTED MEAN VECTOR LENGTH (r)	= .11
ARITHMETIC MEAN VECTOR LENGTH	= 1.21
MEAN ANGULAR DEVIATION (s)	= 76.28
ABSOLUTE MEAN TURN	= 35.07
MEAN MEANDER	= 27.93
DISTANCE AS THE CROW FLIES	= 9.68
INDEX OF STRAIGHTNESS	= .80
TOTAL DISTANCE TRAVELLED	= 12.07

3.8.2 Fractal dimensions and sampling frequency

Fractal dimensions were calculated using a modification of the 'dividers' method. \ln (path length) is regressed on \ln (step size) where step sizes are of varying length (in this study, 1, 2, 4, 8, 10, 16, 40, 80 and 100 s). Using the relationship $\ln(L) = \ln(k) + (1-D)\ln(\lambda)$ where L is the path length, measured at stepsize (λ) and where k is the constant the fractal dimension D , can be calculated (Dicke and Burrough, 1988). Fractal dimensions were calculated for both ERM and *T. pyri* as an alternative parameter for characterising the tortuosity of their locomotory behaviour. Data sets of 3000 points were used and fractal dimensions of mite paths on control leaf discs were calculated for both species. All calculations were done using a special purpose FORTRAN program called FRACTAL (Appendix VI). The program required five step sizes to calculate the fractal dimension, therefore two fractal dimensions were calculated in some experiments (using step sizes 1, 2, 4, 8 and 16 s, for one value and 1, 10, 40, 80 and 100 s for the other).

Path lengths at the various step sizes calculated by the FRACTAL program, were also used to investigate the effect of sampling interval on overall path length. The mean values of the \ln (path length) were plotted against the \ln (step size) to determine the nature of the relationship of the two variables for both ERM and *T. pyri*.

3.8.3 Sample length

To examine the effect of sample length on the behavioral parameters, six randomly chosen TSM sequences of 48 minutes from the "time-course/settling experiment" were broken down into 24, 12, 6, and 3 minute time segments starting from the same point as the original full length 48 minute segment. Using SigmaPlot® 2.0 (Jandel Corporation) the following parameters were plotted versus increasing sample length: weighted mean vector length, mean angular deviation, mean angle, distance travelled, meander, tortuosity (CSL), arithmetic mean vector length and absolute mean turn.

3.8.4 Parameter correlations

To understand the relationships between the parameters used in this study Pearson correlations (Zar, 1984) were used to determine "relatedness" of parameters from the synthetic pyrethroid behavioural study with ERM and *T. pyri* (see behavioural responses of mites to sub-lethal SP). Only the control treatments (nine replicates of each) were used for both species to avoid any interference which may be caused by the arenas that were half-treated with esfenvalerate.

3.8.5 The usefulness and reliability of parameters for measuring tortuosity

To test the ability of the movement parameters to distinguish between different behaviours a 'straight' and a 'tortuous' segment of equal time (100 coordinate pairs) were selected from each of ten TSM tracks for analysis. Fractal dimensions were calculated as described above using step sizes 1, 2, 4, 8, and 16 seconds. Statistics were calculated using the MIKE program and frequency histograms of the two types of behaviour were plotted using Harvard Graphics version 3.0 (Software Publishing Corporation).

3.8.6 Turn and angle aberration

To determine whether there was any behavioural bias caused by unintentional stimuli as a consequence of the methods used, frequency distributions were analysed for their symmetry. The procedure used to investigate turn and angle distributions is described below.

3.8.6.1 Turn distributions

To test for symmetry around 0° , which is expected in unimodal distributions (Casas, 1988; Batschelet, 1981), frequency histograms of turn angles of combined values for five TSM, nine ERM and nine *T. pyri* from control treatments were constructed. Although each individual mite had 3000 turn values, the zero turn values corresponding to a stationary mite were removed from the data to leave the 'true' turn values of moving mites only. The percent frequency for each mid-point at graduations of 15° was calculated and histograms were plotted using Harvard Graphics.

3.8.6.2 Angle distributions

To investigate any bias that may exist within the pixel configuration of the analysis system, percent frequency distributions of the angle values from five TSM each with 3000 data points was calculated and a circular histogram was constructed using a radar plot (MicroSoft Excel[®]) with a resolution of 5° .

3.8.7 Humidity

The humidity experiment was primarily conducted to evaluate arena 2 (described earlier) for the ability to use the behavioural arena at different humidities. However, as the mite behaviour was shown to be highly modified by some humidities it was decided to include the data even though only three replicates were used for each treatment. To test the effects of different relative humidities on behaviour at $25 \pm 1^\circ\text{C}$, three relative humidities were investigated: $10 \pm 5\% \text{RH}$ using saturated sodium hydroxide (NaOH), $50 \pm 5\% \text{RH}$ using the ambient setting of the constant temperature room, and $95 \pm 5\% \text{RH}$ using water. The behavioural parameters (described earlier) were calculated for each of the three replicates of the three humidity treatments.

3.8.8 Edge-effect

To determine the effect of the arena edge on behaviour, two 25 point segments for each of eleven TSM tracks were selected. The first segment included repeated encounters of the edge, the second segment had no edge encounters. With respect to the latter, care was taken not to choose straight walking segments. The fractal dimension using step sizes of 1, 2, 4, 8 and 16 s were calculated. In addition, the standard parameters

calculated were tested by the Wilcoxon rank sum test (Vet and Papaj, 1992) to determine whether the 'edge' and 'non-edge' behaviour differed significantly. Turns resulting from the respective behaviours were combined (excluding stationary zero value turns) and their distributions plotted as histograms using Harvard Graphics.

3.8.9 Behavioural responses of mites to sub-lethal SP

In this experiment ERM and *T. pyri* were placed in the centre of a peach leaf disc and coverslip respectively (see earlier description) in which one half of the arena was sprayed with esfenvalerate at the recommended field concentration (0.015 g a.i. l⁻¹) and the other half with deionised water. Control arenas were completely sprayed with deionised water. A Potter precision spray tower operating at 55kPa with 5 s settling time, was used to produce droplets of uniform size and distribution from a 2.0 ml sample. An acetate strip was used to mask half the leaf disc when spraying the esfenvalerate treatment. Residues were left to dry for ca. 20 minutes before an adult female mite was placed in the centre of each arena. Video recording started immediately and continued for at least 48 minutes at 25±1°C and 50±5% RH. For ERM the experiment was replicated ten times for the insecticide-treated discs and nine times for the water-only control discs. For *T. pyri*, the insecticide-treated discs and control discs were replicated twelve and nine times respectively. The video tapes were stored for analysis at a later date.

All parameters calculated by the MIKE program were computed for the control and half-insecticide-treated leaf discs for both species. The Wilcoxon rank sum test (two sample) was used to determine if significant differences in mite behaviour occurred between the two treatments.

A light pen was used to find the extreme left and right points of the leaf disc to enable the central *x* pixel value between the insecticide-treated and control halves of the leaf discs to be determined. Using Minitab, the known central *x* pixel value was used to sort distance and turn information for the mites into the left and right halves of each leaf disc. This allowed the mite behaviour of treated and untreated halves of the treated arena, and the left and right halves of the control leaf discs to be compared. Frequency distribution histograms of distance travelled and turns exhibited by mites were produced by Harvard Graphics (Figures 11-14). A two-tailed Kolmogorov Smirnov test (Casas, 1988) was used to examine whether the distributions were significantly different ($P < 0.05$).

4.1 Mechanics, processes and data analysis

4.1.1 TSM time-course/settling time

The behaviour of TSM on the leaf disc changed greatly over the 48 minute experimental period (Figures 3a-k). Parameter trends over time were tested using linear regression. The regressions of the mean parameters over time showed a wide variety of trends and that many of the parameters are correlated (e.g., mean distance travelled and stationary time). The distance travelled (Figure 3a) by TSM declined significantly ($r^2=0.88$, $P=0.0001$) over the 48 minutes studied, whereas, the mean stationary time per four minute interval (Figure 3b) more than doubled over the same period ($r^2=0.94$, $P=0.0001$). The mean angular deviation ($r^2=0.80$, $P=0.0001$), arithmetic mean vector length ($r^2=0.79$, $P=0.0001$), mean speed ($r^2=0.88$, $P=0.0001$) and absolute mean turn ($r^2=0.91$, $P=0.0001$) declined slowly but significantly over time (Figure 3c-f). The tortuosity of TSM paths (Figure 3g) measured using the coefficient of a straight line (CSL), mean meander, mean number of movement events and mean duration of movement event (Figs 3h-3j) did not show any obvious trend over time. The mean meander did appear to increase initially before proceeding into a gradual decline. The mean number of movement events and mean duration of movement events declined only slightly over the observation period. The largest change over the 48 minute period was seen in mean duration of stay events (Figure 3k) which increased steadily over time ($r^2=0.80$, $P=0.0001$).

4.1.2 Sampling frequency

The mean distance travelled (path length) versus step size (sampling frequency) of *T. pyri* and ERM are compared in Figure 4. ERM and *T. pyri* gave similar-shaped curves with path length decreasing with increasing step size. *T. pyri* had a considerably longer path length of a step size of 1s ($\ln=0$) than ERM and the decline in mean path length was slightly steeper with increasing step size with *T. pyri*. ERM had a more linear response up to step size 16 s ($\ln = 2.77$). Over 5% of total path length is 'lost' by reducing sampling frequency from one per second to one every two seconds, and if a sampling frequency of one per four seconds then 12% of the total path length is 'lost'.

Figure 3a. Mean distance travelled by TSM over a 48 minute period

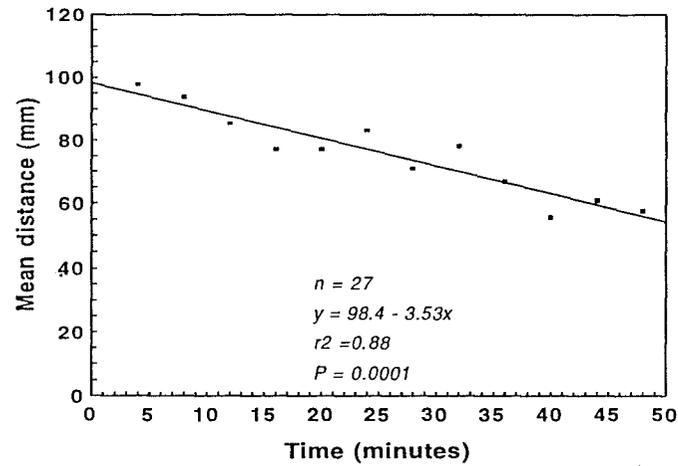


Figure 3b. Mean duration of stationary time by TSM over a 48 minute period

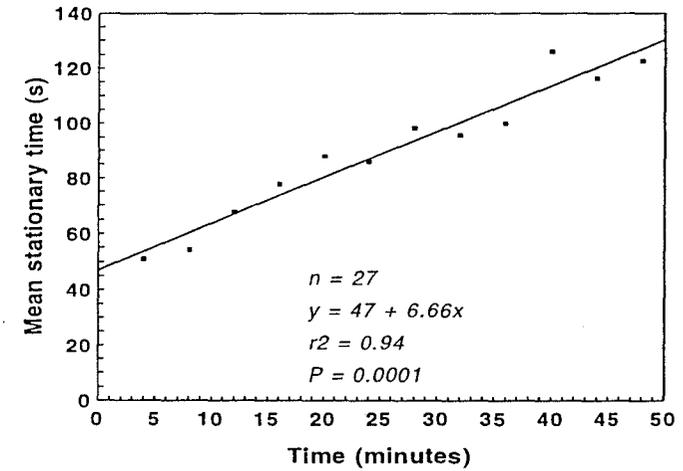


Figure 3c. Mean angular deviation by TSM over a 48 minute period

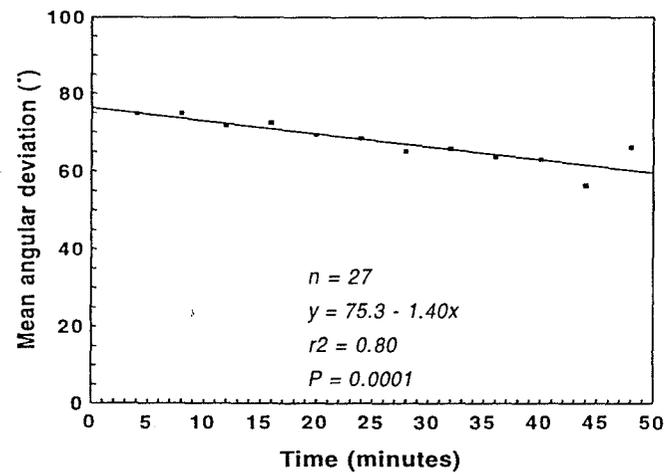


Figure 3d. Mean arithmetic vector length (AMVL) by TSM over a 48 minute period

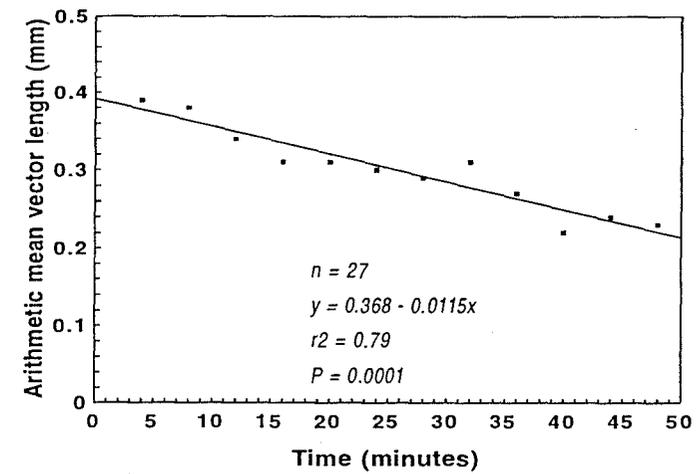


Figure 3e. Mean speed by TSM over a 48 minute period

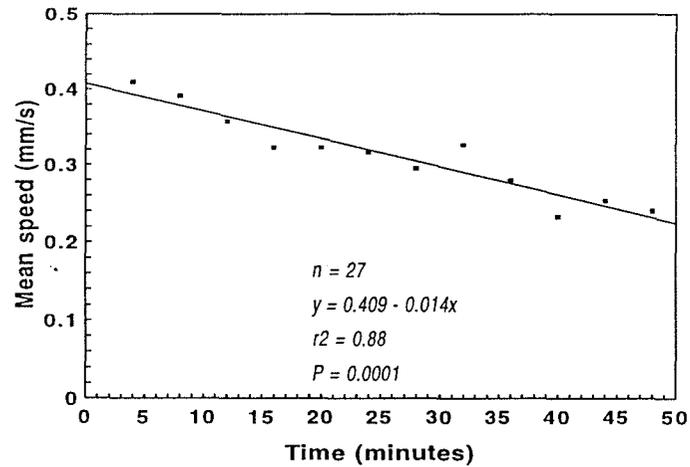


Figure 3f. Mean absolute turn (AMT) by TSM over a 48 minute period

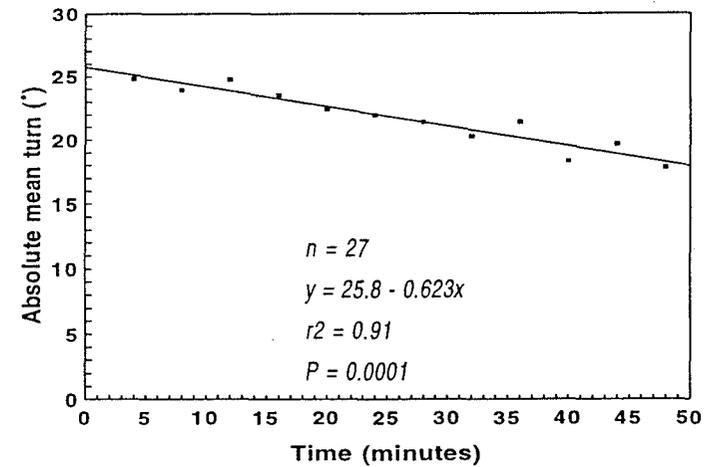


Figure 3g. Mean tortuosity by TSM over a 48 minute period

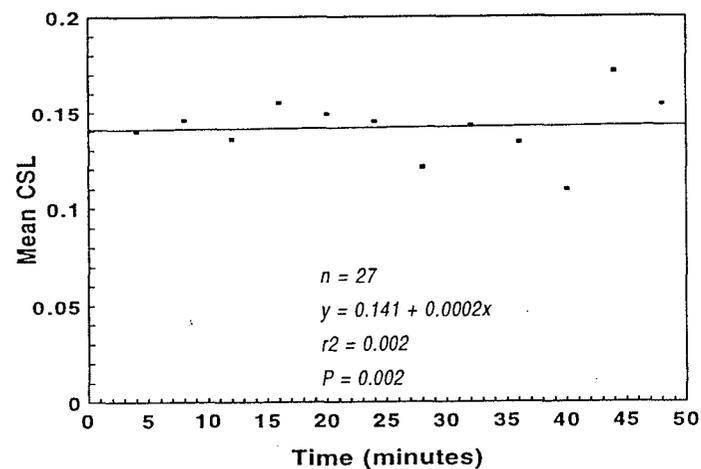


Figure 3h. Mean meander by TSM over a 48 minute period.

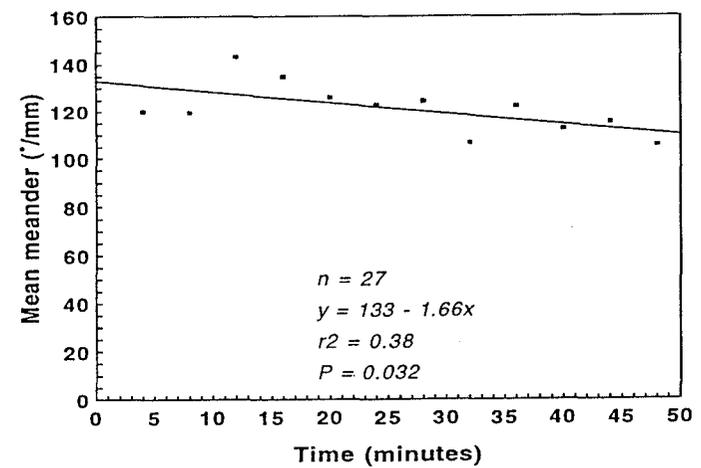


Figure 3i. Mean number of movement events by TSM over a 48 minute period

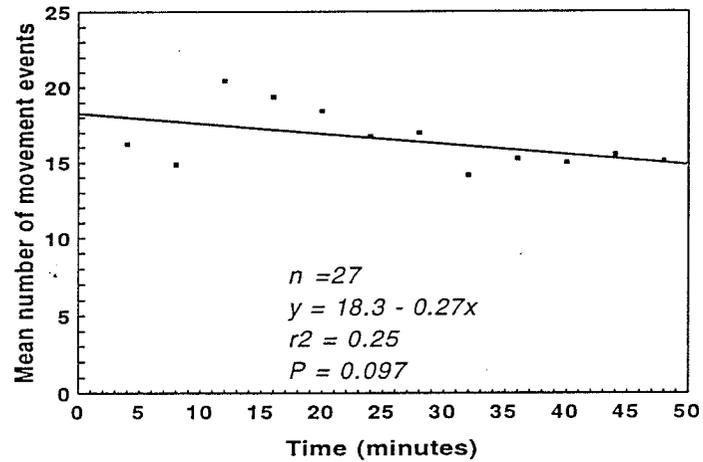


Figure 3j. Mean duration of movement events by TSM over a 48 minute period

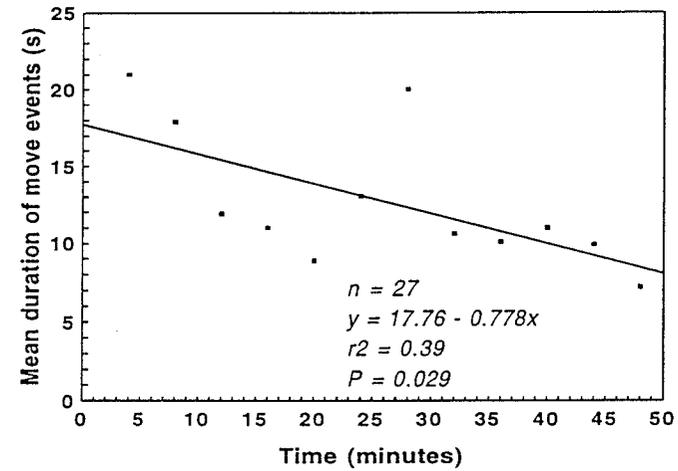


Figure 3k. Mean duration of stationary events by TSM over a 48 minute period

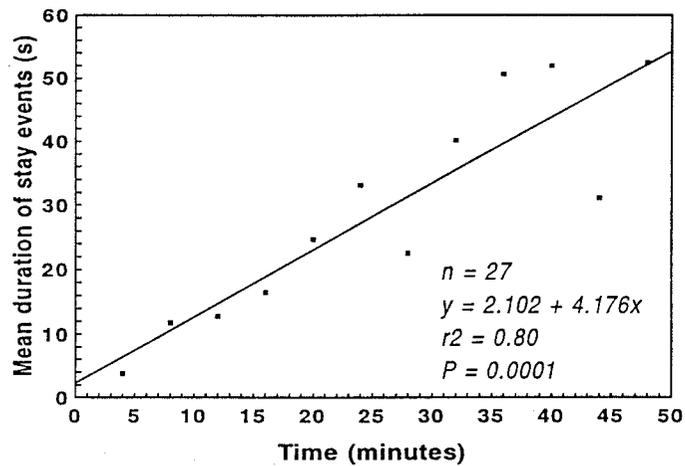
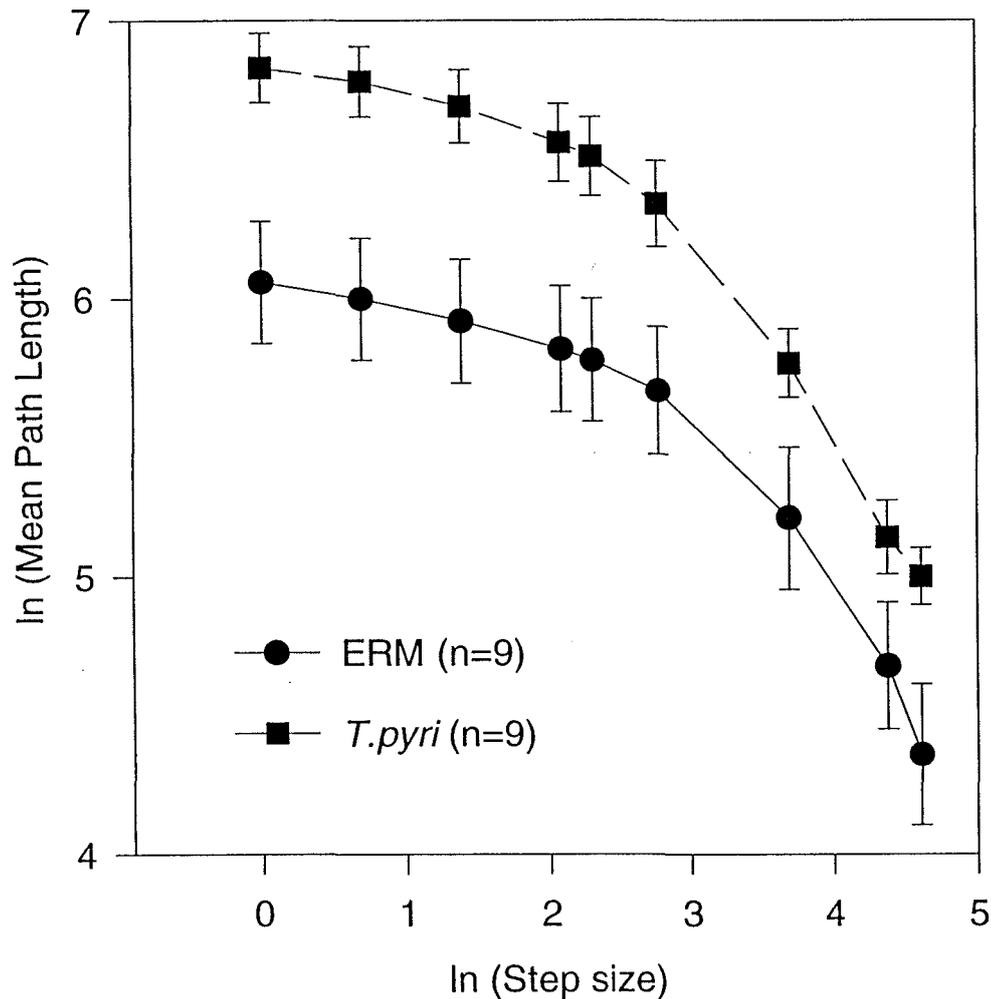


Figure 4. The effect of step size on the mean path length (\pm SEM) for ERM and *T. pyri*.



4.1.3 Effect of sample length on movement parameters

The size of the sample length for analysis greatly influenced the result given for some of the mean parameters calculated for six randomly chosen mites (Figures 5a-h). As expected, the 'index of straightness', mean turning angle and mean number of movement events changed most dramatically with sample length (Figures 5a-c). The mean number of movement events showed a linear response with sample length. The 'index of straightness' decreased greatly with increasing sample length because of the way it is calculated (Figure 5a). The longer the period the mite is observed, the more likely it will reach the edge and turn, thus giving a lower CSL value. Results show that for most parameters, the sample length could be decreased by half to 24 minutes (or 1500 points) without any significant loss in information. Mean angular deviation was the most consistent parameter throughout the experimental period (Figure 5d).

Figure 5a. The effect of sample length on the mean tortuosity (\pm SEM) in TSM.

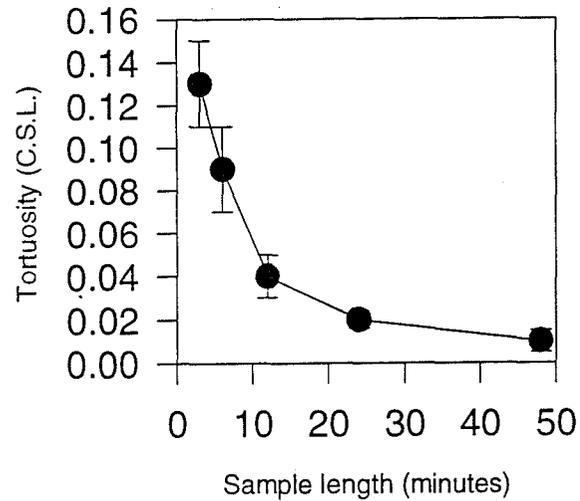


Figure 5b. The effect of sample length on the mean heading angle (\pm SEM) in TSM

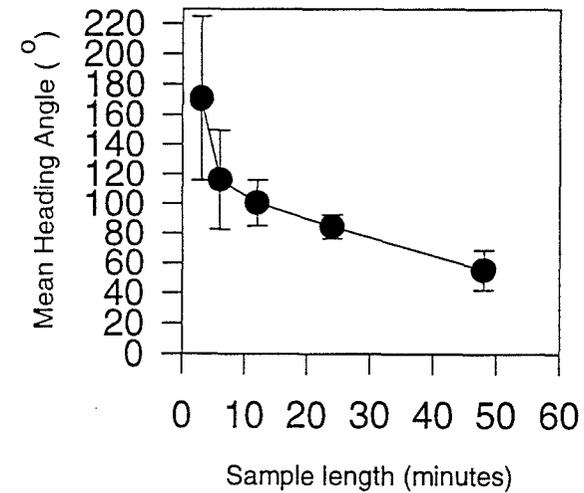


Figure 5c. The effect of sample length on the mean number of movement events (\pm SEM) in TSM

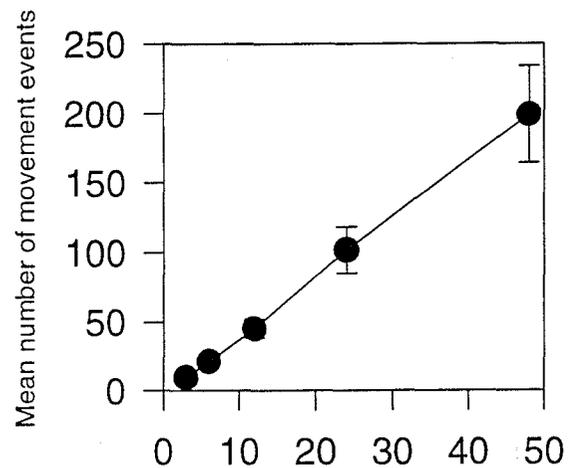


Figure 5d. The effect of sample length on the mean angular deviation (\pm SEM) in TSM

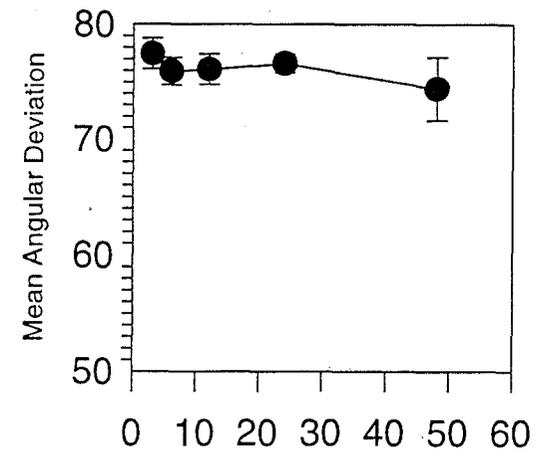


Figure 5e. The effect of sample length on the weighted mean vector length (\pm SEM) in TSM

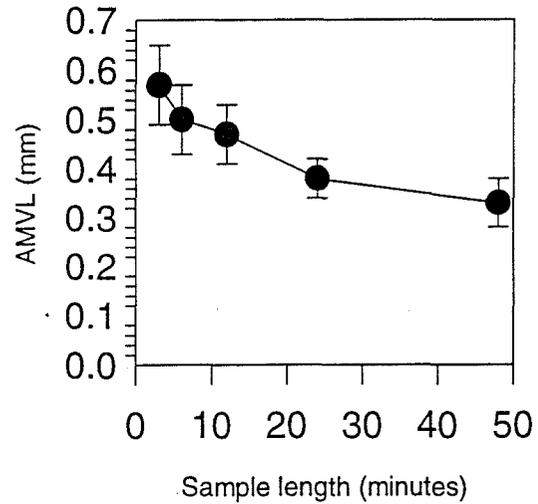


Figure 5f. The effect of sample length on the mean meander (\pm SEM) in TSM

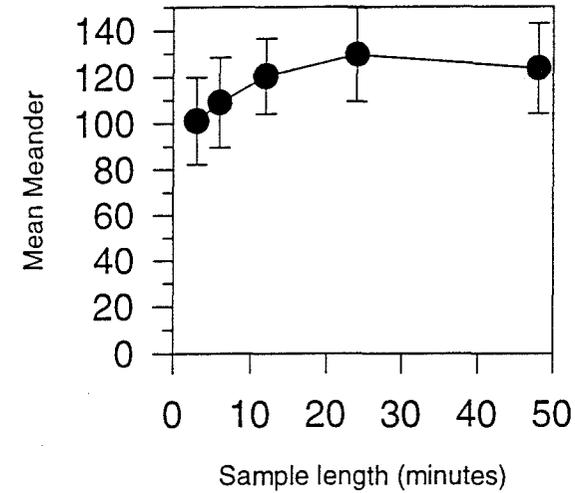


Figure 5g. The effect of sample length on the absolute mean turn (\pm SEM) in TSM

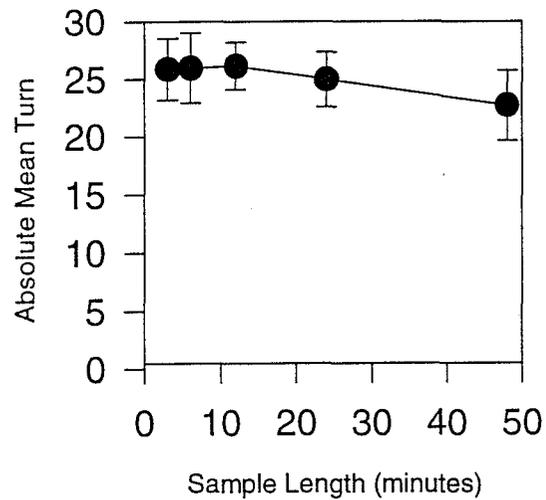
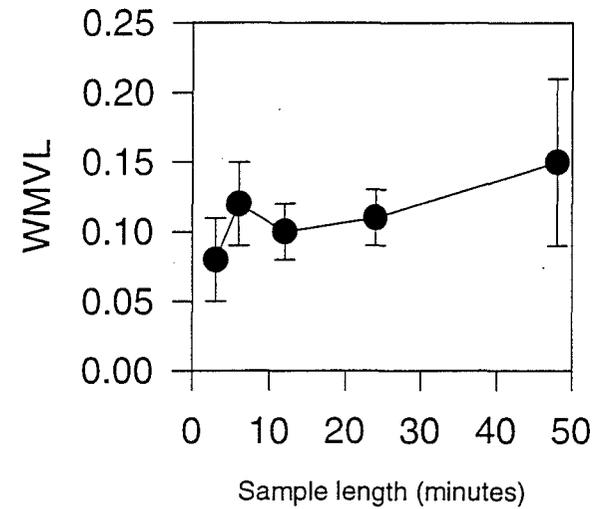


Figure 5h. The effect of sample length on the mean arithmetic vector length (\pm SEM) in TSM



4.1.4 Parameter correlation

Tables 3 and 4 show the correlation matrix between the parameters used in the analysis of ERM and *T. pyri* behaviour on untreated arenas. Consistent significant positive correlations ($P < 0.001$) are shown between speed, total distance and arithmetic mean vector length for both species. Such correlations are expected because both speed and total distance are calculated using arithmetic mean vector length. The other significant (negative) correlation ($P < 0.001$) is between mean angular deviation and weighted mean vector length for both species. Again, this would be expected because both parameters measure the degree of clustering around the mean heading angle. Two other significant correlations between parameters for both species were found between mean meander and movement 'events' (positive), and between absolute mean turn and stationary time (negative). Fractal dimension and coefficient of a straight line were correlated ($P < 0.05$) for both species.

Table 3. Correlations of parameters used in ERM control experiment. Because the correlation matrix is symmetric, only correlations below the diagonal are shown.

	AMT	AMVL	Fractal 100	MAD	Meander	Events	Speed	CSL	Total Dist.	Time Stationary
AMVL										
Fractal										
MAD		*								
Meander					***					
Events						***				
Speed							*			
CSL			*							
TDist								***		
TStat	**	*				*	*		*	
WMVL		*					*		*	*

* P<0.05
 ** P<0.01
 *** P<0.001

Table 4. Correlations of parameters used in *T. pyri* control experiment. Because the correlation matrix is symmetric, only correlations below the diagonal are shown.

	AMT	AMVL	Fractal 100	MAD	Meander	Events	Speed	COL	Total Dist.	Time Stationary
AMVL	*									
Fractal										
MAD										
Meander										
Events					*					
Speed	*	***								
CSL			*							
TDist	*	***				*	***			
TStat	**	***		*			***	***		
WMVL				***						*

* P<0.05
 ** P<0.01
 *** P<0.001

4.1.5 Measuring tortuosity

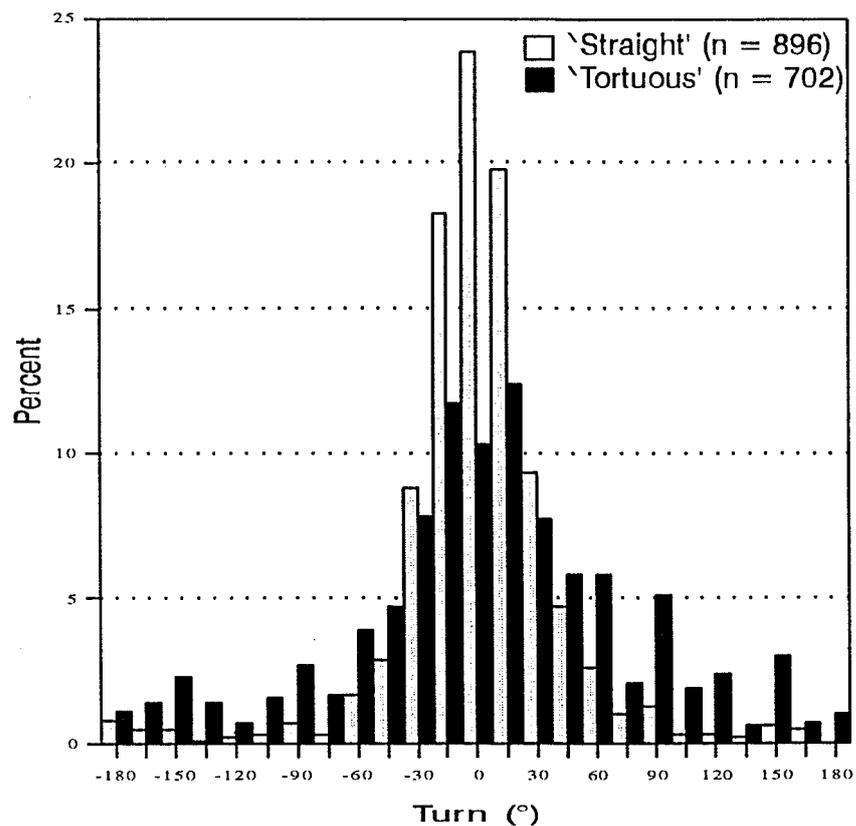
The mean meander and AMT parameters differentiated best between the two behaviours ('tortuous' and 'straight') as shown by their significance values ($P=0.0059$ for both)(Table 5). However, because mean meander incorporates both turning and distance travelled, mean meander was considered to be the more sensitive parameter of the two.

To further illustrate the differences between the two behaviours, frequency histograms of 'tortuous' and 'straight' behaviours were combined to show differences in the turn angle distributions (Figure 6). In the 'straight' behaviour, smaller turns in the -30° to $+30^\circ$ range contributed to 62% of the turns, whereas the same range in the 'tortuous' behaviour contributed only 34% of the turns.

Table 5. The effect of the two types of movement on several behavioural parameters.

Parameter	Movement behaviour				P< 0.05
	'Tortuous'	S.E.	'Straight'	S.E.	
	(N=10)		(N=10)		
Mean speed (mm/s)	0.15	0.03	0.50	0.08	*
Mean stationary time (s)	30.0	5.01	9.9	4.44	*
Weighted mean vector length	0.19	0.02	0.15	0.05	NS
Arithmetic mean vector length	0.14	0.03	0.49	0.08	*
Mean angular deviation (°)	72.9	0.76	74.2	2.21	NS
Absolute mean turn (°)	37.2	1.94	23.7	1.47	*
Mean meander (°/mm)	272.1	21.8	87.8	9.83	*
Mean coefficient of a straight line	0.09	0.03	0.21	0.06	NS
Mean fractal dimension (1-16)	1.36	0.04	1.10	0.02	*

Figure 6. Percent frequency distributions of turns for 'tortuous' and 'straight' TSM movement.

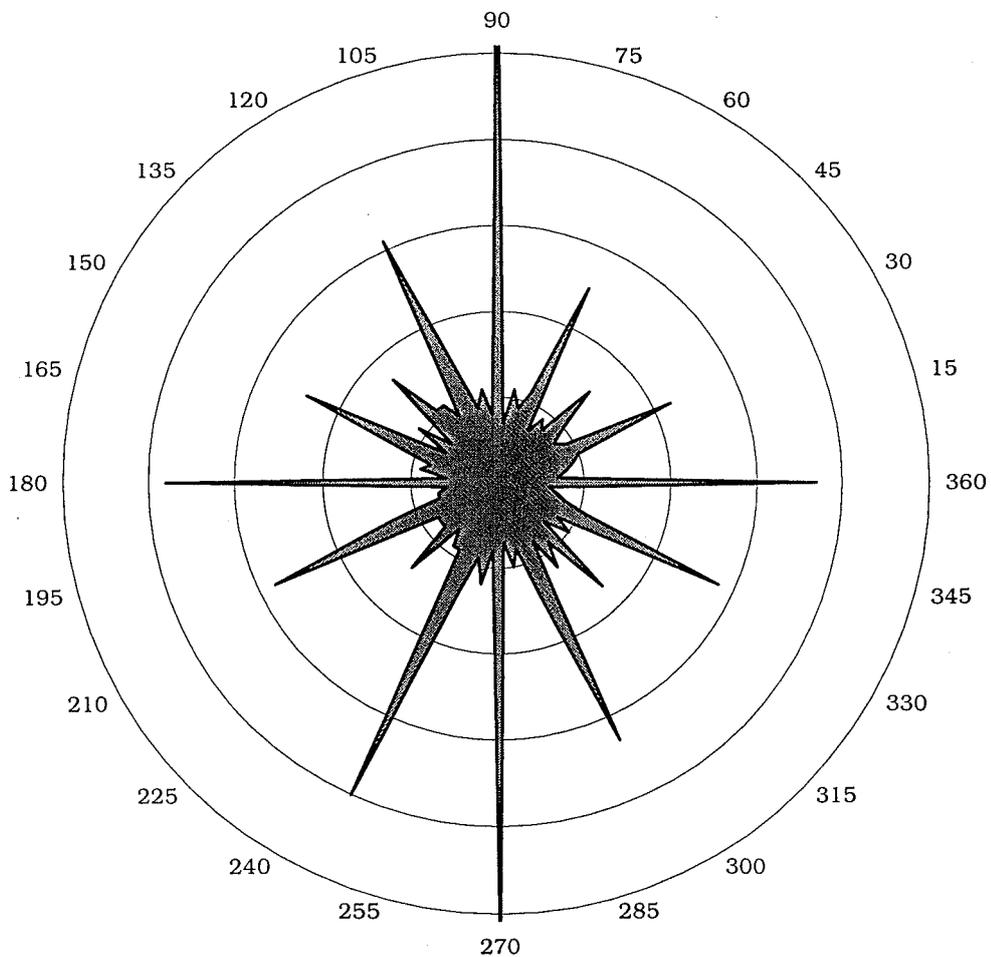


4.1.6 Angle and turn aberrations

4.1.6.1 Angle distributions

Midpoints (with a range of 5°) of 3000 heading angles from five TSM mites were combined and plotted using a radar graph (Microsoft Excel). Figure 7 shows some angles have much greater frequency when compared to others. The points of the 'star' represent the preferred direction and each concentric circle is equivalent to a frequency of one percent. Heading angles 90° and 270° were the most frequently occurring at 7.2% and 5.6% of the time respectively (truncated in Figure 7). Other angles of 1% or greater include: 25°, 65°, 115°, 155°, 180°, 205°, 245°, 295°, 335° and 360°, resulting in an almost symmetrical 'star'.

Figure 7. Percent frequency of angles moved by TSM (total of 15,000 moves from 5 mites). The concentric circles represent a frequency of one percent.



4.1.6.2 Turning distributions

ERM, TSM and *T. pyri* displayed very similar turning distributions (Figures 8a-c). Each species had a positive turn bias which was demonstrated by more turns clockwise to the right (+) than anti-clockwise to the left (-) between the angles of $\pm 105^\circ$ and 0° . The means of ERM ($+2.89^\circ$) and *T. pyri* ($+2.84^\circ$) were almost identical, whereas TSM produced a slightly lower mean turn of $+2.67^\circ$. The standard deviation varied from 55.22 for *T. pyri* to 66.51 for ERM. Compared to the other two species, TSM appear to move in a straighter path, where the majority of turns were in the range between -30 to $+30$. For the three species turns of $\pm 180^\circ$, 150° , 120° , and sometimes 90° occurred more often than adjacent angles creating 'peaks' of higher frequency than would be expected in a 'perfect' normal distribution (Figures 8a-c).

4.1.7 Humidity effect

The use of different salt solutions to control the relative humidity of the arena resulted in identifiable changes in mite behaviour (Figures 9a-c). TSM exposed to 10% RH were more inclined to move around the edge of the arena than when exposed to the other humidities. One replicate (051190A) at 95% RH displayed some unusual circling behaviour. Only three replications were used in this experiment and any test of statistical significance of behavioural parameters between the three humidities was not considered appropriate. At 50% RH, TSM appeared to remain stationary for longer, had the lowest mean meander, mean angular deviation and absolute mean turn compared to the more extreme humidities (10% RH and 95% RH). Such results indicate that at the extreme humidities TSM moved more quickly and turned less, presumably in an attempt to escape unfavourable moisture conditions.

Figure 8a. Percent frequency distributions of ERM turns

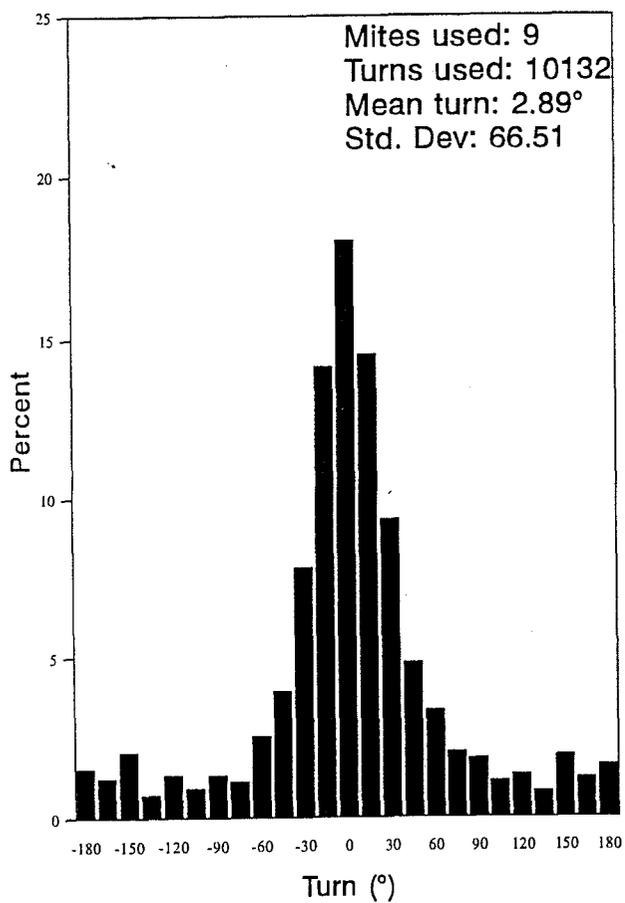


Figure 8b. Percent frequency distributions of *T. pyri* turns

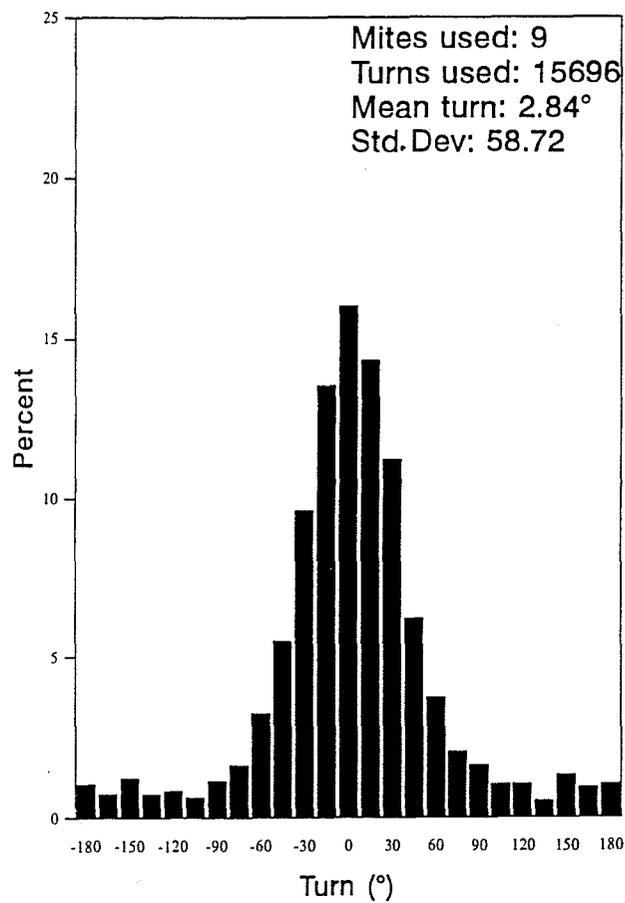


Figure 8c. Percent frequency distributions of TSM turns

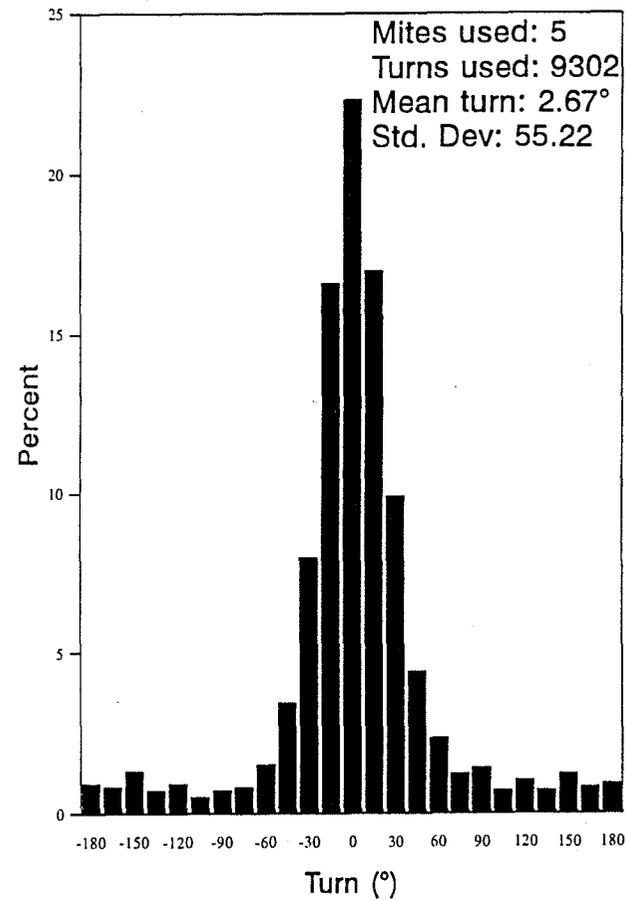
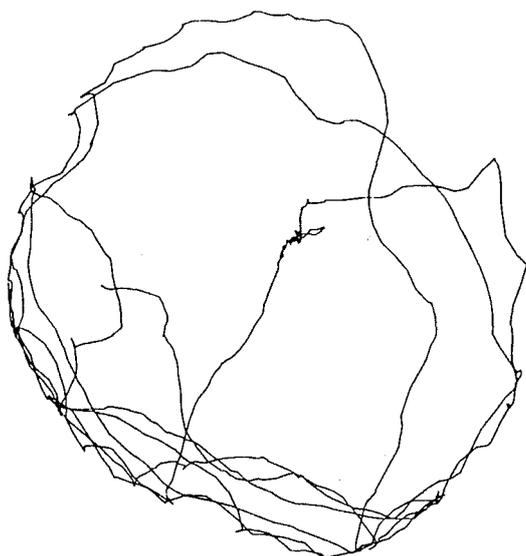
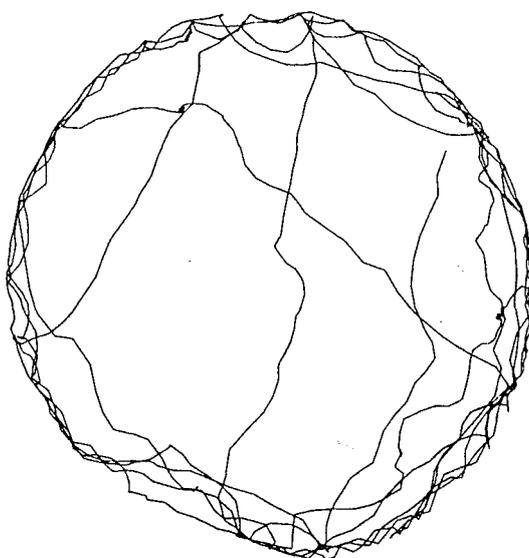


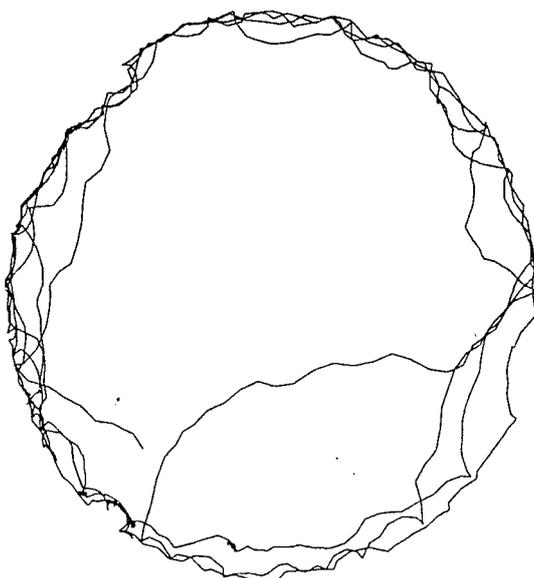
Figure 9a. Pathways of three TSM at 10% R.H. for 20 minutes.



051190E (10%RH)

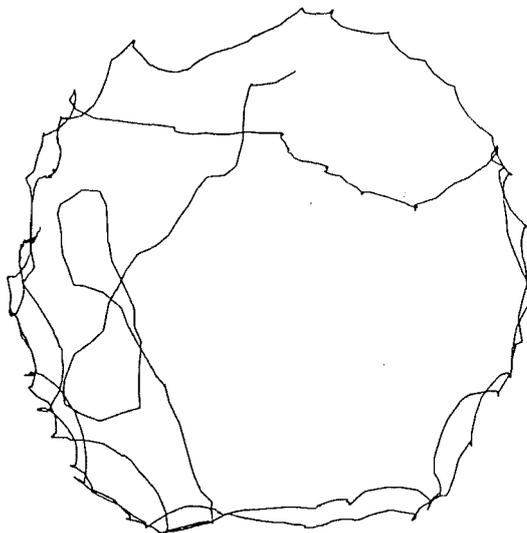


021190I (10%RH)

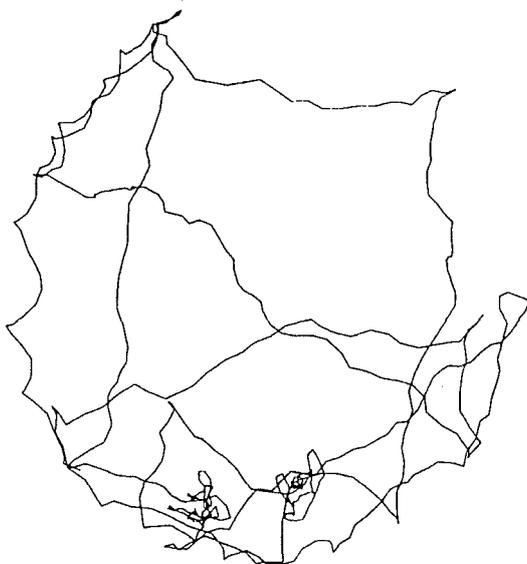


011190B (10%RH)

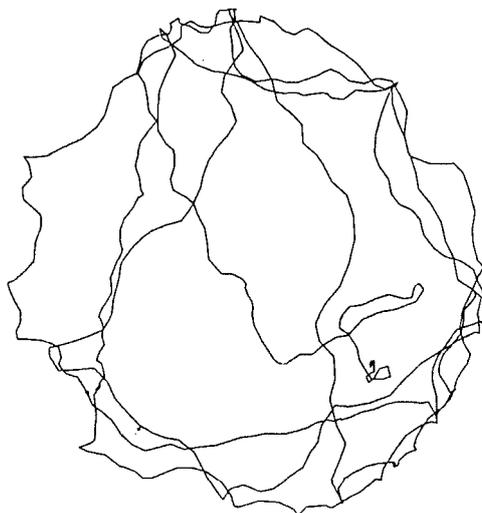
Figure 9b. Pathways of three TSM at 50% R.H. for 20 minutes.



021190A1 (50%RH)

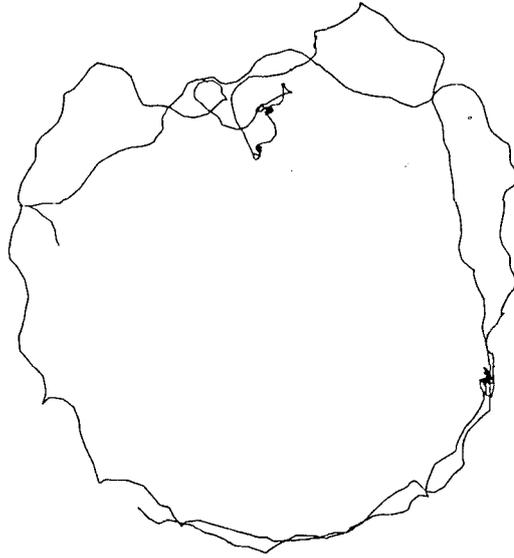


011190A (50%RH)

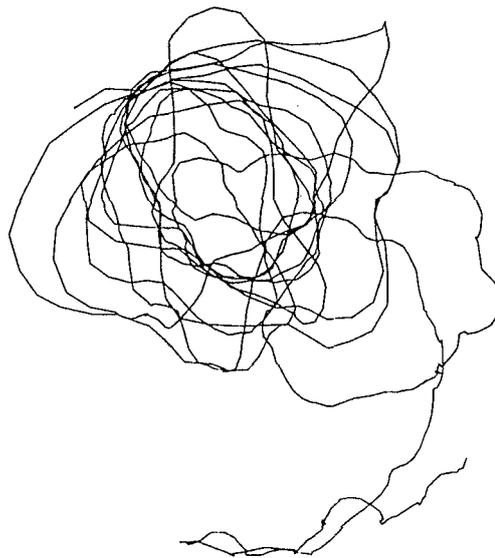


011190E (50%RH)

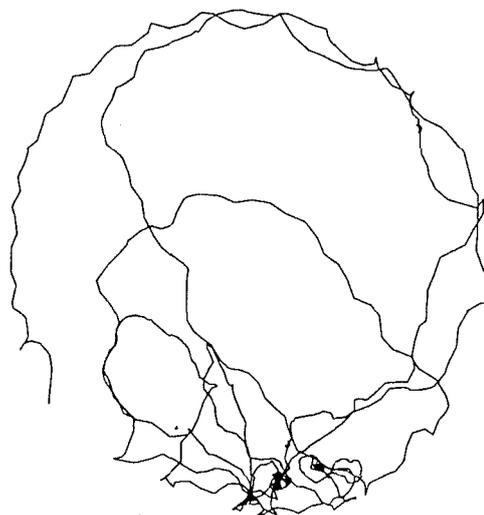
Figure 9c. Pathways of three TSM at 95% R.H. for 20 minutes.



021190B (95%RH)



051190A (95%RH)



011190I (95%RH)

Table 6. The effects of three humidities on the behavioural parameters for TSM.

Parameter	Humidity					
	95% (N=3)	S.E.	50% (N=3)	S.E.	10% (N=3)	S.E.
Mean speed (mm/s)	0.27	0.08	0.24	0.02	0.34	0.04
Mean stationary time (s)	343.4	65.6	416.2	13.0	234.0	17.7
Mean angle (°)	91.8	10.8	82.1	26.4	75.6	12.9
Weighted mean vector length	0.15	0.06	0.28	0.08	0.12	0.02
Mean angular deviation (°)	74.5	2.4	68.6	3.9	76.2	1.0
Absolute mean turn (°)	23.4	3.7	20.5	0.52	27.2	2.75
Mean meander (°/mm)	133.2	32.7	102.1	10.9	136.0	13.8
Coefficient of a straight line	0.053	0.01	0.033	0.01	0.023	0.01
Mean total distance (mm)	260.6	77.9	228.8	13.4	329.9	39.5
Mean stay events	100.7	12.5	61.0	18.0	75.0	10.2
Mean stay duration	3.45	0.61	7.87	1.80	3.18	0.25

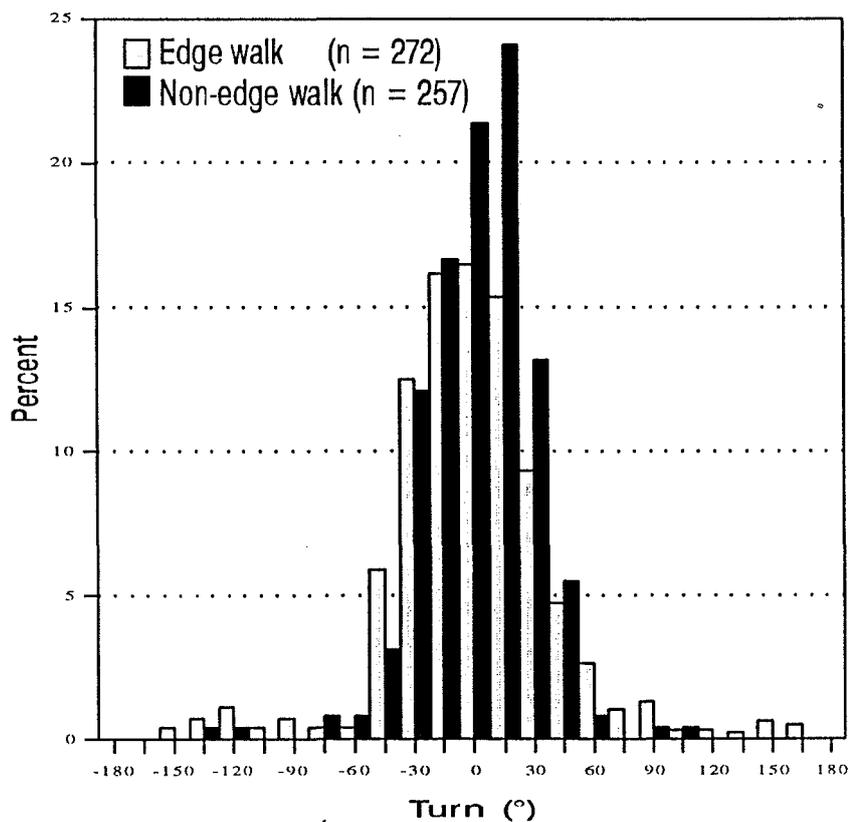
4.1.8 The influence of edge-effect on behavioural measures

The track printouts of mobile mites showed a definite behavioral response to the edges of the arenas; this was particularly noticeable for TSM over a 48 minute period (Figures 9a and b). When mites reached the arena edge the angle of approach was often similar to the angle of departure, much like a pool ball bounces off the cushion. It was thought that the edge of the arena would strongly influence mite behaviour. However, mean meander was the only parameter that could significantly ($P < 0.05$) distinguish between the two behaviours, i.e., 'edge' and 'non-edge' responses. Although differences between the two types of behaviour were evident for AMVL and AMT, they were significant only when combined in the mean meander calculation (Table 7) A histogram of the relative frequency of angles turned show a higher frequency of the smaller angles in the 'non-edge' walk when compared with edge-walking behaviour (Figure 10). 'Non-edge' walking in TSM had an asymmetrical distribution about 0° , where mites preferred turning to the right (clockwise), whereas edge-walking was more symmetrical around zero.

Table 7. The effect of the arena edge on several behavioural parameters for TSM.

Parameter	Behaviour				p<0.05
	'Edge' (N=11)	S.E.	'Non- edge' (N=11)	S.E.	
Mean speed (mm/s)	0.55	0.07	0.74	0.11	NS
Weighted mean vector length	0.23	0.04	0.22	0.03	NS
Arithmetic mean vector length (mm)	0.53	0.07	0.71	0.11	NS
Mean angular deviation (°)	71.0	1.92	71.6	1.18	NS
Absolute mean turn (°)	33.5	2.32	20.2	1.23	NS
Mean meander (°/mm)	114.7	15.8	44.1	6.38	*
Mean coefficient of a straight line	0.78	0.03	0.71	0.08	NS
Mean fractal dimension (1-16)	1.11	0.01	1.07	0.01	NS

Figure 10. Comparison of percent frequency distributions of turns for 'edge' and 'non-edge' TSM behaviour.



4.2 Application

4.2.1 Behavioural responses of ERM to a sub-lethal concentration of esfenvalerate

A summary of behavioural response statistics for ERM and *T. pyri* are shown in Table 8. The distance travelled and time spent stationary on each half of the half-treated and control leaf discs was not significantly different. The total mean distance and fractal dimensions were also not significantly different. The remaining parameters are significantly different ($P < 0.05$). To study more closely the changes in mite behaviour, frequency distributions of the distances travelled and turns made by the mites moving on the arenas were compared. Percentage frequency distributions of the distances and turns made by ERM between each successive coordinates (every second) gave relatively similar patterns. However, the distributions of control and half-treated arenas (left and right sides combined), were significantly different ($P < 0.001$) for distance and turn using two-sample Kolmogorov Smirnov test (Figure 11 and 12).

4.2.2 Behavioural responses of *T. pyri* to a sub-lethal concentration of esfenvalerate

In contrast to ERM, *T. pyri* appeared not to respond in any measurable way to the esfenvalerate residues. For *T. pyri* no significant difference was found in the initial summary statistics (Table 9). To study more closely the changes in mite behaviour, frequency distributions of the distances travelled and turns made by the predator mites moving on the arenas were compared. Percentage frequency distributions of distance travelled, and turns by, *T. pyri* are shown in Figures 13 and 14. The shapes of the distributions of the two halves of the same leaf discs were visually similar, however, both halves of the half-treated leaf disc had different shaped distributions to the control halves. The two-sample Kolmogorov-Smirnov test was used to compare between distances travelled over the whole arena (left and right sides combined) of control and half-treated arenas. This showed that mites on the control arena travelled significantly ($P < 0.001$) greater distances than mites on the half-treated arena.

Table 8. Summary of statistics for behavioural responses of ERM to a sub-lethal concentration of esfenvalerate.

Parameter	Control arenas (N=9)		Half-treated arenas (N=12)		P<0.05 ^w
		S.E.		S.E.	
Mean movement events (/48min.)	143.9	22.7	83.5	17.1	*
% time spent stationary: left	52.3 _{NS}		52.8 _{NS}		NS
right	47.7		47.2 _T		
Mean no. of seconds stationary	1841	145	2273	129	*
Arithmetic vector length	0.161	0.02	0.088	0.021	*
Mean angular deviation (°)	68.8	2.15	59.9	3.42	*
Absolute mean turn (°)	16.6	2.09	9.6	1.86	*
Mean meander (° turned/mm moved)	110.3	17.2	63.1	11.2	*
% of distance travelled: left	56.4 _{NS}		54.2 _{NS}		NS
right	43.6		45.8 _T		
Mean total distance travelled (mm)	485	63.8	264	62.4	NS
Weighted mean vector length (r)	0.292	0.038	0.433	0.052	*
Mean coefficient of a straight line	0.024	0.011	0.063	0.018	*
Mean speed (mm/second)	0.167	0.022	0.092	0.022	*
Fractal dimension (1,10,40,80,100) _s	1.35	0.02	1.31	0.03	NS
Fractal dimension (1,2,4,8,16) _s	1.14	0.01	1.19	0.06	NS

^T is the esfenvalerate-treated half of disc.

^S are the step sizes used to calculate the fractal dimensions.

^w Significance determined by Two-tailed Wilcoxon Rank Sum test.

Table 9. Summary of statistics for behavioural responses of *T. pyri* to sub-lethal concentrations of esfenvalerate.

Parameter	Control arenas (N=9)		Half-treated arenas (N=10)		P<0.05 ^w
		S.E.		S.E.	
Mean movement events (/48min.)	140	10.3	151	28.5	NS
% time spent stationary:					
left	30.6 _{NS}		51.9 _{NS}		NS
right	69.4		48.1 _T		
Mean no. of seconds stationary	1203	153.2	1404	195.8	NS
Arithmetic vector length	0.33	0.04	0.24	0.04	NS
Mean angular deviation (°)	75.2	1.07	71.2	3.73	NS
Absolute mean turn (°)	23.7	1.62	17.9	2.61	NS
Mean meander (° turned/mm moved)	134.0	10.57	109.8	16.19	NS
% of distance travelled:					
left	45.7		47.8 _{NS}		NS
right	54.3		52.2 _T		
Mean total distance travelled (mm)	986	100.70	711	107.99	NS
Weighted mean vector length (r)	0.14	0.03	0.21	0.07	NS
Mean coefficient of a straight line	0.010	0.01	0.016	0.01	NS
Mean speed (mm/second)	0.340	0.04	0.244	0.04	NS
Fractal dimension (1,10,40,80,100) ^s	1.40	0.012	1.38	0.03	NS
Fractal dimension (1,2,4,8,16) ^s	1.17	0.01	1.12	0.01	NS

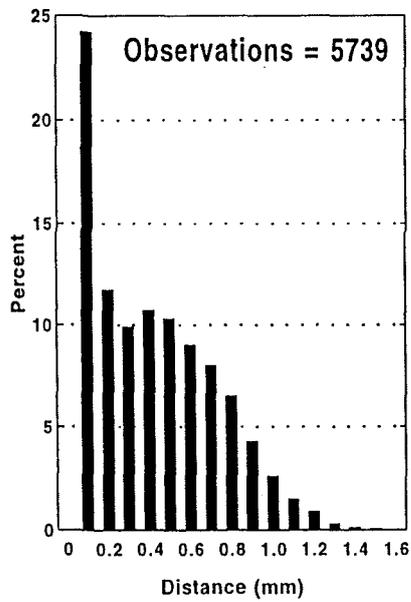
^T is the esfenvalerate-treated half of disc.

^s are the step sizes used to calculate the fractal dimensions.

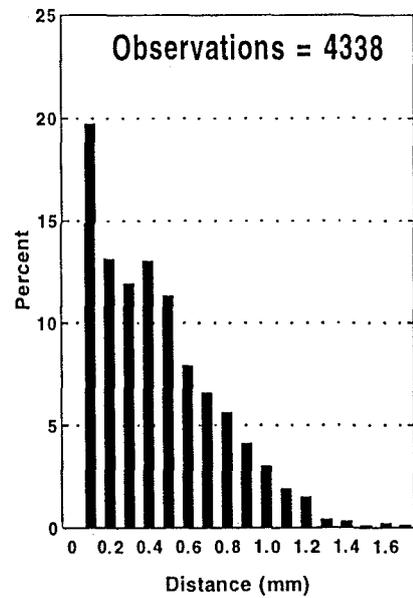
^w Significance determined by Two-tailed Wilcoxon Rank Sum test.

Figure 11. Percent frequency distributions of distances moved between coordinates by ERM on (a) control and (b) half-treated arenas.

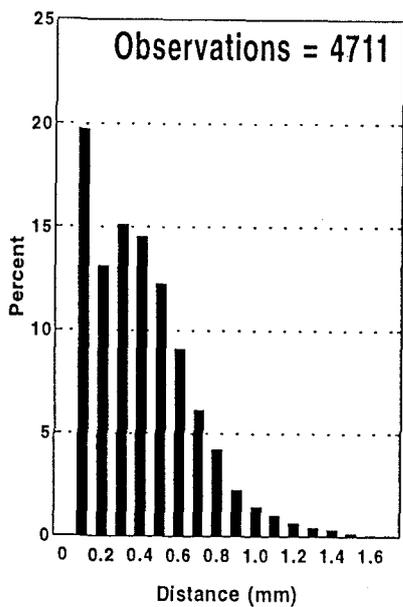
(a) Left half of control arena



Right half of control arena



(b) Untreated half of half-treated arena



Treated half of half-treated arena

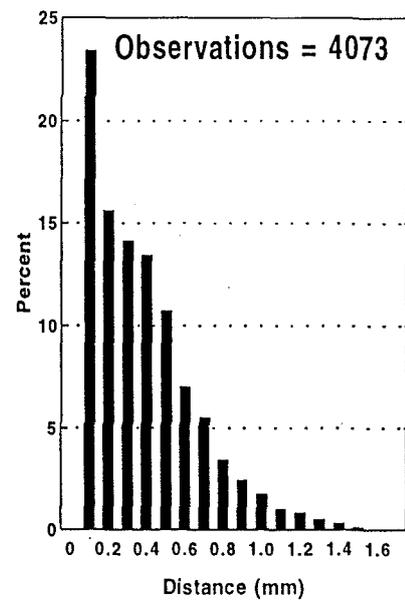
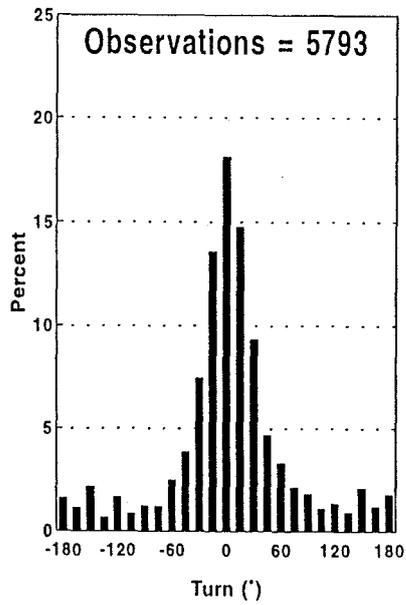
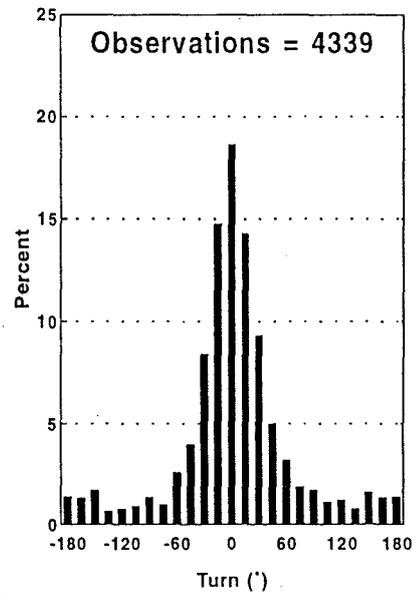


Figure 12. Percent frequency distributions of turns ($^{\circ}$) between coordinates by ERM on (a) control and (b) half-treated arenas. (Left turn = -, right turn = +)

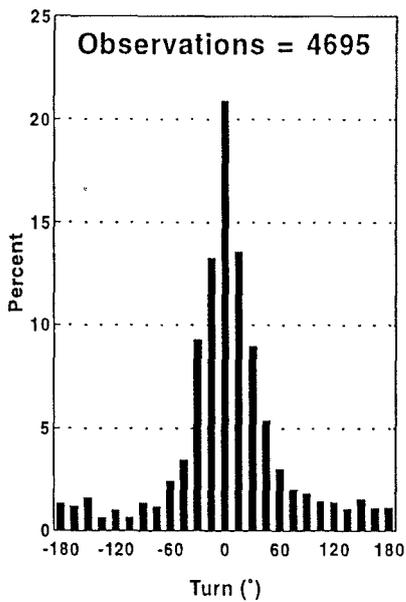
(a) Left half of control arena



Right half of control arena



(b) Untreated half of half-treated arena



Treated half of half-treated arena

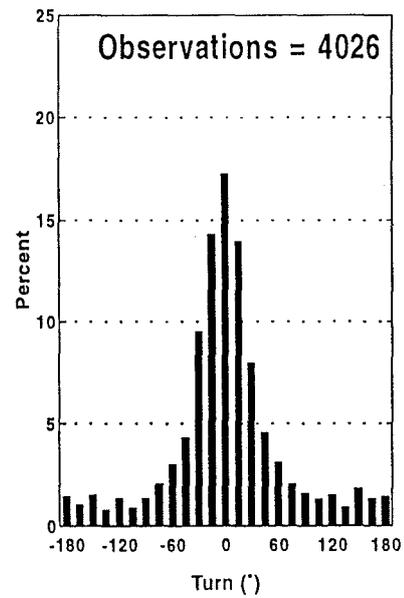
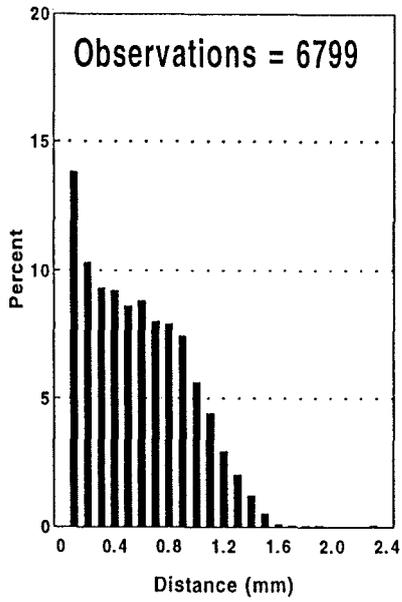
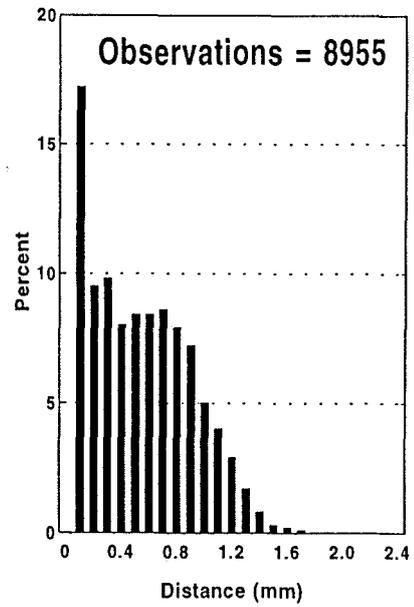


Figure 13. Percent frequency distributions of distances moved between coordinates by *T. pyri* on (a) control and (b) half-treated arenas.

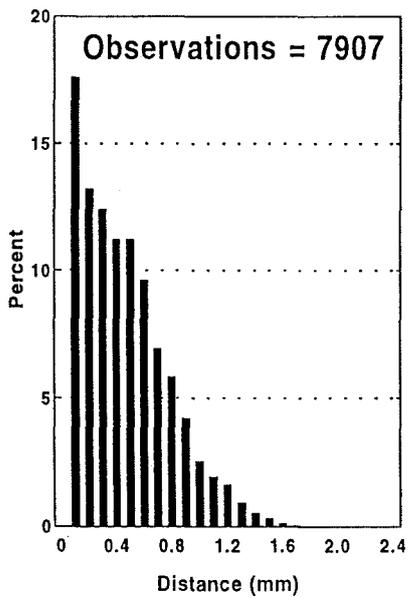
(a) Left half of control arena



Right half of control arena



(b) Untreated half of half-treated arena



Treated half of half-treated arena

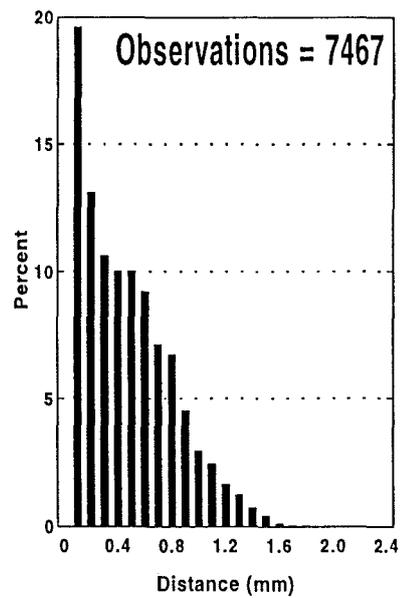
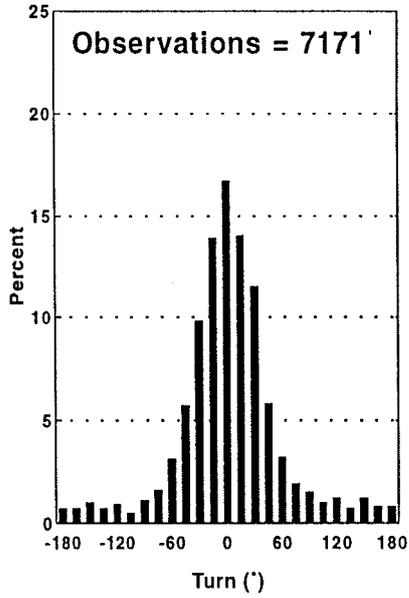
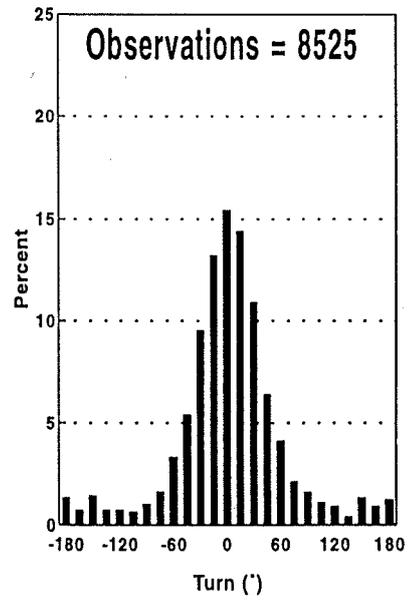


Figure 14. Percent frequency distributions of turns ($^{\circ}$) between coordinates by *T. pyri* on (a) control and (b) half-treated arenas. (Left turn = -, right turn = +).

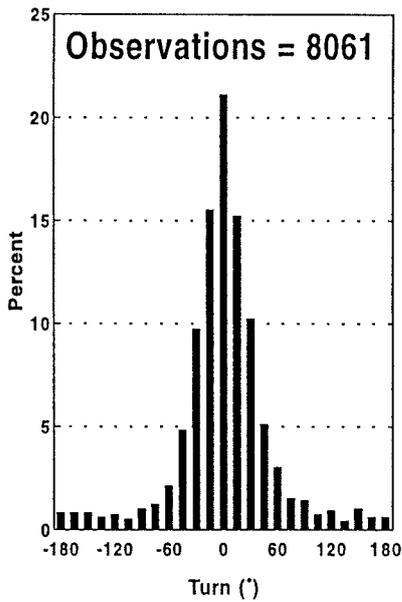
(a) Left half of control arena



Right half of control arena



(b) Untreated half of half-treated arena



Treated half of half-treated arena

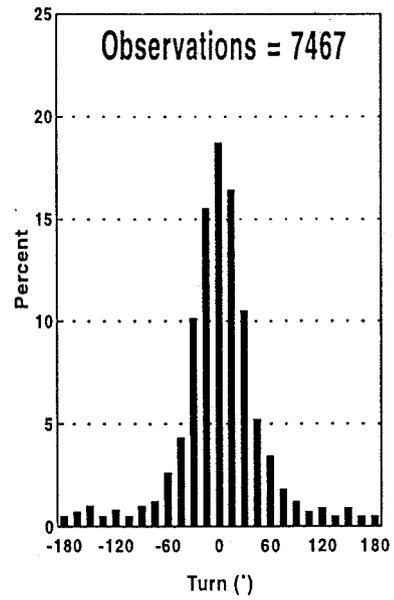
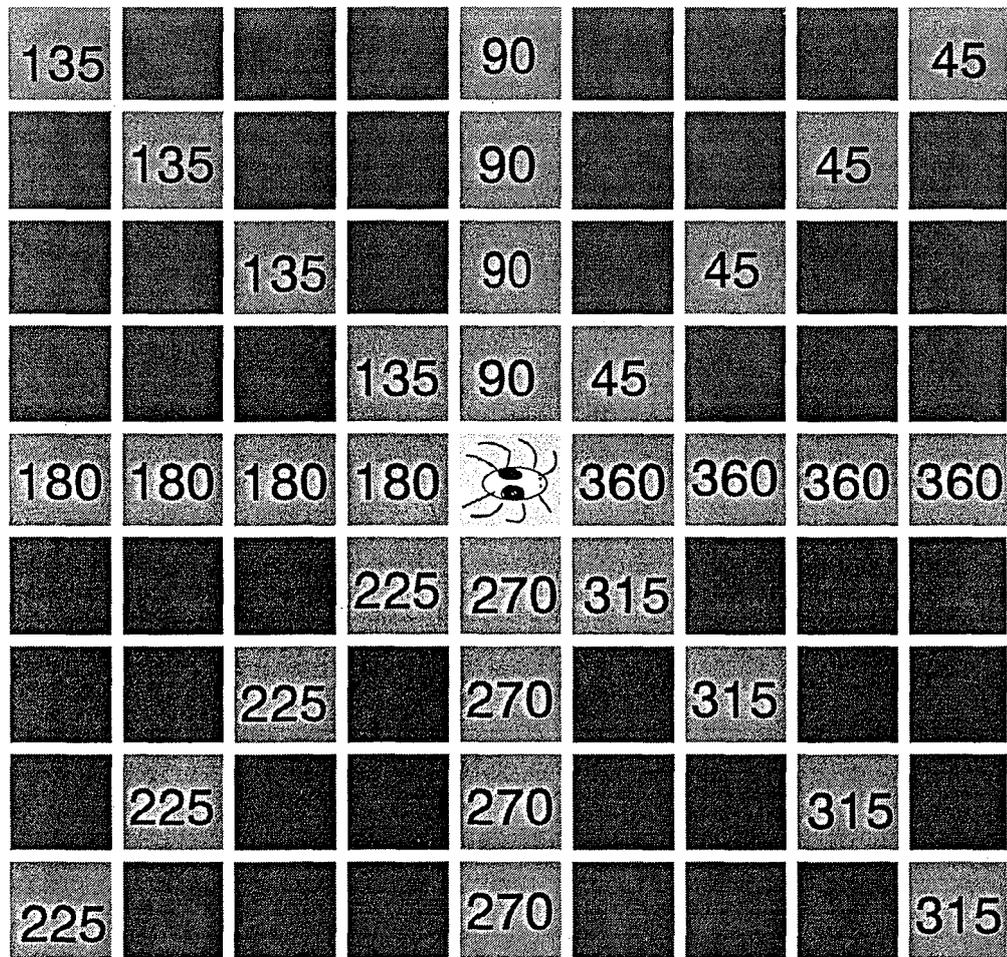


Figure 15. A diagrammatic representation of the pixel configuration showing angles (°) that should be assigned to the direction a mite moves. Eight directions (lightly shaded) should occur more (and about the same frequency) compared to the directions shown by the darker shaded boxes.



CHAPTER 5

DISCUSSION

The image analysis system used in this study was capable of collecting and describing quantitatively large behavioural sequences using x and y coordinates generated from video images of three species of mites on two types of arenas. As with all image analysis, an important requirement is to obtain sufficiently good contrast between the object/s of interest (in this case the mites) and the background (e.g., leaf disc). The teletracker system, based on a brightness threshold, worked well with a single object on a contrasting background, however, non-uniform backgrounds or groups of test subjects give endless trouble (Young, *et al.*, 1994). In addition, the arena must be as large as possible to reduce edge-effect whilst not being so large that the object of interest is lost.

5.1 TSM time course study

This experiment sought to describe the response of TSM behaviour through time. Such information is important to decide when to initiate the analysis of behaviour. Human experimental interactions such as placement on to leaf arena by a sable brush may influence the behaviour of the test individual. Most movement parameters showed a steady decline or increase over time. Holland (1991) also found a significant decrease in TSM movement after release on bean leaf discs, and Saito (1979) found the percentage of female TSM individuals walking on red clover leaves decreased from 100% at 0-6 minutes, to 25% or less at 30-36 minutes. Saito (1979) observed the mites in six minute periods (10 in each hour) at 0-1 hr, 6-7 hrs and 24-25 hrs after release. Although the measure used by Saito (1979) was the percentage of individuals walking (compared with actual time spent stationary or the distance travelled by mites in the present study), the trend for mites to reduce their walking behaviour was the same. One limitation of the present study was that the program BUGSY can only record 3000 data points, restricting the length of behaviour being studied to 48 minutes at a sampling frequency of one position (coordinate set) per second. However, since the experiment was completed, the development of other editing programs (DFILE and CFILE) made longer periods of mite activity easier to analyse. Nevertheless, longer periods were not used because if the mites did not become accustomed to the arena within the 48 minute period then it may not be practical to delay longer before starting to record behaviour. Saito (1979) studied three other spider mite species including *Panonychus citri* which, in contrast to TSM, appeared to settle into feeding immediately without an apparent 'settling down' period. The

relationships of the different spider mite species to their webs was thought to be an important influence on their behaviour.

Unlike the other parameters used in this experiment, the CSL was the only parameter which did not show any significant trend (decreasing or increasing) over time. Penman and Cone (1972) also used the CSL to measure how tortuous TSM movement was in response to various odours over a ten minute period. Male TSM exposed to different odours showed no significant change in the CSL compared to control mites with respect to time (Penman and Cone, 1972). White *et al.* (1984) found pathways of walking housefly searching behaviour to become significantly straighter through time. Such a behavioural response often results directly after feeding (where insects exhibit turning), a 'strategy' that brings the insect back on to a prey or food resource (Evans, 1976; Takabayashi and Takahashi, 1989). If such a resource is not found after a short period of searching, then the insects' path will often straighten (less turning) as a 'strategy' to move the insect into a new area (Evans, 1976). Similar variation in turning rates was observed with aphidophagous syrphids (Chandler, 1969). A change in strategies as described above could partly explain the straightening of tracks found by White *et al.* (1984) over time.

The close relationship between some parameters is shown by their significant correlations (Tables 3 and 4). For example, arithmetic mean vector length and stationary time are significantly negatively correlated for both ERM and *T. pyri* control mites of the esfenvalerate experiment. This is because when distance travelled (AMVL) by mites increases, time spent stationary decreases (and vice versa). Similarly, the arithmetic mean vector length and speed differ only marginally (Figures 3d and 3e), where speed is the arithmetic mean vector length divided by time (mm/sec), but the arithmetic mean vector length is derived from vector lengths calculated at each sample interval. Because of the uneven cyclic variations (see "DIGITIME" column in Table 1) of the computer's digital clock the sample interval only approximates one second thereby calculating similar values for speed and arithmetic mean vector length.

5.2 Length of sample period for observations

It is difficult to assess which sample period for observations is most appropriate for any given species or behaviour. Sample period should be sufficiently long to record a

representative sequence of an individual mites 'normal' behaviour. Alternatively, it should not be an impractical duration for data handling. Forty-eight minutes was chosen simply because it equated to the maximum sample length the BUGSY system could analyse and store. Bakchine *et al.* (1990) believe that sample size (number of individuals) is more important than the length of sample period as it allows for intrapopulation variability. In their olfactometry computer analysis of the mite *Varroa jacobsoni*, a sample length of six minutes 15 s was chosen based on the average time it took the mite "to exhibit oriented behaviour without getting the animal tired". Recording at a sampling frequency of every five seconds for 75 successive positions their experiment was replicated using 20-30 individuals. Olfactometer studies generally test for a tactic response towards or away from a stimulus, and require shorter behavioural sequences than those that are generally used in a kinesis-type study. Although this study primarily assessed the kinesis of mites, a sample period of 48 minutes (3000 points) could be reduced to 24 minutes (1500 points) without a significant loss of information. The number of behavioural bouts (expressed as the mean number of movement events by TSM) is proportional to sample period. After 48 minutes a mean number of 199 movement events occurred, however, after 24 minutes a significant number of behavioural events (mean of 101) still remain (Figure 5c) and therefore, the problem of censoring (removing the first and last bouts) should not be an issue. Other parameters such as stationary time could be greatly influenced by the underlying behaviour over the 48 minute period. For example, mite feeding appears to start after an initial period of locomotory behaviour, as appears to be the case with TSM in the time course experiment of this study and corroborated by Saito (1979). Care should therefore be taken to ensure important behaviours are not omitted through choosing sample sizes that are too short. Based on the above findings, sample length for observations of mites in future studies should be reduced to ca. 24 minutes which would allow more mites to be analysed without a significant loss of information.

5.3 Analysis of mite behaviour to arena edges

Although an edge-effect was clearly obvious on the printouts of most tracks, mean meander was the only parameter that was significantly different between 'edge' and 'non-edge' behaviours. Although large differences between the two types of behaviour were apparent for the parameters AMVL and AMT, they were significantly different only when combined in the form of mean meander. Other authors have noted how a limited arena

can cause edge-effects. Baatrup and Bayley (1993) used an automated computer-tracking system for spider analysis on a rectangular tray with fluon on the sides. The spiders, *Pardosa amentata* (Clerck) preferentially walked around the edges of the arena, a behaviour that Baatrup and Bayley (1993) considered to be distinctly thigmotactic.

Increasing the arena size is often not an option because the test subject must remain within the field of view for video studies, and motion analysis requires a good subject to background contrast. These constraints dictate that the arena should be as large as possible to minimise aberrations caused by the edge, but not so large that the mite becomes undetectable. Such considerations were the basis for the choice of the arena size used in this study and by Noldus *et al.* (1991) in their orientation study on *Trichogramma*. To eliminate the problem of the edge causing modified behavioural patterns the analysis could be stopped before the edge is encountered. In a study to measure attractancy of baits to slugs (Howling, 1991) and the orientation of *Trichogramma* (Noldus *et al.*, 1991) this method was used to measure a tactic behavioural response. Howling (1990; cited in Howling, 1991) in another experiment allowed slugs to move around the arena edge, but considered the edge-effect to be insignificant as the behaviour constituted less than 10% of the total distance travelled. In addition, Bailey (1989) found no difference in slug movements between arena sizes 30 cm² and 93 cm². However, other factors such as speed of movement and complexity of the arena will influence results in such experiments. For example, in a cockroach bait trial the cockroaches travelled around the edge 75% of the time (Bennett and Barclay, 1993). This result is not surprising given that cockroaches are very mobile and seek crevices as refuges.

Some studies have concluded that edge-walking is a natural searching behaviour, where an arthropod species may move along edges or veins of leaves (Noldus *et al.*, 1991; Sabelis, 1981; Sabelis and Dicke, 1985; Berry and Holtzer, 1990). ERM and TSM have also been observed moving along midribs of leaves in the laboratory (pers. obs.). Mueller-Beilschmidt and Hoy (1987) categorised three types of movement, of which edge-walking was considered sufficiently important to isolate and measure. In their study, a computerised video-tracking system was used to evaluate the activity levels of strains of the phytoseiid *Metaseiulus occidentalis*. Edge-walking behaviour by the mites was categorised as a scalloped pattern for at least three-quarters of their running time, with frequent stops and occasional turns. The description given by Mueller-Beilschmidt and Hoy (1987) of the edge-walking behaviour by *M. occidentalis* was identical to that found

in TSM and *T. pyri* in this study.

5.4 Sampling frequency

There was an obvious loss of information when coordinates of the track were sampled less frequently. For example, decreasing the sampling frequency from every second to every other second and every four seconds reduced the path length by 5% and 12% respectively. A reduction in sampling frequency has the effect of 'cutting corners' of the generated track causing a loss of information and resulting in higher (absolute) turn values. This truncation of track and associated loss of information has been demonstrated by Kitching and Zalucki (1982), and Tourtellot *et al.* (1991) who, along with McFarland (1971), stress the importance of move length (= sampling frequency). They suggested that sampling too frequently results in small unit moves representing "gait noise", whereas, sampling too infrequently results in loss of good information. Tourtellot *et al.* (1991) suggested that the sample frequency should be the time taken for the test arthropod to move the distance equivalent to its body length. TSM are about 0.54 mm in length, and the mean mite speed in the present study varied from 0.092 mm/s to 0.74 mm/s depending on the experiment. Although the sampling frequency used was approximately in the middle of the mean speed range for TSM, the large variability in speed shown by the mites means that the bodylength rule is useful as an indicator only. One complicating issue was the way the mean speed was calculated in this program. The mean speed calculation also includes stationary time, so that mites travelling at 1 mm/s should be sampled at every 0.5 s or more frequently. Based on this, the sampling frequency should be at least as frequent as every second. The BUGSY system was operated at its fastest sampling frequency, therefore more frequent comparisons were not possible.

Kitching and Zalucki's (1982) model confirms that there is an increasing loss of information and showed the standard deviation of mean angle (equals turn in this study) increased greatly as the step size increased. They showed a progressive increase from a standard deviation of 33.1 at a step size of one (based on original division of track into 1 cm chords) to a standard deviation of 78.6 at step size seven (i.e., using every seventh coordinate). Concluding remarks by Weins *et al.* (1993) provide a practical guide on how to make sampling frequency decisions: "the resolution with which field data are gathered should be attuned to the study insect and the question being asked". Although mite

speed varied greatly, it was considered that a sampling frequency of 1 s was the best compromise, given the arena size and camera distance (scale). However, had it been possible, a 0.5 s sampling frequency may have been desirable for faster moving mites.

5.5 Reliability and Suitability of Parameters

Computer-assisted analysis of digitised pathways can allow many search variables to be examined simultaneously. The parameters chosen in this study are only a small subset of possible choices available. Collins *et al.*, (1994) suggest the most useful variables are those that describe the physical features of the track: speed, degree of turning, and stopping frequency of the organism concerned and that the parameters used should always reflect the objectives of the study. For example, a behavioural study on pheromone attractancy seeks an index of straightness, such as CSL or a measure of deviation from a mean angle like mean angular deviation or weighted mean vector length. A parameter not used in this study, the thoroughness index (T-index), a measure of search intensiveness (White *et al.*, 1984; Fourcassié and Traniello, 1994) may be a useful addition for predator or parasitoid assessment. Other track parameters such as circling tendency and ground covered (area searched) have been computed for flying insects in the laboratory (Young *et al.*, 1994). Inclusion of one or more of these 'search thoroughness' parameters may be useful for future studies, particularly with studies of predatory arthropods.

5.5.1 Distribution of heading angles

The results of the most frequent heading angles show some unexpected trends. It was expected that the angles 45°, 90°, 135°, 180°, 225°, 270°, 315°, and 360° should have been equally the most frequently occurring angles due to the configuration of the pixels from the digitised image. The configuration can be thought of as a matrix of boxes where the mite has an option of eight boxes directly surrounding any one position, 16 boxes at the next level, 24 at the third, and so on (Figure 15). As the angles corresponding to the eight boxes in level one are the only ones to continue out to the end of the field, it was assumed that these eight angles would occur more often in random movement. In fact the angles 90°, 180°, 270° and 360° are dominant angles, but the angles in between these, 45°, 135°, 225°, and 315° occur considerably less often (Figure 7). Surprisingly, angles 25°, 65°, 115°, 155°, 205°, 245°, 290° and 335° are the second most dominant 'group' after 90°, 180°, 270° and 360°. Because of the relative symmetry of the frequency

plot these results are unlikely to be caused by some sort of behavioural bias, but maybe a result of the hardware configuration. Possible causes of this discrepancy are geometric distortion due to spherical aberrations in the video camera lens, scanning irregularities in the video camera, or a mismatch between the aspect ratios of the video camera and the display device (Inoué, 1986). Geometric distortion was not considered to be a major problem with this work, however, where critical quantitative measurements of shape and size are required, compensation of these errors would be essential (Inoué, 1986). Another possible cause of symmetrical distortion of the angles may be rounding errors of the angles caused by a 5° precision (Figure 7). In the literature, no other work with video-computer tracking systems has shown the heading angle with sufficient resolution to make such comparisons.

5.5.2 Turning distribution

Based on the definition by Batschelet (1981) the frequency distribution of turns by the three species of mites are considered to be circular normal. Another similarity observed were the common 'peaks' of each of the three mite species at turn angles of 180°(±), 150°(±) and 120°(±). The pattern of these peaks was consistent with the angles (discussed above) and this relationship may be due to the way the angles were used to calculate the turn values. A comparison of turn distribution of the three species showed TSM to have the highest frequency of lower turn values followed by ERM, while *T. pyri* had a 'fatter squat' distribution with larger frequencies in the 30°(±) to 75°(±) range. The shape of these circular normal distributions can be described using von Mises distribution, where K is the concentration of the turning angle (Batschelet, 1981). When $K=0$ the distribution is uniform, and as K increases, the paths get straighter and the distribution is more concentrated around the mean direction (Limburg and Pak, 1991). Limburg and Pak (1991) used this K value for describing the turning angle or tortuosity of the egg parasite *Trichogramma* in their study. The von Mises distribution has also been used to describe the degree of directionality in a simulation model of random search by herbivorous insects (Cain, 1985). Based on the unimodal and symmetrical distributions given by Batschelet (1981) the turn distributions of ERM, TSM and *T. pyri* appear to fit a *wrapped Cauchy* distribution with a K value close to one. Kitching and Zalucki (1982) consider that turn angle distributions can be summarised by their mean and standard deviation so long as they are symmetrical and akurtotic. The observed frequency distributions of turns produced by the gastropod *Polinices incei* (Zalucki and Kitching, 1982) were remarkably similar to those of the three mite species, however, the

standard deviations were higher for the mites (55-66) compared with 33 in the snail study, despite the larger sample size in the mites (732 snail turns compared to 9302-15696 mite turns). Another study simulating the searching behaviour of the parasite *Trichogramma* produced a "cosine" type distribution with an estimated K value of two. Such comparisons may be inappropriate across studies unless parameters such as step size, speed and arena specifications are standard. In this study, TSM and ERM movements were not recorded under the same conditions. For *T. pyri*, the arena used was glass rather than a larger leaf disc. Difference between arenas may alone be sufficient to explain the differences between *T. pyri* and the two prey mites, however, the differing search behaviour of the predator compared with the phytophagous mites is likely to contribute to some extent. Regardless of these factors, the mean turns and variances of the three mite species are remarkably similar.

5.5.3 Measuring tortuosity

The use of the CSL parameter has been restricted to mainly repellent or attractant studies where a tactic response is tested. In such cases the test species is usually required to travel only the approximate length or diameter of the arena. The use of servospheres (Vet and Papaj, 1992) may alleviate the influence of restricted arena size on the CSL parameter because the test subject never reaches the edge. Because of the possible influence of the arena size limiting straight walking and causing low CSL values, the usefulness of the CSL parameter was considered inadequate for the measurement of longer periods of mite behaviour such as that used in this study. However, two separate experiments seem to contradict this. The experiment to test sub-lethal effects of esfenvalerate on ERM behaviour showed significant differences for the CSL between the control and half-sprayed arenas. Each CSL replicate consisted of a 48 minute long behavioural sequence (3000 data points). In contrast, no significant difference was found between the CSL values for two contrasting mite behaviours ('tortuous' and 'straight') that were specifically chosen to test for tortuosity. Other tortuosity measures such as the fractal dimension, mean meander, and absolute mean turn were significantly different. Because the behavioural segments used in the 'tortuosity' experiment were considerably shorter (only 100 data points) than the larger sequences in the sub-lethal experiment described above (3000 data points), the CSL measure was expected to be more sensitive in the shorter experiment. Because of the concerns outlined (in the literature review) by Dicke and Burrough (1988) over CSL as a reliable tortuosity measure, and the results found in this study, the use of CSL should be treated with a degree of caution.

Weighted mean vector length, like CSL, is more suited for tactic studies. In contrast to CSL, WMVL is more often used for describing the clustering of a number of heading angles from different individuals of a population rather than the 'wiggleness' of one track from a single individual. However, weighted mean vector length has been used by Bell *et al.* (1989) to measure path straightness of larder beetles in response to wind strength, and; Bradley and Suckling (1995) to measure 'head convergence factor' for codling moth larvae orientating towards α -farnescene. Batschelet (1981) states the sample for the WMVL calculation should be drawn from a population with a von Mises distribution where the sample is assumed to be "reasonably unimodal and symmetric with respect to mode". The distributions of all three mite species (Figures 8a-c) fit this criteria and justify the use of these data for calculating WMVL.

Fractal dimensions were found to be significantly different for the two selected mite behaviours 'tortuous' and 'straight' (Table 5). However, the plots of the natural logs of the mean path lengths and step size was non-linear for both *T. pyri* and ERM (Figure 4) giving some doubt as to whether they are, in fact, true fractals. Although fractals are not necessarily self-similar, a straight line plot of path lengths versus step size would imply self-similarity (Williamson and Lawton, 1991). Another difficulty recognised by Williamson and Lawton (1991) is that non-fractal objects over a range of scales, can wrongly give a fractal result. The complex effects of spatial scaling in ecology has been discussed by Wiens (1989) and arena size is likely to be an important consideration as there is a relationship between the sizes and movement patterns of organisms and the fractal dimensions of their habitats (Morse *et al.*, 1985; Weiss and Murphy, 1988; Wiens and Milne, 1989; Crist *et al.*, 1992; Wiens *et al.*, 1993). Dicke and Burrough (1988) used fractal dimensions to describe the tortuosity of TSM trails and found this method to be a good, discriminative index, even though they considered the recordings of the mite trails not to be true fractals. Smoothing-out procedures during manual data transcription was thought to be responsible for the lack of self-similarity and it was suggested that the use of automatic recording techniques, such as used in this study, would reduce such errors or deviations (Dicke and Burrough, 1988). As shown by the results of this present study on *T. pyri* and ERM (Figure 4), other factors e.g., sampling method/algorithm, components of the experimental conditions or a relationship between walking speed and 'sensory radii' (or mite size) is influencing the mite behaviour to give the non-linear response (Simon Cox, University of Otago, pers. com.).

Meander and AMT parameters best distinguished ($P < 0.01$) between 'tortuous' and

'straight' paths of TSM. Because mean meander uses both turning and distance characteristics and because arthropods usually slow down when turning (Wendler and Scharstein, 1986; Bell *et al.*, 1985; Bell *et al.*, 1989; Obeng-Ofori, 1991; White *et al.*, 1984), the ability to distinguish between 'tortuous' and 'straight' behaviour increases the effect of making mean meander a 'powerful' measure.

5.5.4 Movement events

One problem associated with observing behaviour over a fixed period of time is truncation of first and last bouts (period of movement; Martin and Bateson, 1986) that may occur at the start and end of behavioural observations, so that first and last bouts that are not the 'true' lengths (Haccou and Meelis, 1994). A common way of avoiding these truncated bouts is to remove the first and last bouts (censoring). Such censoring was not carried out in this work because behavioural bouts were only used for the *stay* and *move* events of the parameters used. In addition, the observation times were sufficiently large that the inclusion of first and last bouts would have a negligible effect on the results (Haccou and Meelis, 1994). Another approach to analysing active and inactive behavioural events is to use fractal time (Cole, 1995). This is a measure of the pattern of events with equal relative precision over many different time intervals and should remain the same regardless of measurement scale. Lévy flight patterns of movement, which produces efficient searching, can result from using fractal time (Cole, 1995).

5.5.5 Relatedness of parameters

Clearly, some correlations between parameters are expected because of the way they are calculated. For example, the positive high correlation between AMVL, total distance and speed is likely to occur because AMVL is used to calculate the other two parameters. The strong negative correlation between MAD and WMVL which occurs because both parameters measure the degree of clustering around the mean heading angle. They are negatively correlated because as the mean angular deviation increases, the mean vector length decreases (Batschelet, 1981).

The correlation between mean meander and the number of movement events is positive. Mean meander incorporates both distance and turn measurements, that affect the number of movement events, The surprising result is that distance had no correlation with mean meander even though it contributes in the calculation. However, the other

component of meander, AMT, did have a strong correlation with mean meander and AMVL in ERM only. Stationary time, a parameter that is strongly negatively related to distance travelled, was negatively correlated to AMT for both species probably because of the inclusion of zero turn values in the AMT calculation when mites are stationary. Thus, as stationary time increases AMT decreases. However, the relationship between stationary time and the number of movement events is a little more complex. Stationary time can consist of many short stops, or fewer larger stops, therefore a correlation between movements and stationary time may not always occur. A negative correlation of $P < 0.05$ was found in ERM only. Ettifouri and Ferran (1993) used the number of stops (= events in this study) to evaluate predatory searching characteristics. Using principal component analysis these authors found the number of stops to be related to angular speed (= AMT/time). In this study, a highly significant positive correlation was found between AMT and movement events in ERM only. Had the correlation been with *T. pyri* rather than ERM, then a common predatory searching behaviour might be an explanation. Collins *et al.*, (1994) found speed to be negatively correlated with the number of stops (events), suggesting that flies that walk faster also stop less. Turn bias, the tendency to turn one way more than the other, was also found to be highly correlated with locomotory rate in the flies (Collins *et al.*, 1994).

Both the fractal dimension and CSL measure tortuosity yet they are significantly negatively correlated. For both parameters a perfectly straight linear path gives a value of one, however, with tortuous paths, the fractal dimension increases towards two (Wiens *et al.*, 1993), while CSL decreases towards zero (Gibson *et al.*, 1991), therefore explaining the negative correlation.

Tests of correlation between behavioural parameters similar to those used in this study were completed on *Musca domestica* (Collins *et al.*, 1994) and found to have a highly significant negative correlation between locomotion rate and meander. Other studies with lacewings (Bond, 1980) and flies (Bell, 1985; Bell *et al.*, 1985; and Bell *et al.*, 1989) have found similar patterns; i.e., the more rapidly the arthropods travelled, the slower the turning rate. The results of the tortuosity experiment, where 'tortuous' and 'straight' mite behaviour were analysed, shows this relationship graphically. 'Tortuous' behaviour had a mean speed of 0.15mm/s and AMT of 37.2°, whereas 'straight' moving mites had a faster mean speed (0.50 mm/s) but turned with smaller angles (23.7°).

5.6 Humidity

The results of the humidity experiment suggest that TSM at 'extreme humidities' (95% R.H. and 5% R.H.) travelled further, turned with greater angles, had larger mean angular deviations, and shorter stop events than the TSM at 50% RH. Such results suggest that mites find extreme relative humidities unfavourable and try to move away from the area. Studies on beetles *Tenebrio molitor* L. and *Hylobius abietis* L. have shown significant changes in the turning responses to changes in the humidity (Havukkala, 1980; Havukkala and Kennedy, 1984). In *Hylobius*, the change from moist to dry air caused a decrease in spatial displacement as a result of a decrease in walking speed and an increase in the amount of turning per unit time. In *Tenebrio* the change from dry to moist air caused a decrease in walking speed and a turning-back reaction.

Penman and Chapman (1980) were unable to detect significant variations in locomotor activity (mm/minute) or linearity of travel with TSM at 15, 48, 75 and 95% R.H. However, several other mite studies suggest that feeding and/or movement is inhibited by high (100%) humidity (Hazan *et al.*, 1975; Mori and Chant, 1966; Putman, 1970). The length of time the mites were left to equilibrate may have influenced the results greatly. Mori and Chant (1966) showed the kinetic response of male and female TSM decreased within six hours from 100% active to completely stationary at 100% R.H. Another factor that may affect the interpretation of the humidity experiments carried out using leaf discs is that microclimates are created by the unstirred air layer at the leaf surface. Ferro and Southwick (1984) suggest that in still air conditions the unstirred layer extends by as much as 5-10 mm from the leaf surface and contains a higher water vapour density than outside this layer. In addition, water vapour density may also be influenced by leaf shape, size, hairiness, temperature and placement.

5.7 Sub-lethal pesticide effects on mite behaviour

The results of the study of sub-lethal effects of esfenvalerate on ERM behaviour were greatly affected by the residue on the leaf surface. Although ERM behaviour between half-treated and control (water only) discs were significantly different for several measures, no significant differences were found for percentage stationary time or distance travelled between the left and right halves of arenas in both the control and half-treated treatments. To further test for mite avoidance or some other behavioural change to esfenvalerate, the leaf discs were analysed in halves to give percentage distributions of

turns and distances travelled every second. The Kolmogorov-Smirnov test, used to compare the distributions, also did not detect differences between halves of the same leaf discs, but found control discs to be different to half-treated discs. Although this method of analysis did show some significant differences in the distributions, some caution must be exercised concerning the interpretation. The observations were pooled where a small number of replicates were used, although a large number of observations were made within each replicate. It is possible that aberrant behaviour of just one mite could change the distributions. Clearly, considerable variation was apparent between the mite paths, but visually the paths gave no indication of avoidance of the esfenvalerate-treated half. Holland and Chapman (1994) found ca. 70% run-off occurred when TSM were placed on esfenvalerate residues of almost identical concentrations (0.01 g. ai. l⁻¹ cf. field rate of 0.015 g. ai. l⁻¹ used in this study) after 48 hours.

There are several explanations why ERM and *T. pyri* did not show a preference for the untreated half of arenas when given a choice. Firstly, the mites did not detect the pesticide gradient; secondly, insufficient time was given for the mites to find their 'preferred' resting or feeding site; and thirdly, more replications may be required to detect a difference. Another possible explanation is that chemoreceptors located in the mites' tarsi become saturated with esfenvalerate making it impossible for the mites to detect a gradient between the two halves, therefore preventing the mite avoiding the esfenvalerate residue. Hirano (1987) may give some support to this observation. In this study parafilm was placed over fenprothrin-treated leaf discs so the carmine spider mite *Tetranychus cinnabarinus* (Boisduval) was able to feed without their legs contacting the residues. Mites were stimulated to leave only when their legs contacted the residues. This may explain why significant changes in behaviour on whole leaf discs were found in phytophagous ERM but not in the predatory *T. pyri*.

The *T. pyri* results displayed the same trends as ERM in all parameters except for the mean number of movement events, and no significant difference was detected between the behaviour of *T. pyri* on half-treated pesticide arenas and control arenas. Two factors may have contributed to the contrasting results between *T. pyri* and ERM; the SP-resistance status of the *T. pyri* strain used, and the different arena surfaces used for the two species. Margolies and Kennedy (1988) found significant differences in TSM walk-off and aerial dispersal occurred between control and fenvalerate-treated leaf discs. However, mites showed no significant difference in dispersal when Petri dishes were used. They believed that the difference in TSM dispersal on leaves was a direct anti-feedant

effect of fenvalerate causing them to dehydrate. This hypothesis was supported by the mites on non-feeding surfaces moving more rapidly and in greater numbers at 10% R.H. compared to 95% R.H. in their study.

If esfenvalerate acts as an anti-feedant on ERM then a 48 minute experiment may not be long enough to observe increased mobility through dehydration. Instead, ERM on arenas half-treated with esfenvalerate performed significantly fewer movement events, moved slower and were stationary longer than on control arenas. This result may indicate an early behavioural response of the mites to esfenvalerate, before the more usual repellent or hyperactivity response often seen with synthetic pyrethroids either after 24 or 48 hours (Holland and Chapman, 1994; Iftner *et al.*, 1986), although most repellency of TSM by synthetic pyrethroids occur within the first four hours of treatment (Donahue *et al.*, 1988; Penman and Chapman, 1988; Gerson and Aronowitz, 1981). Whether or not feeding occurred, the mites should have sensed the esfenvalerate via the chemoreceptors that have been found in the pedipalps and tarsi of TSM (Bostanian and Morrison, 1973) and *Phytoseiulus persimilis* (Jagers op Akkerhuis *et al.*, 1985; Dicke *et al.*, 1991).

CHAPTER 6
CONCLUSIONS

1. The image analysis system was shown to be suitable for behavioural analysis of the three mite species: two-spotted spider mite, *Tetranychus urticae* (Koch), European red mite, *Panonychus ulmi* Koch and *Typhlodromus pyri* Scheuten using two experimental arenas. Locomotory behaviour of other arthropod species could also be studied by modifying behavioural arenas.
2. The decrease in two-spotted spider mite locomotion through a 48 minute period was possibly due to the onset of feeding. The influence of these behavioural changes must be considered when designing experiments.
3. Optimum choice of sampling frequency of mite coordinates is dependent on mite speed, however, between one per 0.5 second and one per second is recommended to minimise information 'loss'.
4. The inherent mite behaviour made it difficult to decide on the 'best' sample period length to use, however, 1500 coordinates (ca. 24 minutes) was considered suitable for TSM.
5. Many of the parameters are closely correlated because of the way they are calculated, therefore caution is recommended in interpretation of the biological meaning of the results.
6. Mean meander is the most useful parameter tested for differentiating between 'straight' and 'tortuous' mite movement and should be included in all studies if possible.
7. Discrepancies in frequency distributions of turns and heading angles of three mite species were believed to be caused by spherical aberrations of the hardware or rounding errors of the software. Care must therefore be taken in the biological interpretation of turning or heading angle data.
8. Relative humidity was shown to be an important influence on TSM behaviour. Care should be taken to avoid extreme or changing humidities throughout experiments from modifying mite behaviour.

9. Although all three mite species displayed a characteristic edge-walking behaviour around the arenas, the edge influence was not considered a problem for experiments where relative mobility is the main parameter being measured. However, arena size should be as large as possible given the constraints of the image analysis set up.

10. European red mite locomotory behaviour is altered by esfenvalerate over a 48 minute period. Assessment of ERM behaviour on esfenvalerate residues after 24 to 48 hours may show avoidance as has been found with spider mites and pyrethroids in other studies. A comparison of SP-resistant and susceptible *T. pyri* behaviour under the same experimental conditions would provide useful information.

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REFERENCES

- Allemand, R; Pompanon, F; Fleury, F; Fouillet, P. and Bouletreau, M. 1994. Behavioural circadian rhythms measured in real-time by automatic image analysis: applications in parasitoid insects. *Physiological entomology* 19: 1-8.
- Aluja, M.; Prokopy, R.J; Elkinton, J.S. and Laurence, F. 1989. Novel approach for tracking and quantifying the movement patterns of insects in three dimensions under seminatural conditions. *Environmental entomology* 18(1): 1-7.
- Anon. 1985. *Image analysis. Principles & practice*. A technical handbook produced by Joyce Loeb. 250pp.
- Anon. 1994. *EthoVision*. Video tracking, motion analysis and behaviour recognition system. Noldus information technology. Netherlands. 4pp.
- Baatrup, E. and Bayley, M. 1993. Quantitative analysis of spider locomotion employing computer-automated video tracking. *Physiology and behaviour* 54: 83-90.
- Bailey, S.E.R. 1989. Foraging behaviour in terrestrial gastropods: Integrating field and laboratory studies. *Journal of molluscan studies* 55: 263-272.
- Bakchine, E., Pham-Delegue, M.H., Kaiser, L. and Masson, C. 1990. Computer analysis of the exploratory behaviour of insects and mites in an olfactometer. *Physiology and behaviour* 48: 183-187.
- Batschelet, E. 1981. *Circular statistics in biology*. Academic Press, London. 371pp.
- Bell, W.J. 1985. Sources of information controlling motor patterns in arthropod local search orientation. *Journal of insect physiology* 31(11): 837-847.
- Bell, W.J. and Tobin, T.R. 1982. Chemo-orientation. *Biological reviews* 57: 219-260.
- Bell, W.J.; Tortorici, C.; Roggero, R.J.; Kipp, L.R. and Tobin, T.R. 1985. Sucrose-stimulated searching behaviour in *Drosophila melanogaster* in a uniform habitat: modulation by period of deprivation. *Animal behaviour* 33: 436-448.
- Bell, W.J.; Tobin, T.R. and Sorensen, K.A. 1989. Orientation responses of individual larder beetles, *Dermestes ater*, to directional shifts in wind stimuli. *Journal of insect behaviour* 2(6): 787-801.
- Bennett, G. and Barclay, J. 1993. Cockroach baits: understanding their use and effectiveness. *Pest control* 61(7): 34-36.
- Berg, H.C. 1971. How to track bacteria. *Review scientific instruments* 42: 868-871.
- Berry, J.S. and Holtzer, T.O. 1990. Ambulatory dispersal behaviour of *Neoseiulus fallacis* (Acarina: Phytoseiidae) in relation to prey density and temperature. *Experimental and applied acarology* 8: 253-274.
- Bond, A.B. 1980. Optimal foraging in a uniform habitat: the search mechanism of the green lacewing. *Animal behaviour* 28: 10-19.

- Bornbusch, A.H. and Conner, W.E. 1986. Effects of self-steered turn size and turn bias upon simulated chemoklinotactic behaviour. *Journal of theoretical biology* 122: 7-18.
- Bostanian, N.J. and Morrison, F.O. 1973. Morphology and ultrastructure of sense organs in the twospotted spider mite. *Annals of the entomological society of America* 66(2): 379-383.
- Bradley, S.J. and Suckling, D.M. 1995. Factors influencing codling moth larval response to α -farnesene. *Entomologia experimentalis et applicata* 75: 221-227.
- Cain, M.L. 1985. Random search by herbivorous insects: A simulation model. *Ecology* 66(3): 876-888.
- Casas, J. 1988. Analysis of searching movements of a leafminer parasitoid in a structured environment. *Physiological entomology* 13: 373-380.
- Chandler, A.E.F. 1969. Locomotory behaviour of first instar larvae of aphidophagous syrphidae (Diptera) after contact with aphids. *Animal behaviour* 17: 673-678.
- Cole, B.J. 1995. Fractal time in animal behaviour: the movement activity of *Drosophila*. *Animal behaviour* 50(5): 1317-1324.
- Collins, R.D.; Gargesh, R.N.; Maltby, A.D.; Roggero, R.J.; Tourtellot, M.K. 1994. Innate control of local search behaviour in the house fly, *Musca domestica*. *Physiological entomology* 19: 165-172.
- Crist, T.O.; Guertin, D.S.; Weins, J.A. and Milne, B.T. 1992. Animal movement in heterogeneous landscapes: an experiment with *Eleodes* beetles in shortgrass prairie. *Functional ecology* 6: 536-544.
- Davenport, D.; Wright, C.A. and Causley, C. 1961. Technique for the study of the behaviour of motile microorganisms. *Science* 135: 1059-1060
- Dicke, M. 1986. Volatile spider-mite pheromone and host-plant kairomone, involved in spaced-out gregariousness in the spider mite *Tetranychus urticae*. *Physiological entomology* 11: 251-262.
- Dicke, M. and Burrough, P.A. 1988. Using fractal dimensions for characterising tortuosity of animal trails. *Physiological entomology* 13: 393-398.
- Dicke, M.; Sabelis, M.W.; Bogaers, R.J.F.; Alers, M.P.T. and van Halder, I. 1991. Kairomone perception by a predatory mite: behavioural analysis of chemoreceptor-carrying extremities. *Proceedings of the section experimental and applied entomology, N.E.V. Amsterdam* 2: 179-184.
- Doherty, J.A. and Pires, A. 1987. A new microcomputer-based method for measuring walking phonotaxis in field crickets (Gryllidae). *Journal of experimental biology* 130: 425-432.
- Donahue, D.H.; McPherson, R.M. and Poe, S.L. 1988. Dispersal response of *Tetranychus urticae* to fenvalerate and permethrin residues on soybeans. *Journal of entomological science* 23(4): 350-356.

- Dunsenbery, D.B. 1985. Using a microcomputer and video camera to simultaneously track 25 animals. *Computers in biology and medicine* 15(4): 169-175.
- Escoubas, P.; Lajide, L. and Mitzutani, J. 1993. An improved leaf-disk antifeedant bioassay and its application for screening of Hokkaido plants. *Entomologia experimentalis et applicata* 66: 99-107.
- Ettifouri, M. and Ferran, A. 1993. Influence of larval rearing diet on the intensive searching behaviour of *Harmonia axyridis* larvae. *Entomophaga* 38(1): 51-59.
- Evans, H.F. 1976. The searching behaviour of *Anthocerus confusus* (Reufer) in relation to prey density and plant surface topography. *Ecological entomology* 1: 163-169.
- Ferro, D.N. and Southwick, E.E. 1984. Microclimates of small arthropods: estimating humidity within the leaf boundary layer. *FORUM: Environmental entomology* 13:926-929.
- Fleschner, C.A.; Badgely, M.E.; Ricker, D.W. and Hall, J.C. 1956. Air drift of spider mites. *Journal of economic entomology* 49: 624-627.
- Fourcassié, V. and Traniello, J.F.A. 1994. Food searching behaviour in the ant *Formica schaufussi* (Hymenoptera, Formicidae): response of naive foragers to protein and carbohydrate food. *Animal behaviour* 48(1): 69-79.
- Fraenkel, G.S. and Gunn, D.L. 1961. *The orientation of animals - kinesis, taxes and compass reactions*. Dover Publications Inc., New York, 376pp.
- Franz, E. 1993. Spray coverage analysis using a hand-held scanner. *Transactions of ASAE* 36(5): 1271-1278.
- Gerson, U. and Aronowitz, A. 1981. Spider mite webbing. Part IV: The effect of acaricides on spinning by the carmine spider mite *Tetranychus cinnabarinus* (Boisduval). *Pesticide science* 12: 211-214.
- Gerson, U. and Cohen, E. 1989. Resurgences of spider mites (Acari: Tetranychidae) induced by synthetic pyrethroids. *Experimental and applied acarology* 6: 29-46.
- Gibson, G.; Packer, M.J.; Steullet, P. and Brady, J. 1991. Orientation of tsetse flies to wind, within and outside host odour plume in the field. *Physiological entomology* 16: 47-56.
- Haccou, P. and Meelis, E. 1994. *Statistical analysis of behavioural data. An approach based on the time-structured models*. Oxford University Press, 396pp.
- Hagerup, M.; Sondergaard, I. and Nielsen, J. 1990. Measurements of areas consumed from leaf discs by chewing phytophagous insects: description of a new method involving image processing. *Entomologia experimentalis et applicata* 57: 105-113.
- Havukkala, I.J. 1980. Klinokinetic and klinotactic humidity reactions of the beetle *Hylobius abietis* and *Tenebrio molitor*. *Physiological entomology* 5: 133-140.
- Havukkala, I.J. and Kennedy, J.S. 1984. A programme of self-steered turns as a humidity response in *Tenebrio*, and the problem of characterizing spatial manoeuvres. *Physiological entomology* 9: 157-164.

- Haynes, K.F. 1988. Sublethal effects of neurotoxic insecticides on insect behaviour. *Annual review of entomology* 33: 149-168.
- Hazan, A. Gerson, U. and Tahori, A. 1975. Quantitative evaluation of the feeding of the carmine spider mite *Tetranychus cinnabarinus* (Boisduval). *Bulletin of entomological research* 5: 515-521.
- Heinzel, H. and Böhm, H. 1989. The wind-orientation of walking carrion beetles. *Journal of comparative physiology A* 164: 775-786.
- Hirano, M. 1987. Locomotor stimulant activity of fenpropathrin against the carmine spider mite *Tetranychus cinnabarinus* (Boisduval). *Applied entomology and zoology* 22(4): 499-503.
- Holland, J.M. 1991. *Toxic and sub-lethal effects of selected pyrethroid insecticides on spider mites*. Unpublished Ph.D. thesis, Lincoln University, Canterbury, New Zealand. 202pp.
- Holland, J.M. and Chapman, R.B. 1990. The behaviour and reproductive response of the European red mite to fluvalinate. *Proceedings of the New Zealand weed and pest control conference* 43: 278-281.
- Holland, J.M. and Chapman, R.B. 1991. The effect of fluvalinate on the development and survival of the two-spotted spider mite. *Proceedings of the New Zealand weed and pest control conference* 44: 248-251.
- Holland, J.M. and Chapman, R.B. 1994. A comparison of the toxic and sublethal effects of fluvalinate and esfenvalerate on the twospotted spider mite. *Experimental and applied acarology* 18: 3-22.
- Holland, J.M.; Chapman, R.B.; and Penman, D.R. 1994a. Effects of fluvalinate on twospotted spider mite dispersal, fecundity and feeding. *Entomologia experimentalis et applicata* 71: 145-154.
- Holland, J.M.; Chapman, R.B. and Penman, D.R. 1994b. A comparison of the toxic and sub-lethal effects of fluvalinate and esfenvalerate on the twospotted spider mite (Acari:Tetranychidae). *Experimental and applied acarology* 18: 3-22.
- Howling, G.G. 1991. Slug foraging behaviour: attraction to food items from a distance. *Annals of applied biology* 119: 147-153.
- Hoy, J.B. 1994. Follow that roach!: exploiting data from desktop video chips. *Advanced imaging* 9(1): 44-46.
- Hoy, J.B and Dahlsten, D.L. 1984. Effects of malathion and Staley's bait on the behaviour and survival of parasitic hymenoptera. *Environmental entomology* 13: 1483-1486.
- Hoy, J.B., Globus, P.A. and Norman, K.D. 1983. Electronic tracking and recording system for behavioural observations with application to toxicology and pheromone assay. *Journal of economic entomology* 76: 678-680.

- Hoyt, S.C.; Westigard, P.H. and Burts, E.C. 1978. Effects of two synthetic pyrethroid insecticides on the codling moth, pear psyllia and various mite species in North West apple and pear orchards. *Journal of economic entomology* 71: 431-434.
- Hurkova, J. 1984. Responses of OP-resistant *Tetranychus urticae* (Acarina) to pyrethroids. *Vestnik Ceskoslovenske spoleonusti zoologicke* 48: 102-106.
- Iftner, D.C. and Hall, D.C. 1983. Effects of fenvalerate and permethrin on *Tetranychus urticae* Koch (Acari: Tetranychidae) dispersal behaviour. *Environmental entomology* 12: 1782-1786.
- Iftner, D.C.; Hall, F.R. and Storm, M.M. 1986. Effects of residues of fenvalerate and permethrin on feeding behaviour of *Tetranychus urticae* (Koch). *Pesticide science* 17: 242-248.
- Inoué, S. 1986. *Video microscopy*. Plenum Press, New York. 584pp.
- Jagers op Akkerhuis, J.; Sabelis, M.W. and Tjallingii, F. 1985. Ultrastructure of chemoreceptors on pedipalps and first tarsi of *Phytoseiulus persimilis*. *Journal of experimental and applied acarology* 1: 235-251.
- Jander, R.J. 1975. Ecological aspects of spatial orientation. *Annual review of systematics and ecology* 6: 171-188.
- Jasko, D.J.; Little, T.V.; Smith, K.; Lein, D.H. and Foote, R.H. 1988. Objective analysis of stallion sperm motility. *Theriogenology* 30(6): 1159-1167.
- Jepson, P.C.; Cuthbertson, P.S.; Thacker, J.R. and Bowie, M.H. 1987. A computerised droplet size analysis system and the measurement of non-target invertebrate exposure to pesticides. *Aspects of applied biology (Aspects of pesticide transfer and performance)* 14: 97-112.
- Jones, R.E. 1977. Search behaviour: A study of three caterpillar species. *Behaviour* 60: 237-259.
- Jouen, F. and Lepecq, J.C. 1989. Optical and electronic systems for spatial and temporal analysis of video images. *Behaviour research methods, instruments and computers* 21(1): 2-10.
- Kareiva, P.M. and Shigesada, N. 1983. Analyzing insect movement as a correlated random walk. *Oecologia (Berlin)* 56: 234-238.
- Kennedy, J.S. 1986. Some current issues in orientation to odour sources. pp.11-25 in: *Mechanisms in insect olfaction*. Edited by Payne, T.L.; Birch, M.C. and Kennedy, C.E.J. Oxford University Press, Oxford.
- Kitching, R.L. and Zalucki, M.P. 1982. Component analysis and modelling of the movement process: analysis of simple tracks. *Researches of population ecology* 24: 224-238.
- Kokko, E.G.; de Clerck-floate, R.A. and Leggett, F.L. 1995. Method to quantify leaf beetle consumption of leaf disks using image analysis. *The Canadian entomologist* 127: 519-525.

- Kokko, E.G.; Schaber, B.D. and Entz, T. 1993. Quantification of mandibular wear of female alfalfa leafcutter bees, *Megachile rotundata* (F.) (Hymenoptera: Megachilidae), by image analysis. *The Canadian entomologist* 125: 93-99.
- Kramer, E. 1976. The orientation of walking honeybees in odour fields with small concentration gradients. *Physiological entomology* 1: 27-37.
- Lengwiler, U. and Benz, G. 1994. Effects of selected pesticides on web building behaviour of *Lariniodes scolopetarius*. *Journal of applied entomology* 117: 99-108.
- Limburg, H. and Pak, G.A. 1991. Genetic variation in the walking behaviour of the egg parasite *Trichogramma*. pp.47-44 in: *Proceedings of the 5th workshop of the global IOBC working group "quality control of mass reared organisms"* Wageningen.
- Lönnendonker, U. and Scharstein, H. 1991. Fixation and optomotor response of walking Colorado beetles: interaction with spontaneous turning tendencies. *Physiological entomology* 16: 65-76.
- Mandelbrot, B.B. 1975. *Le objets fractals: forme, hasard et dimension*. Flammarion, Paris.
- Mandelbrot, B.B. 1983. *The fractal geometry of nature*. Freeman, San Francisco.
- Margolies, D.C and Kennedy, G.G. 1988. Fenvalerate-induced aerial dispersal by the twospotted spider mite. *Entomologia experimentalis et applicata* 46: 233-240.
- Martin, P. and Bateson, P. 1986. *Measuring behaviour - an introductory guide*. Cambridge University Press. 200pp.
- McCulloch, C.E. and Cain, M.L. 1989. Analyzing discrete movement data as a correlated random walk. *Ecology* 70(2): 383-388.
- McEwen, P.K.; Clow, S.; Jervis, M.A. and Kidd, N.A.C. 1993. Alternation in searching behaviour of adult female green lacewings *Chrysoperla carnea* following contact with honeydew of the black scale *Saissetia oleae* and solutions containing acid-hydrolysed L-tryptophan. *Entomophaga* 38(3): 347-354.
- McFarland, D.J. 1971. *Feedback mechanisms in animal behaviour*. New York, Academic Press.
- Mori, H. 1962. Seasonal differences of phototactic response in three species of spider mites (Acarina, Tetranychidae). *Journal of the faculty of agriculture, Hokkaido University* 52: 1-9.
- Mori, H. and Chant, D.A. 1966. The influence of humidity on the activity of *Phytoseiulus persimilis* (Athias-Henriot) and its prey, *Tetranychus urticae* (Koch). *Canadian journal of zoology* 44: 863-871.
- Morse, D.R.; Lawton, J.H.; Dodson, M.M. and Williamson, M.H. 1985. Fractal dimension of vegetation and the distribution of arthropod body lengths. *Nature* 314: 731-733.
- Mueller-Beilschmidt, D. and Hoy, M.A. 1987. Activity levels of genetically manipulated and wild strains of *Metaseiulus occidentalis* (Nesbitt)(Acarina: Phytoseiidae) compared as a method to assay quality. *Hilgardia* 55(6): 1-23.

- Noldus, L.P.J.J. 1991. The observer: a software system for the collection and analysis of observational data. *Behavioural research methods, instruments, and computers* 23(3): 415-429.
- Noldus, L.P.J.J.; van Lenteren, J.C. and Lewis, W.J. 1991. How *Trichogramma* parasitoids use moth sex pheromones as kairomones: orientation behaviour in a wind tunnel. *Physiological entomology* 16: 313-327.
- Noldus, L.P.J.J.; van de Loo, E.L.H.M.; and Timmers, P.H.A. 1989. Computers in behavioural research. *Nature* 341: 767-768.
- Notting, S.P. and Edwards, C.R. 1985. Defoliation assessment using video imagery and a microcomputer. *Bulletin of the entomological society of America* 31(4): 38-42.
- Obeng-Ofori, D. 1991. Analysis of orientation behaviour of *Tribolium castaneum* and *T. confusum* to synthetic aggregation pheromone. *Entomologia experimentalis et applicata* 60: 125-133.
- Penman, D.R. and Chapman, R.B. 1980. Effect of temperature and humidity on the locomotory activity of *Tetranychus urticae* (acarina: Tetranychidae), *Typhlodromus occidentalis* and *Amblyseius fallacis* (Acarina: Phytoseiidae) *Ecologica applicata* 1: 259-264.
- Penman, D.R. and Chapman, R.B. 1983. Pesticide-induced distributional imbalances of two-spotted spider mite on bean plants. *Entomologia experimentalis et applicata* 33: 71-78.
- Penman, D.R. and Chapman, R.B. 1988. Pesticide-induced mite outbreaks: pyrethroids and spider mites. *Experimental and applied acarology* 4: 265-276.
- Penman, D.R.; Chapman, R.B. and Bowie, M.H. 1986. Direct and repellent activity of pyrethroids against *Tetranychus urticae* (Acari: Tetranychidae). *Journal of economic entomology* 79: 1183-1187.
- Penman, D.R.; Chapman, R.B. and Jesson, K.E. 1981. Effects of fenvalerate and azinphosmethyl on two-spotted spider mite and phytoseiid mites. *Entomologia experimentalis et applicata* 30: 91-97.
- Penman, D.R. and Cone, W.W. 1972. Behaviour of male twospotted spider mites in response to quiescent female deutonymphs and to web. *Annals of the entomological society of America* 65(6): 1289-1293.
- Putman, W.L. 1970. The effects of water and high humidity on European red mite, *Panonychus ulmi* (Acarina: Tetranychidae). *Canadian entomologist* 102(8): 955-961.
- Royce-Malmgren, C.H. and Watson, W.H. 1987. An interactive video-computer tracking system for quantification of locomotor behaviour. *Journal of chemical ecology* 13(5): 1029-1044.
- Sabelis, M.W. 1981. Biological control of two-spotted spider mites using phytoseiid predators. Part I: Modelling the predator-prey interaction at the individual level. *Agricultural research reports 910*, Pudoc, Wageningen, The Netherlands, 242pp. Cited in: Sabelis and Dicke, 1985.

- Sabelis, M.W. and Dicke, M. 1985. Long-range dispersal and searching behaviour. pp.141-160 In: Helle, W. and Sabelis, M.W. *Spider mites. Their biology, natural enemies and control*. World crop pests 1A. Elsevier.
- Sabelis, M.W.; Vermaat, J.E. and Groeneveld, A. 1984. Arrestment responses of the predatory mite, *Phytoseilus persimilis*, to steep odour gradients of a kairomone. *Physiological entomology* 9: 437-446.
- Saito, Y. 1979. Study of the spinning behaviour of spider mites III responses of mites to webbing residues and their preferences for particular physical conditions of leaf surfaces (Acarina: Tetranychidae). *Japanese journal of applied entomology and zoology* 23: 82-91.
- Samu, F. and Vollrath, F. 1992. Spider orb web as bioassay for pesticide side effects. *Entomologia experimentalis et applicata* 62: 117-124.
- Smitley, D.R. and Kennedy, G.G. 1985. Photo-orientated aerial-dispersal behaviour of *Tetranychus urticae* (Acari: Tetranychidae) enhances escape from the leaf surface. *Annals of the entomological society of America* 78: 609-614.
- Suski, Z.W. and Naegele, 1968. Techniques for the study of light response in the two spotted spider mite. *Advances in acarology* 1: 193-198.
- Takabayashi, J. and Takahashi, S. 1989. Effects of host fecal pellet and synthetic kairomone on host-searching and postoviposition behavior of *Apanteles kariyai*, a parasitoid of *Pseudaletia separata*. *Entomologia experimentalis et applicata* 52: 221-227.
- Thiery, D. and Visser, J.H. 1987. Misleading the Colorado potato beetle with an odour blend. *Journal of chemical ecology* 13: 1139-1146.
- Tourtellot, M.K.; Collins, R.D.; Bell, W.J. 1991. The problem of movelength and turn definition in analysis of orientation data. *Journal of theoretical biology* 150(3): 287-297.
- Tumanov, E.A., Galkin, A.A., Filippov, M.M., Sakovich, V.V. and Karelin, A.A. 1990. Automatic recording and analysis of leukocyte migration based on an image analysis system. *Bulletin of experimental biology and medicine* 109: 796-798.
- Turchin, P.; Odendaal, F.J. and Rausher, M.D. 1991. Quantifying insect movement in the field. *Environmental entomology* 20(4): 955-963.
- van der Steen, W.J. and ter Maat, A. 1979. Theoretical studies on animal orientation I. Methodological appraisal of classification. *Journal of theoretical biology* 79: 223-234.
- van Vorhis Key, S.E. and Barker, T.C. 1982. Trail pheromone-conditioned anemotaxis by the Argentine ant, *Iridomyrmex humilis*. *Entomologia experimentalis et applicata* 32: 232-237.
- Varley, M.J.; Copland, M.J.W.; Wratten, S.D. and Bowie, M.H. 1994. Parasites and predators. pp.33-63 in: Wratten, S.D. (Ed.) *Video techniques in animal ecology and behaviour*. Chapman & Hall, London.

- Vet, L.E.M. and Papaj, D.R. 1992. Effects of experience on parasitoid movement in odour plumes. *Physiological entomology* 17: 90-96.
- Visser, J.H. 1988. Host-plant finding by insects: orientation sensory input and search patterns. *Journal of insect physiology* 34(3): 259-268.
- Weiss, S.B. and Murphy, D.D. 1988. Fractal geometry and caterpillar dispersal: or how many inches can inchworms inch? *Functional ecology* 2: 116-118.
- Wendler, G. and Scharstein, H. 1986. The orientation of grain weevils (*Sitophilus granarius*): influence of spontaneous turning tendencies and of gravitational stimuli. *Journal of comparative physiology A* 159: 377-389.
- White, J.; Tobin, T.R. and Bell, W.J. 1984. Local search in the housefly *Musca domestica* after feeding on sucrose. *Journal of insect physiology* 30(6): 477-487.
- Wiens, J.A. 1989. Spatial scaling in ecology. *Functional ecology* 3: 385-397.
- Wiens, J.A., Crist, T.O. and Milne, B.T. 1993. Forum: on quantifying insect movements. *Environmental entomology* 22(4): 709-715.
- Wiens, J.A. and Milne, B.T. 1989. Scaling of "landscapes" in landscape ecology, or, landscape ecology from a beetle's perspective. *Landscape ecology* 3: 87-96.
- Williamson, M.H. and Lawton, J.H. 1991. Fractal geometry of ecological habitats. pp.69-86 in: *Habitat structure: the physical arrangement of objects in space*. Edited by Bell, S.S.; McCoy, E.D. and Mushinsky, H.R. Chapman and Hall. 438pp.
- Winston, P.W. and Bates, D.H. 1960. Saturated solutions for the control of humidity in biological research. *Ecology* 41(1): 232-237.
- Young, S.; Hardie, J. and Gibson, G. 1994. pp.17-32 in: Wratten, S.D. (Ed.) *Video techniques in animal ecology and behaviour*. Chapman & Hall, London.
- Yu, D.S.; Kokko, E.G.; Barron, J.R.; Schaalje, B.E. and Gowen, B.E. 1992. Identification of ichneumonid wasps using image analysis of wings. *Systematic entomology* 17: 389-395.
- Zalucki, M.P. and Kitching, R.L. 1982. Component analysis and modelling of the movement process: the simulation of simple tracks. *Researches of population ecology* 24: 239-249.
- Zar, J.H. *Biostatistical analysis*. 2nd ed. Prentice Hall, New Jersey. 715pp.

APPENDICES
Appendix I

BUGSY - program developed by Joyce LoebI which can track individual insects or count populations in defined areas.

```
{ BUGSY.TXT }  
{ 28/7/89 }  
{ SB }
```

This is a program for tracking a single insect's movements and for counting insect populations as they move from one defined region to another. The 'environment' options (e.g. threshold levels, calibration, timing method etc.) should be set up before the TRACK options are used. Data analysis gives options for a screen &/or printer listing of the results and a binary print out of the currently displayed image. The ROTATE option will work for anti-clockwise rotations but has not been updated for those in the other direction. The ROTATE routine needs a lot more input to make it completely robust and possibly advice from an entomologist as to exactly what a rotation is defined as !!!

```
{ $INCLUDE : 'MXTYPESI.TXT' }  
{ $INCLUDE : 'TASKSI.TXT' }  
{ $INCLUDE : 'MXRTI.TXT' }  
{ $INCLUDE : 'MXSYSI.TXT' }  
{ $INCLUDE : 'MXVIDEOI.TXT' }  
{ $INCLUDE : 'MXFEATI.TXT' }  
{ $INCLUDE : 'MXGRAPHI.TXT' }  
{ $INCLUDE : 'MXMEASI.TXT' }  
{ $INCLUDE : 'MXFILUI.TXT' }  
{ $INCLUDE : 'MXFILIOI.TXT' }  
{ $INCLUDE : 'BINDISCI.TXT' }
```

PROGRAM BUGSY(INPUT, OUTPUT);

USES

```
MXTYPES, MXRT, MXVIDEO, MXSYS, MXMEAS,  
MXGRAPH, MXFEAT, TASKS, MXFILU, MXFILIO, BINDISC;
```

CONST

```
MAXINDEX = 3000;  
RTOD = 0.0174532; { radians to degrees SF }
```

TYPE

```
VIDEOIN = ( CAMERA, VTR );  
TABLES = ( LINEAR, INVERTED, PSEUDO );  
LOADING = ( TIMING, KEYINPUT );
```

BUGINFO = RECORD

```
COORD :POINT;  
HR, MIN,  
SEC, CS :INTEGER;  
ANGLE :REAL;  
END;
```

BUGARRAY = ADS OF ARRAY [0..MAXINDEX] OF BUGINFO;

COLINFO = RECORD

```
R1, R2, R3,  
HR, MIN,  
SEC, CS :INTEGER;  
END;
```

S100 = LSTRING(100);

VAR

```
BUGDATA :BUGARRAY;  
COLDATA :ADS OF ARRAY [0..MAXINDEX] OF COLINFO;  
GOTDATA :BOOLEAN;  
KEY :CHAR;
```

```

METHOD :LOADING;
INPUT :VIDEOIN;
ST :S255;
UNITS :S20;
INTST :S14;
DATE :DATEREC;
LIMIT,
COLLIMIT,
PORTNO,
TEMP :INTEGER;
SCALEF,
WAIT :REAL;
GREYIM,
TEXTIM,
BINIM,
BINIM2,
ALLIM,
BLOBIM2,
BLOBIM :IMAGE;
INTAB,
OUTTAB :IOTAB;
DISPOUT :TABLES;
BINTDS,
THOLDS :VRANGE;

```

```

PROCEDURE IOWRITE( PORTNO, DATA :INTEGER ); EXTERN; { ASMMX }
PROCEDURE GETTIM( VAR HOUR, MIN, SEC, HUNDRETH : WORD ); EXTERN; { ASMMX }
PROCEDURE GETDAT( VAR YEAR, MONTH, DAY :WORD ); EXTERN;
PROCEDURE ENDXQQ; EXTERN; { PASCAL.LIB }
PROCEDURE SAVETXT( FRMNUM :INTEGER ); EXTERN;
PROCEDURE RESTXT( FRMNUM :INTEGER ); EXTERN;

```

```

{$INCLUDE : 'BUGSETUP.TXT'}
{$INCLUDE : 'BUGUTILS.TXT'}
{$INCLUDE : 'BUGDATA.TXT'}

```

```

PROCEDURE GET_ANGLES;
{ Angles measurements in radians. }
VAR

```

```

    OPPOSITE,
    ADJACENT,
    I :INTEGER;
BEGIN

```

```

    BUGDATA^[0].ANGLE := 0.0;

```

```

    FOR I := 1 TO LIMIT DO
    BEGIN

```

```

        ADJACENT := BUGDATA^[I].COORD.X - BUGDATA^[I-1].COORD.X;
        OPPOSITE := BUGDATA^[I].COORD.Y - BUGDATA^[I-1].COORD.Y;

```

```

        IF ADJACENT = 0

```

```

        THEN

```

```

        BEGIN

```

```

            IF OPPOSITE < 0

```

```

            THEN

```

```

                BUGDATA^[I].ANGLE := -1.5707963 { -90 }

```

```

            ELSE

```

```

                IF OPPOSITE > 0

```

```

                THEN

```

```

                    BUGDATA^[I].ANGLE := 1.5707963 { 90 }

```

```

            ELSE

```

```

                BUGDATA^[I].ANGLE := 0.0;

```

```

        END

```

```

    ELSE

```

```

IF OPPOSITE = 0
THEN
  BUGDATA^[I].ANGLE := 0.0
ELSE
BEGIN
  IF OPPOSITE > 0
  THEN
  BEGIN
    IF ADJACENT > 0
    THEN
      BUGDATA^[I].ANGLE := ARCTAN( OPPOSITE/ADJACENT )
    ELSE
      BUGDATA^[I].ANGLE :=
        3.1415926 + ARCTAN( OPPOSITE/ADJACENT );
    END
  ELSE
    IF ADJACENT < 0
    THEN
      BUGDATA^[I].ANGLE :=
        ARCTAN( OPPOSITE/ADJACENT ) - 3.1415926
    ELSE
      BUGDATA^[I].ANGLE := ARCTAN( OPPOSITE/ADJACENT );
    END;
  END;
END;
END;
END;

PROCEDURE BUG_TRACK;
VAR
  BKEY      :CHAR;
  I         :INTEGER;
  TEMP,
  AREA,
  BUGSIZE   :REAL;
  TRACK_ON,
  ABORT     :BOOLEAN;
  BUGPT,
  COGPT     :POINT;
  THING     :BLOB;
  HR, MIN, SEC,
  HUN       :WORD;
  STB       :S255;

PROCEDURE DRAW_LINES;
VAR
  PREVPT,
  PT1, PT2  :POINT;
BEGIN
  OVLAY( TEXTIM, MAGENTA );
  PT1.X := 0; PT1.Y := 0;
  PT2.X := 1; PT2.Y := 1;
  PREVPT := PT2;
  GET_POINT( 'DRAW 1st POINT:', PT1 );

  WHILE NOT READ_POINT( 'DRAW 2nd POINT:', PT2, TEXTIM) DO
  BEGIN
    WITHIN_IM (PT2);
    DRAWPOINT (PREVPT, TEXTIM, 0);
    DRAWLINE( PT1, PREVPT, TEXTIM, 0 );
    PREVPT := PT2;
    DRAWPOINT (PT2, TEXTIM, -1);
    DRAWLINE( PT1, PT2, TEXTIM, 1 );
  END;
END;
END;

```

```

PROCEDURE DRAW_CROSS;
VAR
  PT1 :POINT;
BEGIN
  OVLAY( TEXTIM, MAGENTA );
  PT1.X := 255; PT1.Y := 255;
  GET_POINT( 'MARK POINT FOR CROSS:', PT1 );
  DRAWCROSS( PT1, 4, TEXTIM, 1 );
END;

BEGIN
  I := 0;
  TRACK_ON := TRUE;

  CLEARIM( ALLIM );
  LIVE( GREYIM, INTAB, OUTTAB );
  (*
  OVLAY( BLOBIM, RED );
  *)
  REPEAT
    COPYLST( 'TRACK BUG: V)isible |DRAW L)ines C)ross G)rid', STB );
    CONCAT( STB, 'S)tart tracking | |<Esc> TERMINATES.' );

    IF TRACK_ON
    THEN
      UPPER_OPTIONS( STB, ['V'] );

    BKEY := READ_MENU( STB );

    CASE BKEY OF
      'C' : DRAW_CROSS;
      'G' : GRIDS;
      'L' : DRAW_LINES;
      'V' : TRACK_ON := NOT TRACK_ON;
      OTHERWISE;
    END; { case }

    IF BKEY IN ['C','G','L','V']
    THEN
      PROMPTSUP( PROMPTX, PROMPTY );

  UNTIL BKEY IN ['S','Q'];

  OVKILL;

  IF TRACK_ON
  THEN
    OVLAY( BINIM, GREEN );

  IF BKEY <> 'Q'
  THEN
    BEGIN
      REPEAT
        BUGSIZE := 0.0;
        PHOTO;
        VDISP( GREYIM );
        GETTIM( HR, MIN, SEC, HUN );
        SLICE( GREYIM.WHOLE, GREYIM, BLOBIM, THOLDS );

        IF FIRSTBLOB( BLOBIM.WHOLE, BLOBIM, THING )
        THEN
          BEGIN
            REPEAT
              SIMPLE_MEASURE( THING, COGPT, TEMP, TEMP, AREA, TEMP );
          END;
        END;
      UNTIL BKEY = 'Q';
    END;
  END;

```

```

    IF BUGSIZE < AREA
    THEN
    BEGIN
        BUGSIZE := AREA;
        BUGPT := COGPT;
    END;
    DRAW( THING.REGION, BLOBIM, 0 );
    UNTIL NOT NEXTBLOB( BLOBIM.WHOLE, BLOBIM, THING );

    BUGDATA^[I].COORD := BUGPT;
    BUGDATA^[I].HR := ORD( HR );
    BUGDATA^[I].MIN := ORD( MIN );
    BUGDATA^[I].SEC := ORD( SEC );
    BUGDATA^[I].CS := ORD( HUN );

    IF I > 0
    THEN
        DRAWLINE( BUGDATA^[I-1].COORD, BUGDATA^[I].COORD,
            BINIM, 1 );
        I := I+1;
    END
    ELSE
    BEGIN
        ERROR_MESS( 'ERROR: NO BUG FOUND. ' );

        IF METHOD = KEYINPUT
        THEN
            ABORT := YES_NO( 'CONTINUE TRACKING ?' ) = 'N'
        ELSE
            ABORT := TRUE;
        END;

        IF NOT ABORT
        THEN
            IF METHOD = TIMING
            THEN
                DELAY( WAIT )
            ELSE
                REPEAT
                UNTIL KEYPRESS;

            IF KEYPRESS
            THEN
                READCHEO( BKEY );

        UNTIL ( BKEY = CHR( ESC ) ) OR ( I > MAXINDEX ) OR ABORT;

        OVLAY( TEXTIM, MAGENTA );

        IF I > 1
        THEN
        BEGIN
            LIMIT := I-1;
            GET_ANGLES;
            GOTDATA := TRUE;

            BKEY := READ_MENU(
                'BUG TRACKED: P)rint track S)ave image to disc' );

            CASE BKEY OF
                'P' : HARDCOPY( BINIM, TEXTIM, TRUE );
                'S' : SAVE_IMAGE( BINIM, TEXTIM );
                OTHERWISE;

```

```

        END; { case }

        IF BKEY IN ['P','S']
        THEN
            PROMPTSUP( PROMPTX, PROMPTY );
        END;

        PROMPTSUP( PROMPTX, PROMPTY );
    END;
END;

PROCEDURE COLONY_TRACK;
VAR
    CKEY      :CHAR;
    THING,
    AREA1,
    AREA2     :BLOB;
    CST       :S255;
    HR, MIN,
    HUN, SEC  :WORD;
    SUM1, SUM2,
    TOTAL, I  :INTEGER;
    A1, A2,
    ABORT     :BOOLEAN;
    SIZES     :VRANGE;

PROCEDURE CONV_TO_BLOB( VAR OBJ : BLOB );
BEGIN
    CLEARIM( BLOBIM2 );
    SLICE( TEXTIM.WHOLE, TEXTIM, BLOBIM2, BINTDS );

    IF FIRSTBLOB( BLOBIM2.WHOLE, BLOBIM2, OBJ )
    THEN
        RESETPS( OBJ.REGION );
    END;

PROCEDURE DEF_DISC( VAR OBJ : BLOB; VAR OK : BOOLEAN );
VAR
    CENTRE     :POINT;
    RADIUS     :INTEGER;

FUNCTION THREE_POINT_CIRCLE :BOOLEAN;
VAR
    X1, Y1, X2, Y2,
    P, Q, S, XC, YC  :REAL;
    PREVPT,
    PTA, PTB, PTC    :POINT;

BEGIN
    PTA.X := 0;   PTA.Y := 0;
    GET_POINT ('1ST CIRCUMFERENTIAL POINT:', PTA);
    PTB.X := 0;   PTB.Y := 0;
    GET_POINT ('2ND CIRCUMFERENTIAL POINT:', PTB);
    PTC.X := 0;   PTC.Y := 0;
    GET_POINT ('3RD CIRCUMFERENTIAL POINT:', PTC);

    X1 := PTB.X - PTA.X;   Y1 := PTB.Y - PTA.Y;
    X2 := PTC.X - PTA.X;   Y2 := PTC.Y - PTA.Y;

    { Find intersection of normal bisectors: }
    S := X2 * Y1 - Y2 * X1; { x product: P.Q.sin0 }

    IF S <> 0.0
    THEN

```

```

BEGIN { Not colinear: }
  P := X1 * X1 + Y1 * Y1;   Q := X2 * X2 + Y2 * Y2;
  XC := 0.5 * (Y1 * Q - Y2 * P) / S;
  YC := 0.5 * (X2 * P - X1 * Q) / S;
  RADIUS := ROUND (SQRT (XC * XC + YC * YC));
  CENTRE.X := ROUND (XC) + PTA.X;
  CENTRE.Y := ROUND (YC) + PTA.Y;
  THREE_POINT_CIRCLE := TRUE;
END
ELSE
BEGIN
  CENTRE := PTA; RADIUS := 0;
  THREE_POINT_CIRCLE := FALSE;
END;
END; { THREE_POINT_CIRCLE }

BEGIN { DEF_DISC }
  OK := FALSE;

  IF THREE_POINT_CIRCLE
  THEN
  BEGIN
    CIRCGEN (OBJ.BOUNDARY, CENTRE, RADIUS);
    DRAW (OBJ.BOUNDARY, TEXTIM, -1);
    DIVEST (OBJ.BOUNDARY);
    CONV_TO_BLOB( OBJ );
    OK := TRUE;
  END
  ELSE ERROR_MESS ('Your points lie in a straight line. ');
END; { DEF_DISC }

PROCEDURE FREEHAND( VAR OBJ : BLOB; VAR OK :BOOLEAN );
BEGIN
  IF MYPENLINE (OBJ.BOUNDARY, TEXTIM, TRUE, TRUE, -1)
  THEN
  BEGIN
    DIVEST (OBJ.BOUNDARY);
    CONV_TO_BLOB( OBJ );
    OK := TRUE;
  END
  ELSE
  BEGIN
    RESETIM( GREYIM );
    OBJ.REGION := GREYIM.WHOLE;
    OK := FALSE;
  END;
END;

PROCEDURE DRAW_AREAS( VAR OBJ : BLOB; VAR DONE : BOOLEAN );
VAR
  AKEY :CHAR;
BEGIN
  REPEAT
    AKEY := READ_MENU( 'DRAW AREA: F)reehand M)ake circles' );

    CASE AKEY OF
      'F' : FREEHAND( OBJ, DONE );
      'M' : DEF_DISC( OBJ, DONE );
      OTHERWISE;
    END; { case }

    IF AKEY <> 'Q'
    THEN
      PROMPTSUP( PROMPTX, PROMPTY );

```

```

CLEARIM( TEXTIM );
DRAW( OBJ.BOUNDARY, BINIM, 1 );

UNTIL AKEY = 'Q';
END;

PROCEDURE CHECK_AREAS( VAR KEY :CHAR );
VAR
  PATCH :BLOB;
  TEMP,
  A1, A2 :REAL;
  PT :POINT;
BEGIN
  CLEARIM( BLOBIM );
  DRAW( AREA1.REGION, BLOBIM, -1 );

  IF FIRSTBLOB( BLOBIM.WHOLE, BLOBIM, PATCH )
  THEN
  BEGIN
    SIMPLE_MEASURE( PATCH, PT, TEMP, TEMP, A1, TEMP );
    DIVEST( PATCH.REGION );
    DIVEST( PATCH.BOUNDARY );
  END
  ELSE
    A1 := 0.0;

  DRAW( AREA1.REGION, BLOBIM, 0 );
  DRAW( AREA2.REGION, BLOBIM, -1 );
  (*
  IF FIRSTBLOB( BLOBIM.WHOLE, BLOBIM, PATCH )
  THEN
    SIMPLE_MEASURE( PATCH, PT, TEMP, TEMP, A2, TEMP )
  ELSE
    A2 := 0.0;
  *)
  IF FIRSTBLOB( BLOBIM.WHOLE, BLOBIM, PATCH )
  THEN
  BEGIN
    SIMPLE_MEASURE( PATCH, PT, TEMP, TEMP, A2, TEMP );
    DIVEST( PATCH.REGION );
    DIVEST( PATCH.BOUNDARY );
  END
  ELSE
    A2 := 0.0;

  IF ( A1 = 0.0 ) OR ( A2 = 0.0 )
  THEN
  BEGIN
    ERROR_MESS(
      'ERROR: Either one or both areas have not been defined. ');
    CKEY := 'X';
  END
  ELSE
  BEGIN
    IF A1 > A2
    THEN
    BEGIN { swap 'em over ! }
      PATCH.REGION := AREA1.REGION;
      PATCH.BOUNDARY := AREA1.BOUNDARY;
      AREA1.REGION := AREA2.REGION;
      AREA1.BOUNDARY := AREA2.BOUNDARY;
      AREA2.REGION := PATCH.REGION;
      AREA2.BOUNDARY := PATCH.BOUNDARY;
    END;
  END;

```

```

    END;
END;

BEGIN
  ABORT := FALSE;
  CLEARIM( ALLIM );
  I := 0;
  AREA1.REGION := GREYIM.WHOLE;
  AREA2.REGION := AREA1.REGION;
  A1 := FALSE;
  A2 := FALSE;

  LIVE( GREYIM, INTAB, OUTTAB );
  CKEY := READ_OPTION( 'ANY KEY TO PHOTO.' );
  PHOTO;
  VDISP( GREYIM );
  OVLAY( BINIM, RED );
  OVLAY( TEXTIM, GREEN );

  REPEAT
    COPYLST( 'POPULATION COUNTING: | DEFINE AREA 1) 2) ', CST );

    IF A1 AND A2
    THEN
      CONCAT( CST, 'D)raw grid S)start counting || <Esc> TERMINATES.' );
      CKEY := READ_MENU( CST );

      CASE CKEY OF
        '1': DRAW_AREAS( AREA1, A1 );
        '2': DRAW_AREAS( AREA2, A2 );
        'D': GRIDS;
        OTHERWISE;
      END; { case }

      IF CKEY IN ['1','2','D']
      THEN
        PROMPTSUP( PROMPTX, PROMPTY )
      ELSE
        IF YES_NO( 'ARE THESE AREAS SEPARATE ?.' ) = 'N'
        THEN
          { a quick check to get the areas the right way around ! }
          CHECK_AREAS( CKEY );

    UNTIL CKEY IN ['S','Q'];

    IF CKEY = 'S'
    THEN
      BEGIN
        CLEARIM( BINIM );
        DRAW( AREA1.BOUNDARY, TEXTIM, 1 );
        DRAW( AREA2.BOUNDARY, TEXTIM, 1 );
        SIZES.MIN := 0;
        SIZES.MAX := 1000;

        REPEAT
          SUM1 := 0;
          SUM2 := 0;
          TOTAL := 0;
          PHOTO;
          VDISP( GREYIM );
          GETTIM( HR, MIN, SEC, HUN );
          SLICE( GREYIM.WHOLE, GREYIM, BLOBIM, THOLDS );

          BLOBCOUNT( AREA1.REGION, BLOBIM, SIZES, SUM1 );

```

```

BLOBCOUNT( AREA2.REGION, BLOBIM, SIZES, SUM2 );
BLOBCOUNT( BLOBIM.WHOLE, BLOBIM, SIZES, TOTAL );

IF (TOTAL + SUM1 + SUM2) = 0
THEN
BEGIN
    ERROR_MESS( 'ERROR: NO BUGS FOUND. ' );

    IF METHOD = KEYINPUT
    THEN
        ABORT := YES_NO( 'CONTINUE COUNTING ?:' ) = 'N'
    ELSE
        ABORT := TRUE;
END
ELSE
BEGIN
    COLDATA^[I].R1 := SUM1;
    COLDATA^[I].R2 := SUM2;
    COLDATA^[I].R3 := TOTAL;
    COLDATA^[I].HR := ORD( HR );
    COLDATA^[I].MIN := ORD( MIN );
    COLDATA^[I].SEC := ORD( SEC );
    COLDATA^[I].CS := ORD( HUN );
    I := I+1;
END;

IF NOT ABORT
THEN
    IF METHOD = TIMING
    THEN
        DELAY( WAIT )
    ELSE
        REPEAT
            UNTIL KEYPRESS;

    IF KEYPRESS
    THEN
        READCHEO( CKEY );

UNTIL ( CKEY = CHR( ESC ) ) OR ( I > MAXINDEX ) OR ABORT;

IF I > 1
THEN
BEGIN
    COLLIMIT := I-1;
    GOTDATA := TRUE;
    SLICE( GREYIM.WHOLE, GREYIM, BINIM, THOLDS ); { Hardcopy }
END;

    PROMPTSUP( PROMPTX, PROMPTY );
END;
END; { COLONY_TRACK }

BEGIN
    SYSINIT;
    TASKSINIT;

    WITH DATE DO
    BEGIN
        DAY := 28;
        MONTH := 'Jun';
        YEAR := 89;
    END;
    TITLEBOX( 'BUGSY', '2.0', DATE );

```

```

BUGINIT;

REPEAT
  REPEAT
    COPYLST( 'MAIN MENU: V)ideo input', ST );

    IF INPUT = CAMERA
    THEN
      CONCAT( ST, ' CAMERA' )
    ELSE
      CONCAT( ST, ' VTR' );
    CONCAT( ST, ' O)utput tables ' );

    CASE DISPOUT OF
      LINEAR: CONCAT( ST, 'LINEAR' );
      INVERTED: CONCAT( ST, 'INVERTED' );
      PSEUDO: CONCAT( ST, 'PSEUDO COLOUR' );
    END; { case }

    CONCAT( ST, 'Threshhold levels [ ' );
    INT2STRING( THOLDS.MIN, INTST );
    CONCAT( ST, INTST );
    CONCAT( ST, ' ' );
    INT2STRING( THOLDS.MAX, INTST );
    CONCAT( ST, INTST );
    CONCAT( ST, ' ] Calibrate ' );
    REAL_FORMAT( SCALEF, '**.*****', INTST );
    CONCAT( ST, INTST );
    CONCAT( ST, 'I)nput port no. S)ampling method ' );
    INTEGER_INSERT( ST, PORTNO, 1, 'T' );

    IF METHOD = KEYINPUT
    THEN
      CONCAT( ST, 'KEYPRESS' )
    ELSE
      CONCAT( ST, 'TIMED DELAY' );

    CONCAT( ST, ' || TRACK B)ug P)opulation R)ead data file' );

    IF GOTDATA
    THEN
      CONCAT( ST, 'D)ata analysis' );

    KEY := READ_MENU( ST );

    CASE KEY OF
      'B' : BUG_TRACK;
      'C' : CALIBRATE;
      'D' : DATA_ANALYSIS;
      'I' : SET_PORTNO;
      'O' : TABLE_MENU;
      'P' : COLONY_TRACK;
      'R' : READDATA;
      'S' : SAMPLING;
      'T' : SET_THOLDS;
      'V' : IF INPUT = CAMERA
        THEN
          BEGIN
            INPUT := VTR;
            METHOD := KEYINPUT; { forced option }
          END
        ELSE
          INPUT := CAMERA;
    OTHERWISE;

```

```
END; { case }  
  
IF KEY <> 'Q'  
THEN  
    PROMPTSUP( PROMPTX, PROMPTY );  
  
    UNTIL KEY = 'Q';  
    UNTIL ( YES_NO( 'ARE YOU SURE ?.' ) = 'Y' );  
  
DOFF;  
END.
```

Appendix II

MIKE - program to calculate movement data from BUGSY x,y output

C MIKES PROGRAM FOR IMAGE ANALYSIS

```
INTEGER*4 IND(3002),IX(3002)*4,IY(3002)*4,IHR(3002)*4,
*IMIN(3002)*4,ISEC(3002)*4,IHSEC(3002)*4
DIMENSION DIST(3002),ANGLE(3002),ATURN(3002),SSTAY(3002),
*FFMOVE(3002)
CHARACTER DUMMY*60,INPUT*40,OUTPUT*40,RESP*1,ARESP*5
CHARACTER*40 STATFIL,MOVFIL*40,TFIL

WRITE(*,*)'Type in input file name '
READ(*,1)INPUT
WRITE(*,*)'Type in file name statistics file'
READ(*,1)STATFIL
WRITE(*,*)'Type in name movement duration file'
READ(*,1)MOVFIL
WRITE(*,*)'Type in filename for full report file'
READ(*,1)OUTPUT
WRITE(*,*)'Type in name of table file'
READ(*,1)TFIL
WRITE(*,*)'Type in number of records in file (NREC)'
WRITE(*,*)'NREC = last index no.- first index no. + 1'
READ(*,*)N
WRITE(*,*)'Type in scale factor, (TYPE 1. IF NOT REQUIRED)'
READ(*,*)SCALE
50 WRITE(*,*)'Do you want time reported in minutes or'
WRITE(*,*,ERR=50)'seconds? [S/M]'
READ(*,1)RESP
1 FORMAT(A)
IF(RESP.EQ.'M'.OR.RESP.EQ.'m')ARESP='(min)'
IF(RESP.EQ.'S'.OR.RESP.EQ.'s')ARESP='(sec)'
OPEN(UNIT=1,FILE=INPUT,STATUS='OLD')
OPEN(UNIT=2,FILE=OUTPUT,STATUS='NEW')
OPEN(UNIT=3,FILE=STATFIL,STATUS='NEW')
OPEN(UNIT=4,FILE=MOVFIL,STATUS='NEW')
OPEN(UNIT=7,FILE=TFIL,STATUS='NEW')
C WRITE FILE INFORMATION
WRITE(2,*)
WRITE(2,36)OUTPUT
36 FORMAT(1X,THIS FILE IS:           ',A/)
WRITE(2,37)INPUT
38 FORMAT(1X,TABLE FILE IS:         ',A/)
WRITE(2,38)TFIL
37 FORMAT(1X,INPUT FILE IS:         ',A/)
WRITE(2,22)STATFIL
22 FORMAT(1X,NAME OF STATISTICS FILE IS: ',A/1X,
***INDEX X Y DIST ANGLE TURN SPEED TDIST DTIME**/)
WRITE(2,23)MOVFIL
23 FORMAT(1X,NAME OF MOVEMENT DURATION FILE IS: ',A/1X,
***COL1= STAY PERIODS COL2= MOVE PERIODS**//)
WRITE(2,*)
WRITE(2,*)
WRITE(2,*)'SCALE FACTOR = ',SCALE
WRITE(2,*)
WRITE(2,*)
WRITE(2,24)ARESP
24 FORMAT(1X,TIME IS EXPRESSED IN: ',A5//)
C WRITE HEADING
WRITE(2,2)ARESP
2 FORMAT(1X,'INDEX',3X,'X',3X,'Y',4X,'DIST',3X,'ANGLE',
*4X,'TIME',8X,'TURN',4X,'SPEED',4X,'TDIST',2X,'DTIME ',
```

```

*A5/1X,80('_)/

C READ DATA FILE
  READ(1,1)DUMMY
  READ(1,1)DUMMY
  DO 500 I=1,N
    READ(1,10,END=200)IND(I),IX(I),IY(I),
    *DIST(I),ANGLE(I),IHR(I),IMIN(I),ISEC(I),IHSEC(I)
    IX(I)=IX(I)
    IY(I)=IY(I)
  500 CONTINUE
  10 FORMAT(I5,I6,I4,F11.5,F11.5,2X,I2,1X,I2,1X,I2,1X,I2)
  200 CONTINUE
    DIST(1)=0

C TEST FOR PIXEL MOVEMENT
  DO 502 I=2,N
    IF((ABS(IX(I)-IX(I-1)).EQ.1.AND.ABS(IY(I)-IY(I-1)).EQ.1)
    *.OR.(ABS(IX(I)-IX(I-1)).EQ.1.AND.ABS(IY(I)-IY(I-1)).EQ.0)
    *.OR.(ABS(IX(I)-IX(I-1)).EQ.0.AND.ABS(IY(I)-IY(I-1)).EQ.1)
    *.OR.(ABS(IX(I)-IX(I-1)).EQ.0.AND.ABS(IY(I)-IY(I-1)).EQ.0)
    *)THEN
      IX(I)=IX(I-1)
      IY(I)=IY(I-1)
      DIST(I)=0.
    END IF
  502 CONTINUE
C RECALCULATE DISTANCE
  DO 556 I=2,N
    DIST(I)=SQRT((IX(I)-IX(I-1))**2+(IY(I)-IY(I-1))**2)*SCALE
  556 CONTINUE
C RECALCULATE ANGLE
  DO 557 I=2,N
    X=IX(I)-IX(I-1)
    Y=IY(I)-IY(I-1)
    IF(X.GT.0.0)ANGLE(I)=(360/6.28319)*ATAN(Y/X)
    IF(X.GT.0.0.AND.Y.LT.0.0)ANGLE(I)=360+ANGLE(I)
    IF(X.GT.0.0.AND.Y.EQ.0.0)ANGLE(I)=360.
    IF(X.LT.0.0)ANGLE(I)=180+((360/6.28319)*ATAN(Y/X))
    IF(X.EQ.0.0.AND.Y.GT.0.0)ANGLE(I)=90.
    IF(X.EQ.0.0.AND.Y.LT.0.0)ANGLE(I)=270.
    IF(X.EQ.0.0.AND.Y.EQ.0.0)ANGLE(I)=ANGLE(I-1)
    SUMX=SUMX+(COS(ANGLE(I)))
    SUMY=SUMY+(SIN(ANGLE(I)))
  557 CONTINUE
C WRITE FIRST LINE
  WRITE(2,15)IND(1),IX(1),IY(1),DIST(1),ANGLE(1),IHR(1),
  *IMIN(1),ISEC(1),IHSEC(1)
  15 FORMAT(1X,I5,I6,I4,F7.2,F7.2,2X,I2,':',I2,':',I2,':',I2,
  *F8.2,F9.3,F8.2,F7.4)
  16 FORMAT(1X,I5,I6,I4,F7.2,F8.2,2X,
  *F8.2,F9.3,F9.2,F7.4)
C CALCULATE ANGLE TURNED
  DO 503 I=2,N
    RADIANS=(6.28319/360)*(ANGLE(I)-ANGLE(I-1))
    ATURN(I)=(360/6.28319)*ACOS(COS(RADIANS))
    IF(ANGLE(I)-ANGLE(I-1).GT.0.)ATURN(I)=-ATURN(I)
    IF(ANGLE(I).GT.270.0.AND.ANGLE(I-1).LT.90.0)
    *ATURN(I)=-ATURN(I)
    TANGLE=TANGLE+ABS(ATURN(I))
  503 CONTINUE
C CALCULATE SPEED. RESTRICTIONS: TIME MUST NOT BE RECORDED
C NEAR MIDNIGHT
  DO 504 I=2,N

```

```

DDIST=DIST(I)
TDIST=TDIST+DDIST

C TRY ANOTHER CALCULATION OF TIME
  DMIN1=IMIN(I-1)+(ISEC(I-1)/60.) + (IHSEC(I-1)/6000.)
  DMIN2=IMIN(I)+(ISEC(I)/60.) + (IHSEC(I)/6000.)
  IF(RES.P.NE.'M'.AND.RES.P.NE.'m')GOTO 700
  IF(DMIN2.LT.DMIN1)THEN
    TIME=60.-DMIN1+DMIN2
  ELSE
    TIME=DMIN2-DMIN1
  END IF
  GOTO 800

700 CONTINUE
  DSEC1=ISEC(I-1)+(IHSEC(I-1)/100.)
  DSEC2=ISEC(I)+(IHSEC(I)/100.)
  IF(DSEC2.LT.DSEC1)THEN
    TIME=60.-DSEC1+DSEC2
  ELSE
    TIME=DSEC2-DSEC1
  END IF

800 CONTINUE
  TTIME=TTIME+TIME
C CALCULATE SPEED
  SPEED=DDIST/TIME
  TSPEED=TSPEED+SPEED

C CALCULATE STATIONARY TIME
  IF(DIST(I).EQ.0)ST=ST+TIME
C CALCULATE STATIONARY PERIODS AND PERIODS OF MOVEMENT
C MOVEMENT FIRST

  IF(DIST(I).GT.0.0.AND.DIST(I-1).EQ.0.0)THEN
    IF(J.EQ.0)J=1
    SSTAY(J)=STAY
    K=K+1
    STAY=0.
  END IF
  IF(DIST(I).GT.0.0)FMOVE=FMOVE+TIME
  IF(I.EQ.N.AND.FFMOVE(K).EQ.0.0)FFMOVE(K)=FMOVE

C TEST AND RECORD STATIONARY PERIODS
  IF(DIST(I-1).GT.0.0.AND.DIST(I).EQ.0.0)THEN
    IF(K.EQ.0)K=1
    FFMOVE(K)=FMOVE
    J=J+1
    FMOVE=0.
  END IF
  IF(DIST(I).EQ.0.0)STAY=STAY+TIME
  IF(I.EQ.N.AND.SSTAY(J).EQ.0.0)SSTAY(J)=STAY
C WRITE RESULTS
  WRITE(2,15)IND(I),IX(I),IY(I),DIST(I),ANGLE(I),IHR(I),
  *IMIN(I),ISEC(I),IHSEC(I),ATURN(I),SPEED,TDIST,TIME
  WRITE(3,16)IND(I),IX(I),IY(I),DIST(I),ANGLE(I),
  *ATURN(I),SPEED,TDIST,TIME

C CALCULATE MEANDER
  IF(DIST(I).GT.0.0)THEN
    TMEANDER=TMEANDER+(ABS(ATURN(I))/DIST(I))
  ELSE
    TMEANDER=TMEANDER+0.
  END IF

```

504 CONTINUE

```
C CALCULATE MEAN SPEED
  FMSPEED=TSPEED/(N-1)
C CALCULATE MEAN ANGLE
  FMX=SUMX/(N-1)
  FMY=SUMY/(N-1)

  IF(FMX.GT.0.0)FMANGLE=(360/6.28319)*ATAN(FMY/FMX)
  IF(FMANGLE.LT.0)FMANGLE=360+FMANGLE
  IF(FMX.LT.0.0)FMANGLE=180+((360/6.28319)*ATAN(FMY/FMX))
  IF(FMX.EQ.0.0.AND.FMY.GT.0.0)FMANGLE=90.
  IF(FMX.EQ.0.0.AND.FMY.LT.0.0)FMANGLE=270.
C CALCULATE MEAN VECTOR LENGTH (WEIGHTED)
  R=SQRT(FMX**2+FMY**2)
C CALCULATE MEAN ANGULAR DEVIATION
  S=(360/6.28319)*SQRT(2*(1-R))
C CALCULATE ARITHMETIC MEAN VECTOR LENGTH
  AMVEC=TDIST/(N-1)
C CALCULATE MEAN ABSOLUTE TURNING RATE
  FTANGLE=TANGLE/(N-1)
C CALCULATE MEAN MEANDER
  FMEANDER=TMEANDER/(N-1)
  WRITE(7,38)TFIL
  WRITE(7,*)
  WRITE(7,*)
  IF(RESPEQ.'M'.OR.RESPEQ.'m')THEN
    WRITE(7,90)FMSPEED
90 FORMAT(1X,'MEAN SPEED PER MINUTE           = ',F8.2)
    WRITE(7,91)ST
91 FORMAT(1X,'STATIONARY TIME (MINS)           = ',F8.2)
    ELSE
    WRITE(7,92)FMSPEED
92 FORMAT(1X,'MEAN SPEED PER SECOND           = ',F8.2)
    WRITE(7,93)ST
93 FORMAT(1X,'STATIONARY TIME (SECS)          = ',F8.2)
    END IF
C CALCULATE DISTANCE AS THE CROW FLIES
  IX3=IX(1)-IX(N)
  IY3=IY(1)-IY(N)
  CROW=SQRT(IX3**2+IY3**2)*SCALE

  WRITE(7,100)FMX
100 FORMAT(1X,'MEAN X (XBAR)                   = ',F8.2)
  WRITE(7,101)FMY
101 FORMAT(1X,'MEAN Y (YBAR)                   = ',F8.2)
  WRITE(7,102)FMANGLE
102 FORMAT(1X,'MEAN ANGLE                       = ',F8.2)
  WRITE(7,103)R
103 FORMAT(1X,'WEIGHTED MEAN VECTOR LENGTH (r) = ',F8.2)
  WRITE(7,104)AMVEC
104 FORMAT(1X,'ARITHMETIC MEAN VECTOR LENGTH   = ',F8.2)
  WRITE(7,105)S
105 FORMAT(1X,'MEAN ANGULAR DEVIATION (s)      = ',F8.2)
  WRITE(7,106)FTANGLE
106 FORMAT(1X,'ABSOLUTE MEAN TURN              = ',F8.2)
  WRITE(7,107)FMEANDER
107 FORMAT(1X,'MEAN MEANDER                    = ',F8.2)
C CALCULATE INDEX OF STRAIGHTNESS
  STRGHT=CROW/TDIST
  WRITE(7,*)
  WRITE(7,*)
  WRITE(7,108)CROW
108 FORMAT(1X,'DISTANCE AS THE CROW FLIES     = ',F8.2)
```

```
WRITE(7,109)STRGHT
109 FORMAT(1X,'INDEX OF STRAIGHTNESS      = ',F8.2)
WRITE(7,*)
WRITE(7,110)TDIST
110 FORMAT(1X,'TOTAL DISTANCE TRAVELLED   = ',F8.2)
WRITE(7,*)
```

```
C 600 WRITE(2,*)'FILE READ ERROR'
```

```
C WRITE MOVEMENT DATA TO FILE
```

```
  JJ=MAX(J,K)
  DO 650 I=1,JJ
    WRITE(4,25)SSTAY(I),FFMOVE(I)
  25 FORMAT(1X,2F9.4)
650 CONTINUE
  STOP
  END
```

Appendix III

DFILE - program to edit data sets to smaller usable units

C MIKES PROGRAM for cutting up buggy files

```
CHARACTER INPUT*40,OUTPUT*40,DUMMY1*52,DUMMY2*52
CHARACTER TIME*11

WRITE(*,*)'Type in input file name '
READ(*,1)INPUT
WRITE(*,*)'Type in output file'
READ(*,1)OUTPUT
WRITE(*,*)'Type in first record'
READ(*,*)N
WRITE(*,*)'Type in last record'
READ(*,*)M
1 FORMAT(A)
OPEN(UNIT=1,FILE=INPUT,STATUS='OLD')
OPEN(UNIT=2,FILE=OUTPUT,STATUS='NEW')
C READ DATA FILE
READ(1,1)DUMMY1
READ(1,1)DUMMY2
WRITE(2,1)DUMMY1
WRITE(2,1)DUMMY2
DO 500 I=0,N-1
  READ(1,10,END=200)IND,IX,IY,
  *DIST,ANGLE,TIME
500 CONTINUE
DO 600 I=N,M
  READ(1,10,END=200)IND,IX,IY,
  *DIST,ANGLE,TIME
  WRITE(2,10)IND,IX,IY,
  *DIST,ANGLE,TIME
600 CONTINUE
10 FORMAT(I5,I6,I4,F11.5,F11.5,2X,A)
200 CONTINUE
STOP
END
```

Appendix IV

CFILE - program to choose certain parameters to input into statistical packages

```
DIMENSION DATA(9), ICOL(9), FDATA(9)
CHARACTER*40 OUTFIL, INFIL
CHARACTER*10 LOC
WRITE(6,*) 'TYPE IN INPUT FILE'
READ(5,1)INFIL
1 ← FORMAT(A)
OPEN(UNIT=1,FILE=INFIL,STATUS='OLD')
WRITE(6,*) 'TYPE IN OUTPUT FILE'
READ(5,1)OUTFIL
OPEN(UNIT=2,FILE=OUTFIL,STATUS='NEW')
WRITE(6,*) 'TYPE IN NUMBER OF COLUMNS'
READ(5,*)NCOL
WRITE(6,*) 'TYPE IN COLUMN NUMBERS'
READ(5,*)(ICOL(M),M=1,NCOL)

DO 20 I=1,4000
READ(1,10,END=100)(DATA(J),J=1,9)
  DO 30 K=1,NCOL
    FDATA(K)=DATA(ICOL(K))
30 CONTINUE
  WRITE(2,11)(FDATA(K),K=1,NCOL)
10 FORMAT(F6.0,F6.0,F4.0,F7.2,F8.2,F10.2,F9.3,F9.2,F7.4)
11 FORMAT(1X,9F10.3)
20 CONTINUE
100 STOP
END
```

Appendix V

BUG2EPS - program to view insect movement from coordinates stored in .bug files (produced by BUGSY). Also enables movement tracks to be converted to a suitable format for plotting on various graphic output devices of laser quality. The program can not be compiled without some auxillary files (not included).

```
#include <graphics.h>
#include "realgfx.h"
#include <string.h>

#define LINELEN 120

char linein[LINELEN]="",outname[LINELEN]="",iname[LINELEN]="";
FILE *infile, *outfile;
int x,y,ox,oy,num,start,count=-1,count1=-1,slow=1;
rgReal dummy;
char theTime[80];

char header[]={
#include "pshead.h"
};

char footer[]={
#include "psfoot.h"
};

void main(int argc, char **argv) {

    char c;

    clrscr();

    printf(
    " BUGSEY bug track to Encapsualted Postscript converter \n"
    " by Terry Brown, 1992 \n"
    "\n"
    " Converts BUGSEY .BUG files to encapsulated postscript \n"
    "\n"
    " Usage: bug2eps [infile [outfile]] \n"
    "\n"
    " Runs faster if infile and outfile are on separate disks \n"
    "\n"
    " While viewing\n"
    "   Press 'g' to run continuously\n"
    "   Press 'q' to quit\n"
    "   Any other key for step-by-step movement\n"
    "\n"
    );
    rg_ps_xo = rg_ps_yo = 0.0; /* postscript output parameters */
    rg_ps_xr = 620.0;
    rg_ps_yr = 620.0;

    if (argc>1) {
        strcpy(inname,argv[1]);
    } else {
        printf("Name of input file : ");
        scanf("%s",&iname);
    }

    infile=fopen(inname,"r");
    if (infile==NULL) {
        printf("Can't open %s\n",iname);
    }
}
```

```

    exit(1);
}

printf("Counting lines in file, please wait ... \n\n");

fgets(linein,LINELEN,infile);
fgets(linein,LINELEN,infile); /* drop 2 line header */

while (fscanf(infile,"%d %d %d",&num,&x,&y)==3) {
    fgets(linein,LINELEN,infile); /* drop rest of line */
    count++;
}

fclose(infile);

printf("Start at point (%d-%d) : ",0,count);
scanf("%d",&start);
printf("End at point (%d-%d) : ",start,count);
scanf("%d",&count);
getchar();
if (argc>2) {
    strcpy(outname,argv[2]);
} else {
    printf("Name of EPS output file (just RETURN to view only) : ");
    fgets(outname,LINELEN,stdin);
    outname[strlen(outname)-1]='\0'; /* kill eol */
}

#define DOOUT (strlen(outname)!=0)
#define EMIT(file,string) if (1) {long i=0; while (string[i]!='\0') \
    fputc(string[i++],file); }

if DOOUT {
    outfile=fopen(outname,"w");
    if (outfile==NULL) {
        printf("Can't open %s\n",outname);
        exit(1);
    }
    rg_ps_out=outfile;
    EMIT(outfile,header);
}

infile=fopen(inname,"r");

fgets(linein,LINELEN,infile);
fgets(linein,LINELEN,infile); /* drop 2 line header */

OpenDisplay();

xOrg=yOrg=0.;
xRng=yRng=520.;

fscanf(infile,"%d %d %d",&num,&ox,&oy);
count1++;
fscanf(infile,"%lf %lf",&dummy,&dummy);
fgets(theTime,LINELEN,infile); /* drop rest of line */
theTime[strlen(theTime)-1]='\0';

if ( count1>=start && count1<=count && !(ox==x && oy==y) ) {
    rgMode(PS_OFF); Plot((rgReal)ox,(rgReal)oy); rgMode(PS_ON);
    setfillstyle(SOLID_FILL,BLACK);
    bar(0,0,300,20);
    sprintf(theTime,"%d%c",count1,0);
    outtextxy(1,10,theTime);
}

```

```

}

while (fscanf(infile,"%d %d %d",&num,&x,&y)==3 && count1<=count) {

    fscanf(infile,"%lf %lf",&dummy,&dummy);
    fgets(theTime,LINELEN,infile); /* drop rest of line */
    theTime[strlen(theTime)-1]='\0';

    count1++;

    if ( count1>=start && count1<=count && !(ox==x && oy==y) ) {
        rgMode(PS_OFF); Plot((rgReal)ox,(rgReal)oy); rgMode(PS_ON);
        DrawLine((rgReal)x,(rgReal)y);
        setfillstyle(SOLID_FILL,BLACK);
        bar(0,0,300,20);
        sprintf(theTime,"%d%c",count1-1,0);
        outtextxy(1,10,theTime);

        if (kbhit()) slow=1;

        if (slow) {
            c=getch();
            if (c=='g' || c=='G') slow=0;
            if (c=='q' || c=='Q') {
                fclose(infile);
                if (outfile!=NULL) {
                    EMIT(outfile,footer);
                    fclose(outfile);
                }
                CloseDisplay();
                exit(0);
            }
        }
    }

}

ox=x; oy=y;
}

if DOOUT {
    EMIT(outfile,footer);
    fclose(outfile);
    rg_ps_out=outfile=NULL;
}

PutText(20.,30.,"Press RETURN");

getchar();

CloseDisplay();

fclose(infile);}

```

Appendix VI

FRACTAL - program to calculate fractals and distance travelled at various step sizes

```
C MIKES PROGRAM FOR IMAGE ANALYSIS
  INTEGER*4 IND(3002),IX(3002)*4,IY(3002)*4,
  *STEP(5)
  DIMENSION FDIST(5)
  CHARACTER DUMMY*60,INPUT*40,OUTPUT*40

  WRITE(*,*)'Type in input file name '
  READ(*,1)INPUT
  WRITE(*,*)'Type in filename for report file'
  READ(*,1)OUTPUT
  1 FORMAT(A)
  WRITE(*,*) 'TYPE IN SCALE FACTOR'
  READ(*,*)SCALE
  OPEN(UNIT=1,FILE=INPUT,STATUS='OLD')
  OPEN(UNIT=2,FILE=OUTPUT,STATUS='NEW')

C WRITE FILE INFORMATION
  WRITE(2,*)
  WRITE(2,36)OUTPUT
  36 FORMAT(1X,'THIS FILE IS:           ',A/)
  WRITE(2,37)INPUT
  37 FORMAT(1X,'INPUT FILE IS:         ',A/)
  WRITE(*,*)'INPUT NUMBER OF DATA POINTS TO READ'
  READ(*,*)NDAT

C INPUT STEPS
  WRITE(*,*)'INPUT 5 STEPS. THE FIRST SHOULD BE 1'
  DO I=1,5
  READ(*,*)STEP(I)
  END DO

C INPUT STEP LENGTH FOR RENORMALISATION
  WRITE(*,*)'INPUT STEP LENGTH FOR RENORMALISATION E.G. 36'
  READ(*,*)LSTEP

C READ FIRST LINES OF DATA FILE
C  READ(1,1)DUMMY
C  READ(1,1)DUMMY
C READ IN DATA LINES

C  15 FORMAT(1X,I5,I6,I4,F7.2,F7.2,2X,I2,':',I2,':',I2,':',I2,
C  *F8.2,F9.3,F8.2,F7.4)
  16 FORMAT(1X,I5,I6,I4,F7.2,F8.2,2X,
  *F8.2,F9.3,F9.2,F7.4)
  J=0
  DO I=1,NDAT
C  READ(1,15,ERR=600,END=100)IND(I),IX(I),IY(I),DDIST,ANGLE,IHR,
C  *IMIN,ISEC,IHSEC,ATURN,SPEED,TDIST,TIME
  301 READ(1,16,ERR=600,END=100)IND(I),IX(I),IY(I),DDIST,ANGLE,
  *ATURN,SPEED,TDIST,TIME
C  IF(I.GT.1.AND.DDIST.EQ.0.0)GO TO 301
  J=J+1
  END DO
  100 CONTINUE
  WRITE (*,*)'DATA READ SUCCESSFULLY'
C CALCULATE DATA TO DETERMINE THE MEAN FRACTAL DIMENSION FOR THIS
C INSECT
C PASS 1
  DIST1=0
  K=0
  DO I=2,J
  K=K+1
```

```

DIST= SQRT((IX(I)-IX(I-STEP(1)))**2+(IY(I)-IY(I-STEP(1)))**2)
WRITE(*,*)'DIST=',DIST
TDIST1=TDIST1+DIST
END DO
WRITE(*,*)'TDIST1 =',TDIST1
FDIST(1)=TDIST1*SCALE
WRITE(*,*)'FDIST(1)=' ,FDIST(1)
WRITE(2,*)'PASS 1 COMPLETED. STEPS = ',K
C PASS 2
K=0
DO I=STEP(2),J,STEP(2)
K=K+1
IF(I.EQ.STEP(2))THEN
DIST=SQRT((IX(I)-IX(I-STEP(2)+1))**2+(IY(I)-IY(I-STEP(2)+1))**2)
ELSE
DIST=SQRT((IX(I)-IX(I-STEP(2)))**2+(IY(I)-IY(I-STEP(2)))**2)
END IF
TDIST2=TDIST2+DIST
END DO
FDIST(2)=TDIST2*SCALE
WRITE(2,*)'PASS 2 COMPLETED. Steps = ',K
C PASS 3
K=0
DO I=STEP(3),J,STEP(3)
K=K+1
IF(I.EQ.STEP(3))THEN
DIST=SQRT((IX(I)-IX(I-STEP(3)+1))**2+(IY(I)-IY(I-STEP(3)+1))**2)
ELSE
DIST=SQRT((IX(I)-IX(I-STEP(3)))**2+(IY(I)-IY(I-STEP(3)))**2)
END IF
TDIST3=TDIST3+DIST
END DO
FDIST(3)=TDIST3*SCALE
WRITE(2,*)'PASS 3 COMPLETED. STEPS = ',K
C PASS 4
K=0
DO I=STEP(4),J,STEP(4)
K=K+1
IF(I.EQ.STEP(4))THEN
DIST=SQRT((IX(I)-IX(I-STEP(4)+1))**2+(IY(I)-IY(I-STEP(4)+1))**2)
ELSE
DIST=SQRT((IX(I)-IX(I-STEP(4)))**2+(IY(I)-IY(I-STEP(4)))**2)
END IF
TDIST4=TDIST4+DIST
END DO
FDIST(4)=TDIST4*SCALE
WRITE(2,*)'PASS 4 COMPLETED. STEPS = ',K
C PASS 5
K=0
DO I=STEP(5),J,STEP(5)
K=K+1
IF(I.EQ.STEP(5))THEN
DIST=SQRT((IX(I)-IX(I-STEP(5)+1))**2+(IY(I)-IY(I-STEP(5)+1))**2)
ELSE
DIST=SQRT((IX(I)-IX(I-STEP(5)))**2+(IY(I)-IY(I-STEP(5)))**2)
END IF
TDIST5=TDIST5+DIST
END DO
FDIST(5)=TDIST5*SCALE
WRITE(2,*)'PASS 5 COMPLETED. STEPS = ',K
C WRITE RESULTS
WRITE(*,*)'NUMBER OF DATA POINTS = ',J
WRITE(2,*)'NUMBER OF DATA POINTS = ',J
WRITE(2,*)'DATA FOR CALCULATION OF FRACTAL DIMENSIONS'

```

```

WRITE(2,25)(STEP(I),I=1,5)
25 FORMAT(1X,'STEP SIZE IN SECONDS',5(I10,1X))

WRITE(2,24)(FDIST(I),I=1,5)
24 FORMAT(21X,5(F10.2,1X))
C DO REGRESSION
CALL REGRESS(STEP,FDIST,5)

C RENORMALISE VECTORS
C K=0
C DO I=1,J,LSTEP
C RSUM=0
C K=K+1
C NN=I
C DO N=NN,J,LSTEP
C RSUM=RSUM+SQRT(IX(N)**2+IY(N)**2)
C END DO
C R(K)=RSUM*SQRT(INT(J/LSTEP))
C IIX(K)=
C IIY(K)=
C END DO
600 WRITE(2,*)'FILE READ ERROR'
STOP
END

C SubPROGRAM TO CALCULATE REGRESSION EQUATIONS
SUBROUTINE REGRESS(IX,Y,N)
DIMENSION IX(5),Y(5), X(5)
WRITE(*,*)'IN REGRESSION'
SUMY=0.
SUMX=0.
SUMYY=0.
SUMXX=0.
SUMXY=0.
FN=N
DO J=1,N
X(J)=LOG(IX(J))
Y(J)=LOG(Y(J))
END DO
DO 20 I=1,N
C READ(1,3,END=30)IX(I),Y(I)
3 FORMAT(F7.3,1X,F10.2)
SUMX=SUMX+X(I)
SUMY=SUMY+Y(I)
SUMXX=SUMXX+X(I)**2
SUMYY=SUMYY+Y(I)**2
SUMXY=SUMXY+(X(I)*Y(I))
JJ=JJ+1
20 CONTINUE
30 CONTINUE

B=((FN*SUMXY)-(SUMX*SUMY))/((FN*SUMXX)-(SUMX**2))
A=((SUMY*SUMXX)-(SUMX*SUMY))/((FN*SUMXX)-(SUMX**2))
R=((FN*SUMXY)-(SUMX*SUMY))/SQRT(((FN*SUMXX)-(SUMX**2))*
*((FN*SUMYY)-SUMY**2))
RR=R**2
FRACT=ABS(B-1)
WRITE(2,*)
WRITE(2,4)A
WRITE(2,5)B
WRITE(2,6)RR
WRITE(2,7)FRACT
WRITE(2,*)
DO 25 I=1,N

```

```
YY=A+B*X(I)
WRITE(2,3)X(I),YY
25 CONTINUE
4 FORMAT(1X,'A = ',F9.2)
5 FORMAT(1X,'B = ',F9.2)
6 FORMAT(1X,'R2 = ',F9.2)
7 FORMAT(1X,'FRACTAL DIMENSION = ',F5.2)
WRITE(2,*)
RETURN
END
```