Injury and Population Assessment Tools for the Eucalyptus Gall Wasp *Ophelimus maskelli* (Ashmead)

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Malkie Spodek

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כלים להערכת אוכלוסיות ונזק של צרעת עפצי החטטים, *Ophelimus maskelli* (Ashmead) לעצי איקליפטוס

עבודת גמר

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This work was carried out under the supervision of :

Professor Zvi Mendel

and

Dr. David Nestel

in the Department of Entomology, ARO, Volcani Research Institute

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SUMMARY

The gall wasp, *Ophelimus maskelli* (Ashmead) (Hymenoptera: Eulophidae), recently invaded the Mediterranean region. The wasp spread throughout this area in a very short period of time; the first detection in Israel was in 2003. The injury inflicted by this wasp has prompted urgent action programs to deal with the pest. *O. maskelli* produces galls on the leaves of several eucalyptus tree species. In Israel, its major host is the river red gum, *Eucalyptus camaldulensis*. The galls are pimple-like, nearly round and occur on both surfaces of the leaf. This galling eventually can lead to heavily injured trees that are revealed by the desiccation of large parts of their crowns leading to the eventual premature shedding of the leaves.

The present study investigated several assessment tools that could potentially be incorporated into management regimes for this gall wasp. An examination of evaluation methods of levels of tree injury was introduced by a visual grade (V.G.) assessment currently used by professional observers. The visual assessment is based on a one to five ordinal scale of level of injury and it was compared to an Injury Index (I.I.) derived from sampled foliage with non-galled and galled leaves from 13 *E. camaldulensis* stands throughout Israel. Digital analysis of sampled leaves was employed in order to determine the degree of leaf galling per stand (P.G.), one of the two Injury Index parameters (the other parameter was the frequency of galled leaves per stand (F.G.). Assessments were carried out in the Winter 2006 and Winter 2007.

The comparison between the I.I., the developed "objective" index based on quantitative sampled data, and the "subjective V.G., based on the valuation of a "subjective" observer, revealed overall correspondence between the two injury assessment methods. Significant differences between the two assessment methods were found when tree injury levels were low. Other findings showed that the F.G. for all the stands in 2006 and 2007 was statistically similar. In contrast, differences between the two years in P.G. were significantly different, suggesting that the drastic drop in I.I. between years was mainly affected by the proportion of leaf area covered with galls and not by the frequency of galled leaves.

The viability of using remote sensing as a detection and monitoring tool for gall wasp management was also explored in this study. The evaluation of the spectral signatures of galled and non-galled leaves and saplings was determined by using a hand held spectrometer. This level of analysis is usually recommended in the early understanding and testing of methods leading to remote sensing of forest pest injury levels. Investigation of the spectral properties at the leaf level provides a basic understanding of the link between the health condition of the leaf and spectral properties of the investigated phenomena. This level of analysis is needed for the development of remote sensing sampling strategies and data interpretation methods.

This study also included the characterization of the spectra of galled leaves as related to the age of galling, density of galling per leaf and level of necrosis per leaf. The analysis for the resulting spectral curves focused on the near infrared region with an emphasis in the red edge region (680-780 nm) mostly used in remote sensing to detect stress in vegetation.

The results from this study indicate that there are significant differences between galled and non-galled leaves in the red edge region. These differences are expressed in the wavelength where the maximum value of the first derivative curve in the red edge region (680-780 nm) occurs (to be referred to as λ_{re} in the rest of the paper). Wavelengths tended to be generally lower in galled leaves and saplings compared to non-galled leaves and saplings.

These results suggest that galling is inducing stress upon the leaf. In addition, average λ_{re} from leaves bearing one to two month old galls revealed no significant differences in the red edge region compared to non-galled leaves. However, significant differences were found between wavelengths on leaves bearing galls older than three months compared to non-galled leaves, suggesting that stress is expressed closed to the time of wasp emergence, when the wasp is more developed. The association between density of galling on the leaf surface and the average λ_{re} was statistically significant. Increased density of galling on the leaf surface led to lower wavelengths in the red edge region.

These findings suggest that although remote sensing may not be viable as an early detection tool for the gall wasp (due to the required need of a threshold damage level and age), it seems to have potential use for differentiating between galled and non-galled trees in eucalyptus stands under certain conditions (e.g. high galled densities and developed galls). The results also suggest that discerning between gall stress and other sources of stress through remote sensing, may be difficult. In any event, these results provide the initial basis for future applications of remote sensing to detect the stress of galling in eucalyptus stands, at least regarding the location, spatial extent and injury levels.

In addition to the injury assessments examined in this study, population trends of adult *O. maskelli,* were investigated. Sticky traps and accumulated degree-day models were tools used for advancing our understanding of the pest. There was an attempt to link adult wasp emergence trends and geo-climatic variation of Israel. It was found that spring, from March 1st to April 15th is the main period of adult wasp emergence. The variation in emergence was determined for different regions throughout Israel. Early adult emergence (between March 1st and March 15th) was detected in the Western Negev and Coastal Plain. Adult wasps in the Jerusalem area, the Galilee and the Golan emerged a month later.

The time of adult emergence in the different regions seems to be driven by the accumulation of temperatures, since accumulated degree-days (from January 1st) vary between regions. The average number of accumulated degree-days required by a wasp to emerge from the pupae was 388.82 ± 85.21. This estimation had an approximate variation of 20% throughout the different regions. Depending on the region, this level of uncertainty corresponds to a possibility of error that ranges between five to ten days to mistakenly determine the time of adult emergence. The implications of this forecasting error will depend on the management and pest control techniques applied against the pest.

INTRODUCTION

Eucalyptus trees in new habitats have been under constant assault by a stream of phytophagous insect pests that originated in the trees' homeland, Australia (Withers, 2001). The gall wasp, *Ophelimus maskelli* (Ashmead) (Hymenoptera: Eulophidae), recently invaded the Mediterranean Region, reaching the Iberian Peninsula, the Middle East (including Israel), parts of Africa, Southern Europe and Asia (Viggiani et al. 2000, Pujade-Villar and Riba-Flinch 2004, Mendel et al. 2004). Similar to the previous invasions of other eucalypt pests, this gall wasp was not accompanied by its principal natural enemies, which occur in Australia, and therefore, they quickly reached epidemic levels (Protasov et al. 2006). The wasp invaded and spread to these new environments in a very short period of time; the first detection in Israel was in 2003. Since then, reports on new detections in Israel and other areas have increased at a rapid pace and has prompted urgent action programs to deal with the pest.

Eucalyptus trees are the backbone of afforested areas in the low altitude arid and semi-arid lands of the Middle East and North Africa. Eucalyptus plantations are a major source of timber, firewood and honeybee foraging, and are used as recreation areas, as shelterbelts from drifting sands, and as windbreaks surrounding cultivated and residential areas (Mendel et al. 2004). *O. maskelli* induces galls on the leaves of eucalyptus trees. *Eucalyptus camaldulensis* and *Eucalyptus tereticornis* (=*mbellata* Smith), belonging to the Exsertaria section of the genus are more susceptible to attack by *O. maskelli* than other tested species that belong in the Latoangulata and Maidenaria sections (Protasov et al. 2007). *Eucalyptus camaldulensis* are found on 9,000 ha in Israel accounting for 8% of the country's afforested areas (KKL, 2003).

The wasp prefers to lay her eggs on the leaves of the tree's lower canopy (Protasov et al. 2006). Eggs are usually laid in actively growing leaf tissue. Galls are abnormal growths of a plant in response to egg laying by adult insects. The irritated plant tissue quickly surrounds the egg, protects and provides food for the gall-maker until it matures (Buss, 2003). The galls are pimple-like, nearly round and usually occur on both surfaces of the leaf. The gall diameter ranges from 0.9 to 1.2 mm, and recorded gall densities on leaves ranges from 11.5 to 36.0 galls/cm^2. Under epidemic conditions the entire leaf surface is usually densely covered with galls. *O. maskelli* develops in a single gall on the leaf surface, over a period of about three to six months. Five stages of wasp development have been observed; egg, three larval instars, a non-feeding pre-pupa that fills the entire gall cavity and adult. The adult wasp emerges by cutting a circular hole in the gall wall close to the plane of the leaf. *O. maskelli* produces three generations per year in Israel and high flight occurs among the spring generation, starting in early March (Protasov et al. 2006). Newly emerged adult wasps begin to oviposit on young eucalyptus leaves. High density galling eventually can result in heavily injured trees that are revealed by the desiccation of large parts of their crowns leading to the eventual premature shedding of the leaves (Protasov et al. 2006).

Similar observations have been made for *Ophelimus eucalypti* (Hymenoptera: Eulophidae), by Withers and Raman, 2003 in New Zealand. *O. eucalypti* is another eucalyptus gall wasp that induces similar injury to eucalyptus leaves. Observations showed that heavy galling can induce leaf abscission within a few weeks, although less densely galled foliage remains intact for many months. Continuous attacks result in the death of young and newly planted trees and decline in growth in older trees (Withers and Raman, 2003). Intense defoliation of eucalyptus species and consequent economic loss in timber quality as recorded by Withers and Raman 2003, can be expected to be induced by *O. maskelli* on susceptible trees in other invaded regions of the world. When the pest was first found in Israel an action plan was immediately initiated by the appropriate authorities for management and control options (Zvi Mendel, ARO, personal communications).

The planned management actions were aimed to prevent or reduce the damage inflicted by the wasps by adopting various strategies, mainly classical biological control (importation of natural enemies from Australia), silvicultural control (resistant breeding stocks) and chemical control in nurseries and newly established forested areas. Initial forest assessments of main infested areas were also conducted based on subjective visual evaluations. These evaluations were supported by the utilization of green sticky traps that were suspended on heavily galled trees. The color green was chosen for traps based on a previous study showing that capture of *O. maskelli* was greatest on the green sticky traps compared to traps of five other colors; black, red, gray, white and yellow (Protasov et al. 2006). The intent of the study was to assess population levels and trends in relevant areas and to consider geo-climatic factors driving emergent patterns. These initial evaluations revealed a need for a more extensive and quantifiable injury assessment approach. Given the fact that these initial evaluations were conducted using a subjective assessment system, there was a requirement to confirm the assessment technique to provide an objective basis to evaluate the effect of future management.

Over the years, a variety of methods to assess and monitor forest pest damage have been employed and evaluated by forest authorities. A challenge for forest health teams is to quantify the relationship amongst the observed symptoms of damage, the causal agents, impact on the "health and condition" of affected trees, and tree growth at the broader management scale (Stone and Coops, 2004). Forest pest assessments include the traditional estimation of forest health that employs visual assessments or aerial surveys coupled with aerial photographic interpretation (Stone et al. 2000). Tree crown condition is used as a key indicator of forest health in many forest health surveillance programs (e.g. USA Department of Agricultural Forest Service, 2002, Zarnoch et al. 2004). Based on categorization of crown size and shape, branching, crown density and coloration and extent of epicormic growth, several subjective visual scoring systems have been developed (e.g. Grimes, 1978, Kile et al. 1981, Stone et al. 2003b). This concept has been undertaken to assess the damage inflicted by the wasp in Israel. However, given the subjectivity of this assessment tool, we initiated an evaluation of the accuracy of this simple methodology by leaf sampling and digital technology. The idea is that in the long run this approach may provide baseline and health trend information that is statistically precise and accurate.

The present study evaluated the existing visual assessment tool by concomitantly visually grading trees and forests and taking samples of branches with galled leaves for digital analysis and injury determination.

Remote sensing is a technologically advanced forest injury assessment technique, which is currently increasing in importance around the world (Riley, 1989). Remote sensing is based on images produced by photography from satellites, aircrafts, or other means of airborne equipment, and has been used as a viable technology for detection of forest injury inflicted by insects (Wulder et al. 2006). It is well demonstrated that remote sensing techniques can detect and map the extent of unhealthy vegetation canopies by, for example, color infrared (CIR) aerial photography or satellite digital imagery (Stone et al. 2000). Remote sensing utilizes sensors that distinguish different reflectance levels of electromagnetic energy of the targets being observed.

Light incident to the leaf surfaces of plants or trees is either absorbed, transmitted, or reflected and the pattern of reflected light or spectral reflectance is the outcome of the leaf chemical, physical, and physiological situations (Stone et al. 2001) (Appendix, Figure I). Previous studies, based principally on northern hemisphere tree species, have identified diagnostic features from the spectral responses (in the visible and near-infrared regions of the electromagnetic spectrum) of leaves exhibiting symptoms of injury from a range of stress inducing agents, including insect damage (Stone et al. 2001). Early effects of stress are often manifested as pre-visual symptoms at the finest structural scale, for example, disruption of cellular function and organization in young leaves or in the fine root system. These responses have been detected in laboratory and near-range studies of reflectance spectra of stressed leaves and saplings (Stone et al. 2000).

Several studies, (Ahern, 1988, Rock et al. 1988, Carter et al. 1996 and others), have demonstrated that certain regions of the reflected electromagnetic spectrum of leaves, such as at the inflection point between the red and near infrared (NIR) wavelengths (690 - 740 nm) and at the NIR shoulder and plateau (750 -1000 nm), are sensitive to changes arising from the initial cellular response to specific stress inducing agents. Leaves under stress show a shift in the NIR region of the spectrum (Appendix, Figure II).

Studies to date reveal varying reports of the effects of galling on the physiological processes of leaves. Galling insects are usually not considered pests and most are harmless to their vegetation hosts (Buss, 2003). However, under epidemic conditions gallers can physically or aesthetically damage plants or trees by reducing photosynthesis and seed production, discoloring foliage, causing defoliation, branch dieback, and rarely, lead to plant death (Buss, 2003). Conversely, it has also been shown that gall-formers do not directly damage host tissues, but act as phloem parasites by inducing the host plant to allocate resources to gall development and the feeding of the gall-formers. As a result, galls function as strong metabolic sinks that may result in compensatory increases in photosynthesis rates (Larson, 1998). It is therefore expected that the galls of *O. maskelli* could significantly influence leaf and sapling spectral reflectance curves thus creating a substantive basis for remote sensing of infested forests.

In the present study I evaluated the spectral signatures on two main organism levels of *E. camaldulensis*; the leaf and the entire plant (as a young sapling) of healthy and infested galled leaves and saplings. This level of analysis is usually necessary and recommended in the early understanding and establishment of methods and technique leading to remote sensing. Investigation at the leaf level provides a basic understanding of the physiological controls and spectral properties of the investigated phenomena, and is the foundation for the development of remote sensing sampling strategies and data interpretation methods. Diagnostic features identified in the leaf spectra have the potential to form the basis of future spectral indices or algorithms of canopy health (Stone et al. 2001). This study also included the characterization of the spectra resulting from galling as related to the age of galling, density of galling per leaf and level of necrosis per leaf.

We also determined the population trends of *O. maskelli* in Israel, specifically emergent periods of adult wasps. With this information, an attempt was made at developing a forecasting model for management strategies based on preliminary results of accumulated degree-days.

The main objectives of this study were:

1) To validate a current field visual assessment of galling injury using a sampling method based on a digital protocol.

2) To explore the near infrared (NIR), specifically the red edge region, spectra of gall injured eucalyptus leaves and saplings as a first step in the development of remote sensing technology to assess eucalyptus gall wasp injury.

3) To assess the population trends of adult *O. maskelli* wasps and to link the observed emergent trends to the geo-climatic variation in Israel.

MATERIALS AND METHODS

I. Visual Grade of stand injury and Injury Index assessment using sampling and digital tools

A) Sampling stands:

Eucalyptus camaldulensis stands were selected throughout the country where *O. maskelli* infested trees were found. Stands were also chosen based on their location in six geo-climatic regions of Israel: Golan, Hula Valley, Galilee, Coastal Plain and the Western Negev (Table 1). The co-ordinates of each stand were recorded by a mobile navigation system (Mitac Mio 169, Victoria, Australia) and their geo-climatic locations are shown in Appendix, Map I. These stands were sampled throughout the study for injury assessment and estimation of adult wasp emergence trends. Assessment analysis was performed on stand and regional levels.

Table 1: *Eucalyptus camaldulensis* stand locations and regions used throughout the study

Region	Sample Stand	Lat. (N)	Long. (E)	Nearest Meteorological Station	Elevation (m)
Golan	1. Alonei Habashan	33° 02' 5	35° 50' 15	Avnei Eitan	375
	2. Keshet	32° 58' 54	35° 48' 8	Gamla	203
	3. Nov	32° 50' 11	35° 47' 11	Avnei Eitan	375
Hula Valley	4. Hula Valley	33° 04' 3	35° 35' 55	Kfar Blum	75
Galilee	5. El Roi Junction	32° 42' 33	35° 06' 17	Neve Yaar	125
	6. Alon Hagalil	32° 45' 39	35° 13' 56	Neve Yaar	125
	7. Golani Junction	32° 46' 13	35° 24' 35	Tavor	150
Coastal Plain	8. Hadera	32° 26' 11	34° 54' 4	Ein Hachoresh	18
	9. Ilanot	32° 17' 43	34° 53' 54	Ein Hachoresh	18
	10. Beit Dagan	32° 0' 50	34° 49' 49	Beit Dagan	35
Judean Hills	11. Jerusalem	31° 71' 0	35°10' 0	Zovah	700
Western Negev	12. Eitan	31°33' 53	34° 44' 52	Gat	130
	13. Gvaram	31° 35' 41	34° 36' 31	Negba	90
	14. Nahal Phora	31° 27' 36	34° 45' 51	Gat	130
	15.Nahal Snaiim	31° 20' 24	34° 39' 36	Gilat	145

B) Visual injury assessment, sampling and digital estimation of galling injury:

Each stand was visited once in January 2006 and again the following year in January 2007. The stands were assigned a visual inspection grade with the assistance of a professional forest entomologist familiar with the type of injury associated with the galling wasp. The assessments for both years were conducted with the help of the same person. The grading system was from one to five; one representing a healthy forest stand and five representing a forest stand where trees were severely injured by galling. The grading is based on the percentage of treetops of several trees that bore necrotic leaves. A grading of one had no necrotic leaves, while two had up to 20% desiccation, three had 20-50% desiccation, four had 50-80% desiccation and a grading of five means that the entire tree had necrotic leaves (Figure 1). Canopy evaluation was performed from a 3 m distance from each stand in order for the entire tree structures to be included in the grading evaluation.

In order to associate visual assessments with the digital determination of injury, small branches were taken from each stand at the same time of the visual evaluation in January 2006 and January 2007. Each branch was approximately fifty cm in length with 250-350 leaves. Ten sample branches were removed from the lower canopy of ten randomly selected trees per stand; one branch per tree. The lower canopy was chosen for convenience, but it is also known that the wasps prefer to infest the lower canopy of eucalyptus trees (Mendel et al. 2006). Leaves were removed from each branch per stand and classified into two groups according to the presence or absence of galls. Two parameters were determined from these leaf samples; the average frequency of galled leaves per stand and the average proportion of leaf surface area galled on galled leaves for each stand. The frequency of galled leaves in each stand was determined by dividing the number of galled leaves by the number of total leaves from each sample branch. In order to determine the average proportion of leaf surface galled for each forest stand, ten galled leaves were randomly chosen from the sample branches and scanned using a scanner (HP Scanjet 4370, HP Technologies, Massachusetts, USA).

These ten selected leaves for each stand were scanned at one time and then individually examined for the proportion of the surface galled. Scanning was performed in the RGB color space (red, green, blue) with 24 bits color resolution per pixel (8 bits per color) with a spatial resolution of 300 dpi, resulting in 0.0846 mm per pixel. Each scanned leaf image was evaluated using Image Pro (Image Pro, Maryland, USA), an image analysis software. This software determined the total area of each leaf in pixels, A_{leaf} , and the area of the leaf covered with galls, in pixels, A_{galled}. The percentage area of galling per leaf, P_{galled} was then calculated as the ratio between galled area and area of leaf, $P_{galled} = A_{galled}/A_{leaf}$. The percentage area of galling per leaf was performed on the basis of color differentiation analysis. Using the eyedropper function in Image Pro (Image Pro, Maryland, USA), several galls were manually selected on each leaf image. This manual selection of galls determined an accurate threshold of color range for other galls on the same leaf to be detected. The color analysis incorporated Hue, Saturation and Intensity allowing for a wider range of color to be detected. Once galled areas on the leaf were detected, they were highlighted by the program in red pixels and then the total area of galling was calculated (Figure 2). Ten individual leaves were analyzed per forest stand and an average percentage of galling for each stand was calculated.

In order to associate between the two injury assessment techniques, an Injury Index was derived from the sampled material in each stand. The Injury Index was calculated by multiplying the average frequency of galled leaves in the stand by the average proportion of leaf surface galled, which were determined from ten randomly sampled and scanned galled leaves per stand. The average Injury Index values for stands in specific regions were then used to determine the average Injury Index per region. The regions evaluated were the Southern Golan, Galilee, Hula Valley, Coastal Plain and Western Negev. This methodology was repeated for both sampling periods (January 2006 and January 2007). Changes between 2006 and 2007 in the level of injury as assessed with the Injury Index, frequency of galled leaves and proportion of galled leaf surface was inferred from the non-parametric Wilcoxon Matched-Pairs Signed Rank Test (Siegel, 1956).

IMAGE

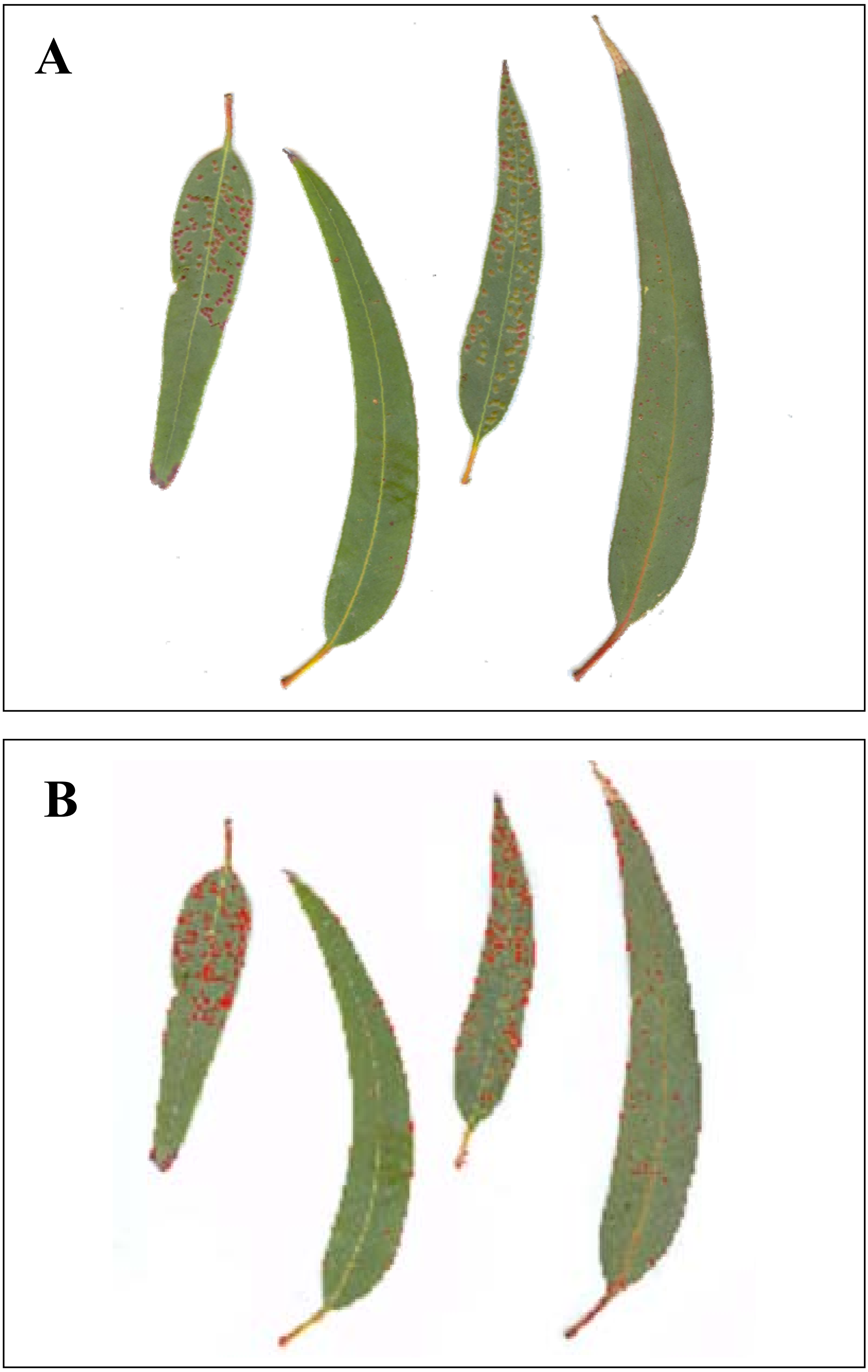

Figure 2: Gall identification on eucalyptus leaves using Image Pro software (Maryland, USA). A: Scanned galled leaves performed in RGB (red, green, blue) and 24 bits/pixel with a spatial resolution of 300 dpi and B: the same leaves whose galls have been emphasized by red pixels, based on Image Pro's (Maryland, USA) color analysis function.

C) Association between field visual inspections (Visual Grade) and digital determination of injury (Injury Index)

The Injury Index was used to characterize the accuracy of the Visual Grading inspection technique. The ability of the visual inspection ranking technique to separate between different levels of injury, as assessed with the Injury Index, was tested using a non parametric one-way ANOVA (Kruskal–Wallis) (Sokal and Rohlf, 1981). Visual inspection values were also contrasted with the two components of the Injury Index: frequency of galled leaves and proportion of leaf surface galled. The separation of visual grading scores by these two components of the Injury Index was inferred using a non-parametric one-way ANOVA tests (Kruskal–Wallis). Medians were separated by the medium notch option.

II. <u>Spectral signature of galled and non-galled leaves as an injury assessment tool</u>

A) General procedures:

1) Sapling material and controlled infestations with O. maskelli:

Non-infested *Eucalyptus camaldulensis* saplings were obtained from the Jewish National Fund's (KKL) Eshtaol nursery in the summer of 2006. All saplings were about 1 m in height. Saplings were maintained in net greenhouses at the Volcani Research Center of the Ministry of Agriculture in Bet Dagan, located in the central Coastal Plain of Israel. Saplings were regularly irrigated and when required, were fertilized. Saplings were separated into two groups: one undergoing wasp infestation and the second kept free of wasps. Saplings undergoing induced infestation were exposed to laboratory reared female gall wasps, *O. maskelli*. Infestation of saplings involved placing an open vial containing wasps (approximately 500-1000 individuals) in the center of a group of saplings. Infested saplings (15 per exposure date) were then maintained in separate net greenhouses to avoid contamination of the control non-infested saplings. In order to investigate the spectra of different development stages of the gall, the infestation procedure was repeated at different times during the summer. Thus, saplings were exposed to *O. maskelli* females in the beginning of July, August and September 2006. Spectral analysis was then conducted in October 2006. This allowed for the investigation of the spectral signature of galls of different stages of development.

2) Spectral instrument and general measuring methods:

A commercial mobile hand held spectrometer (Ocean Optics 001 Inc., Dunedin, Florida, USA), was used to measure and record reflected electromagnetic energy of leaves that remained attached to eucalyptus saplings and entire whole saplings. The spectral range of the spectrometer used is 250-1000 nm and it has a 50 µm wide slit. The spectral resolution is 1.8 nm, with an optical fiber with a field of view of 25 degrees.

The light was dispersed to its spectral components by diffraction grating and sensed by a linear diode array. The source of illumination was a 50 W halogen light. All of the measurements were taken in the same laboratory with the same light conditions. In order to take leaf measurements, the reflection probe (optical fiber) was inserted into the top of a black cone and the leaf was placed at the open base of the cone. There was a 4 cm distance between the light probe and the leaf for every sampling. The diameter of the measured section on the leaf was 1 cm. Leaves remained attached to the saplings throughout all of the spectral measurements. Measurements were taken from the adaxial (upper) leaf surface, in the center of each leaf and the leaves measured were from the middle of the sapling (see standardization of measurements in IIB). For each leaf sample, five reflectance measurements were taken in the same position and later averaged to obtain a mean reflectance spectrum for the leaf (Figure 3A).

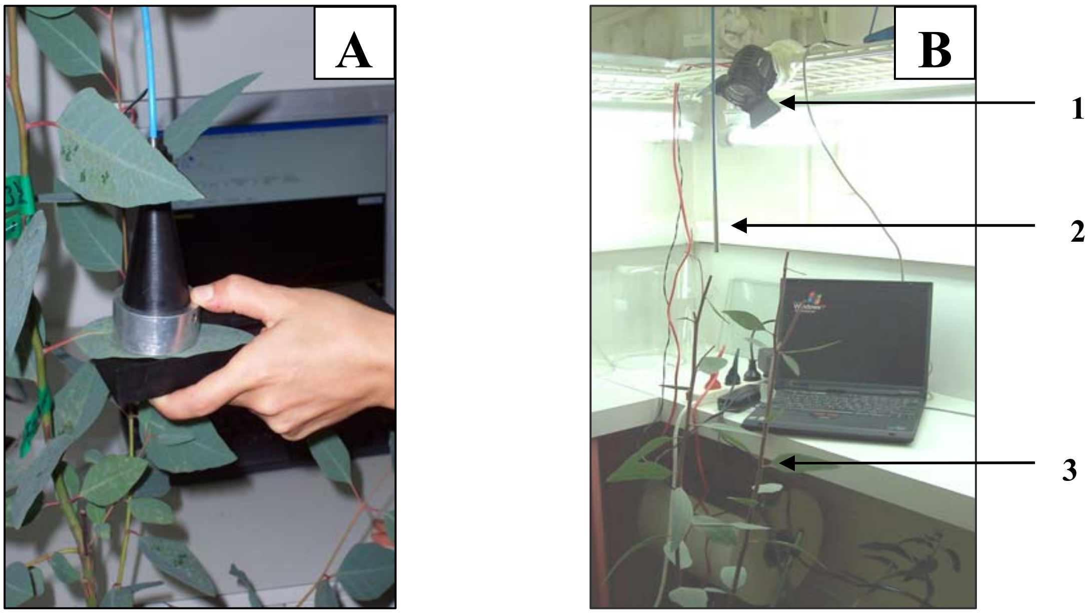

Figure 3: Measuring reflected energy with hand held spectrometer at leaf level and at sapling level. A: Measuring reflected energy of leaf attached to *E. camaldulensis* sapling using a hand held spectrometer and B: Measuring reflected energy from one whole *E. camaldulensis* sapling; 1. One of two, 150 W light bulbs used as a source of light. Bulbs were mounted at 45^{o} angles towards the sapling, 2. Collecting optical fiber of spectrometer hung 5 cm above the sapling, 3. *E. camaldulensis* sapling being measured.

The reflectance curves of whole saplings were also measured using the same equipment and calibration procedure. In order to obtain optimal measurement conditions with the whole sapling, a dark room was conditioned for measurements. The sensor assembly inside the room was designed to provide the necessary geometrical and optical conditions for non-contact reflectance measurements of leaves. A dark mat was placed in the floor of the room to omit unwanted sources of reflected light. Saplings were placed individually on the same spot in the room in order to keep the same measurement geometry and conditions.

The sapling was illuminated by two 150 W light bulbs. Bulbs were mounted at 45° angles towards the sapling (Figure 3B). The collecting optical fiber was placed in a vertical position (aimed downwards), 5 cm above the sapling. Leaves that were not in the path of the illumination beam were expected not to reflect any light. Leaves that intercepted the illumination beam but were closer or further away from the designed sampling distance were outside the field of view of the optical fiber and therefore not seen by the sensor. Reflectance from the potted soil background was also filtered out from the sensor's field of view since it was not illuminated by the light beam. Therefore, the signals acquired by the sensing system were only from leaves at the designed distance from the illumination source and the optical fiber (Alchanatis et al. 2005). Prior to every measuring session (leaf and sapling), white and dark reference signals were taken. A white, ceramic, 3 cm diameter disk was used as a white reference signal. The dark reference signal was sampled by turning the illumination off and covering the sensing fiber. The spectrometer was operated with 25 ms integration time and averaged three spectra per acquisition.

3) Data analysis:

In order to contrast the spectral reflectance obtained from galled leaves and healthy leaves ("non-galled") in the visible and near infrared (NIR) ranges of the electromagnetic spectrum, the rate of change in reflectance throughout all the spectrum were derived from measuring reflectance changes for small section of the spectrum (10 nm sections). This was determined by "First Derivative Analysis", a well known method of spectral analysis (Stone et al. 2001). The method is based on calculating the change rate of average reflectance at each wavelength position throughout the region of the spectrum of interest. Changes in reflectance are derived from the slope obtained by analyzing the observed reflectance on continuous sections of the spectra (usually, sectors of 10 nm). Once the change in reflectance is derived, they were plotted against the whole continuous wavelength, providing the pattern of changes in reflectance throughout the spectrum and as affected by leaf or sapling condition.

In this way, we detected the wavelength at which the maximal value of the first derivative curve in the red edge region (in the NIR range-between 690 and 740 nm) (to be referred to as λ_{re} in the rest of the paper). The average λ_{re} were used for further analysis and comparisons between leaves and sapling conditions.

B) Standardization of measuring techniques at the leaf level:

1) Preliminary measurements:

General sapling infestation condition on individual leaf spectrum:

In order to acquire a general conception of the characteristic reflectance signatures of galled and non-galled leaves, more than 50 leaves of various saplings were randomly selected for measuring. These saplings were infested prior to the start of the study and their galling age was unknown. Four saplings of each condition (galled and non-galled) were used and four leaves from the middle of each sapling were measured for reflectance using a hand held spectrometer. Various positions on the leaf adaxial surface were used for these measurements. 1^{st} Derivative analysis was then applied to the average spectral curves for each condition.

2) Determining position on leaf for measuring:

As mentioned in the previous section (IIA2), the stimulation and reflectance area in the leaf was approximately 1 cm in diameter. In order to standardize the measuring location on the leaf surface and exclude effects of measuring position in the leaf surface, an experiment was run to investigate the reflectance variability between different sections of the same leaf. The study was conducted both for galled and non-galled leaves. For this purpose, six galled saplings with different ages of gall infestation (one, two and three months) were chosen. Within each sapling, four leaves were selected. The leaves were then divided into five sections, proximal, proximal-center, center-distal and distal (Figure 4). Readings were taken from the central point in each of the sections while keeping the leaf attached to the sapling. All measurements were taken from the adaxial (upper) leaf surfaces. Similar measurements were also conducted for non-galled leaves on non-galled saplings. Four non-galled saplings were chosen and two leaves from the middle of each sapling were measured using the hand held spectrometer, following the same protocol as for the galled leaves. The average λ_{re} were calculated for each section and leaf condition (i.e. non-galled and galled) using all the measurements per section in the replicate leaves. Differences between the average λ_{re} of different sections within each of the leaf conditions was inferred using a parametric one-way ANOVA (Sokal and Rohlf, 1981).

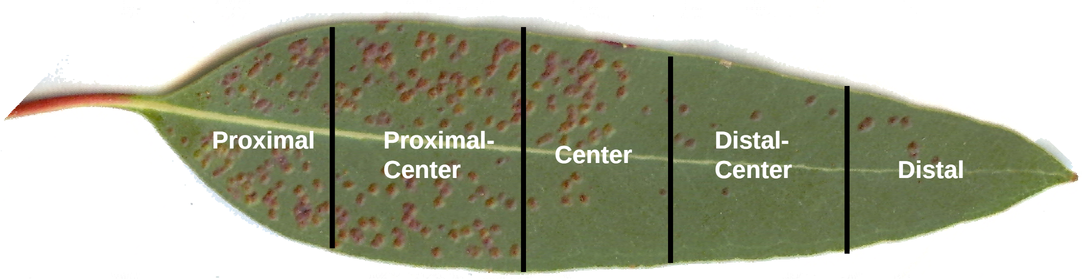

Figure 4: *E. camaldulensis* galled leaf divided into five sections for measuring reflectance with a hand held spectrometer.

3) Establishment of a reference spectrum:

Throughout the study we required non-galled leaves as a reference to the effect of galling upon the reflectance spectrum. Non-galled leaves can be found in completely "healthy saplings" (i.e. sapling with no signs of galling or necrosis), or in saplings with partial infestation of leaves ("mixed saplings"). The effect of neighbor galled leaves on the reflectance spectrum of non-galled leaves was not known at the start of the study. In order to select which type of non-galled leaves to utilize further as reference leaves, we conducted an experiment contrasting the reflectance spectrum of these two types of non-galled leaves (those originating from completely healthy saplings and those originating from mixed saplings). Completely non-galled saplings were represented by six healthy saplings. In each of these saplings, five leaves in the middle of the sapling were selected and the reflectance spectrum measured as specified in the previous section (IIA2). Non-galled leaves on mixed saplings were selected from ten saplings that underwent controlled infestations at different times (one, two and three months post-infestation). Two galled leaves from the middle of each sapling were selected and their reflectance spectrum measured. Average λ_{re} was registered and variance was calculated from all the measurements in the non-galled leaves of healthy saplings, and from all non-galled leaves from mixed saplings having been infested at different stages of development.

C) Spectral signature variability:

1) Spectral signature of leaves as affected by gall color:

It has been observed and reported that when the position of the leaf is unchanged throughout the development of the galls, galls on the adaxial face of the leaf exposed to the sun turn pinkish, whereas galls in the shaded adaxial face of leaf remain green (Mendel et al. 2004). In order to investigate if the gall-color influences the red edge reflectance spectrum of the galled leaves, we measured the spectra of galls showing these two extreme colorations. Four saplings with the same age of gall development (older than two months) were selected. In order to maintain the green color of galls, two saplings were housed in a shaded area in the net greenhouse. To obtain pinkish colored galls, the two other saplings were kept in areas of the net greenhouse with high sunlight intensity. Reflectance measurements were conducted on three leaves from the middle of every sapling. Average λ_{re} were calculated for each situation, and differences in average λ_{re} between the two colors were tested using t-test (Sokal and Rohlf, 1981).

2) Spectral signature of leaves as affected by gall development:

Five stages of gall development on *E. camaldulensis* leaves have been defined in initial studies of the biology of *O. maskelli* (Mendel et al. 2006). Stage one is from oviposition to the first visible signs of gall development that continues for about 30-35 days. Stage two lasts 15-25 days. In this stage the galling tissue is slightly swollen. Towards the end of this stage (2^{nd} larval instar) the gall acquires its typical color (greenish yellow, or light or dark pink). Stage three lasts 16-27 days, at the end of which the gall reaches its maximal dimensions. Stage four lasts 20-26 days, during which the larva reaches it maximum size and pupates while occupying most of the gall chamber volume. Stage five lasts 10-16 days, during which emergence of an adult wasp is complete.

For this experiment, the first three stages of gall development (one, two and three months) were examined. Galling preparation for this experiment was performed as described earlier (IIA1). For each developing stage, ten infested and non-infested saplings were used. From each sapling, three leaves from the middle section of each sapling were selected and used for spectral reflectance measurements. In addition, three leaves from ten non-galled saplings were used as reference. Spectra measurements were conducted as described earlier (IIA). The average λ_{re} were registered in both groups (i.e. galled and reference leaves) for the different ages and differences in the average λ_{re} were determined using t-test (Sokal and Rohlf, 1981).

3) Spectral signature of leaves as affected by a. gall density (without necrosis) and b. leaf necrosis:

Other factors that could affect the reflectance pattern of individual leaves are the proportion of galling on the leaf surface and the degree of necrosis on each leaf. Leaf necrosis in galled leaves is a symptom that develops from the interaction between galling and other undetermined factors (in many cases, galling does not induce clear necrosis in the leaves, at least for a certain period of time). In order to determine the effect of the galled leaf proportion and the degree of necrosis on each leaf upon the reflectance spectrum of the leaf, two separate measuring sessions were performed.

The first group of leaves tested exhibited a range of galling densities and showed no sign of necrosis while the second group of leaves had a range of galling densities with different sized necrotic areas. Five saplings that had been infested for three months and five saplings with three to six month infestations were selected for both groups of measuring. Three leaves from the middle of each sapling were selected based its degree of galling and necrotic attributes. In order to estimate the proportion of leaf surface occupied by galling and necrotic areas, leaves were removed from the saplings after spectral measurements and scanned (HP Scanjet 4370, HP Technologies, Massachusetts, USA). The proportion of the leaf surface that was galled or necrotic in the scanned image was then determined using Image Pro (Image Pro, Maryland, USA) utilizing a similar system as described in an early section (IB). Spectra measurements were conducted as described earlier (IIA2) and the average λ_{re} were used for analysis.

The relationship between the proportion of leaf galled surface (in leaves with no signs of necrosis) and the average λ_{re} was analyzed using Pearson's correlation statistics (Sokal and Rholf, 1981). Multiple regression analysis of two independent variables (degree of gall density and area of necrotic surface) was used to inquire on the combined effects of the proportions of necrotic leaf area and galled leaf area on the spectral signatures (Sokal and Rohlf, 1981). Since the degree of necrosis combined with galling did not show a linear relationship with the average λ_{re}, the average λ_{re} observed in galled leaves with necrotic patches were contrasted with reference leaves using a t-test (Sokal and Rohlf, 1981).

4) A step further: spectral signature of whole saplings; galled and non-galled:

The reflectance of whole saplings (galled and non-galled) were investigated as a step further in the spectral characterization of *O. maskeli* infestations. For this, a total of 20 saplings were selected; ten non-galled saplings and ten galled saplings (IIA1). Galled saplings ranged from four to six month old infestation and nearly every leaf on the saplings was galled. The reflectance spectrum of the whole sapling was measured five times and averaged. The average λ_{re} observed in non-galled and galled saplings were calculated and differences between them inferred from a t-test (Sokal and Rohlf, 1981)

III. Assessment of adult wasp emergence trends

A) Characterization of seasonal wasp emergence using sticky traps:

In each forest stand, two green, double sided sticky plate traps, 15 x 15 cm, covered with non-drying glue, were suspended from January 2006 until January 2007, on two representative galled trees (Table 1). Traps were serviced every two weeks throughout this period. Total counts of *O. maskelli* per sampling date and trap were recorded for each stand and averaged per region.

B) Association of wasp emergence with temperature: exploring accumulation of Degree-Days (DD) as a forecasting tool:

Emergence trends of *O. maskelli* in specific stand and region, as derived from the trapping data, were associated with accumulated degree-days (DD). Accumulated DD for each stand was derived from the daily maximum and minimum temperatures using the sine function (Baskerville and Emin, 1969). Daily climatic information was received from the Israel Meteorological Service from meteorological stations that were situated close to the selected forest stands (Table 1). Accumulated (DD) were calculated from the arbitrary date of January 1st 2006. DD were accumulated using 10^{o}C as the lowest threshold (based on personal communication with Dr. Protasov). January 1st was selected since it is expected that due to low temperatures during that time of year accumulation of DD would be small or negligible, providing a higher certainty that effective DD were not omitted from the model (Baskerville and Emin, 1969).

RESULTS

1. Visual Grade and Injury Index assessments:

A) Correlation between field visual grading inspections (V.G.) and digital determined Injury Index (I.I.)

Figure 5 shows the relationship between the Visual Grade (V.G.) assessment and the Injury Index (I.I). This figure describes this relationship for the combined data set for all the assessed stands from the two sampling periods, January 2006 and January 2007. The figure was constructed by associating the given Visual Grade with the resulting average Injury Index. A non-parametric one way ANOVA test (Kruskal-Wallis) (Sokal and Rohlf, 1981) was applied to the combined data set for the two years. The results suggest that the four visual grade categories significantly differed between them (H = 12.5, $P < 0.01$). V.G. of 3 and 4 were significantly separated when assessed by I.I.: V.G. of 4 corresponded to an I.I. with an average of 0.4, while a V.G. of 3 corresponded with an average I.I. of 0.2. However, the V.G. of 1 and 2 were not separated by the I.I., which show a similar index for these two V.G. grades (I.I. approximately of 0.1). The I.I. was derived from the frequency of leaves in the sample branches with galls and from the average percent leaf surface covered with galls. Figures 6 and 7 show the association between these parameters taken separately from the I.I. and the V.G assessments. Similar results were obtained when investigating the leaf-surface proportion covered with galls (P.G.) and the V.G. (Figure 6). The four V.G. categories significantly differed between them (H = 9.15, $P < 0.05$). Similar to the I.I. and F.G., V.G. of 3 and 4 were significantly separated when assessed by P.G. In contrast, V.G. of 1 and 2 were not significantly separated by P.G. A V.G. of 4 corresponded to the P.G. with an average of 0.4, while a V.G. of 3 corresponds with an average P.G. of 0.2. V.G. of 2 and 3 corresponded with P.G. of approximately of 0.35. The frequency of galling (F.G.) significantly separated the V.G. scores (H = 12.1, $P < 0.01$). V.G. of 3 and 4 were significantly separated while, similarly to the I.I., the V.G. of 1 and 2 where not well separated by the F.G.. V.G. of 4 corresponded to a F.G. with an average of 0.95, while a V.G. of 3 corresponds with a F.G of 0.8. V.G. of 1 and 2 corresponded with F.G.’s around 0.35 (Figure 7).

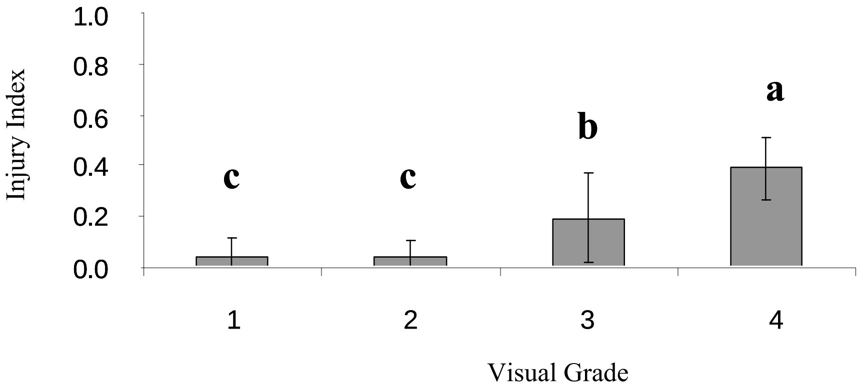

Figure 5: The association between Visual Grade and Injury Index (Injury Index is derived from frequency of galled leaves X average gall density of leaves per forest stand) Medians with different letters are significantly different (P < 0.01).

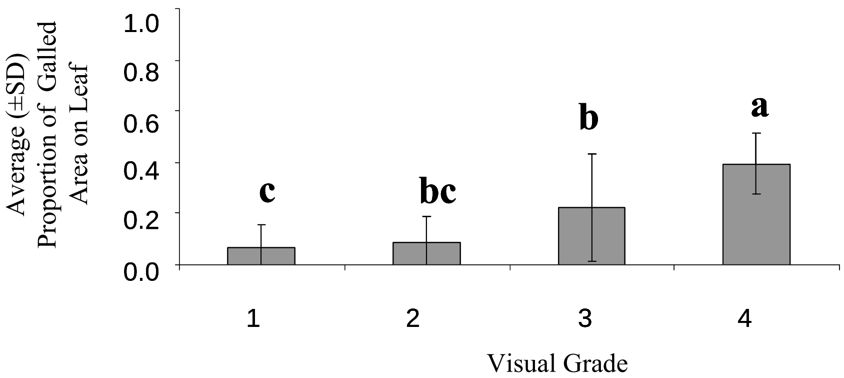

Figure 6: The association between Visual Grade and the average proportion of galled area on leaf surfaces. Leaves were sampled from the lower canopies of eucalyptus trees. Medians with different letters are significantly different (P < 0.05)

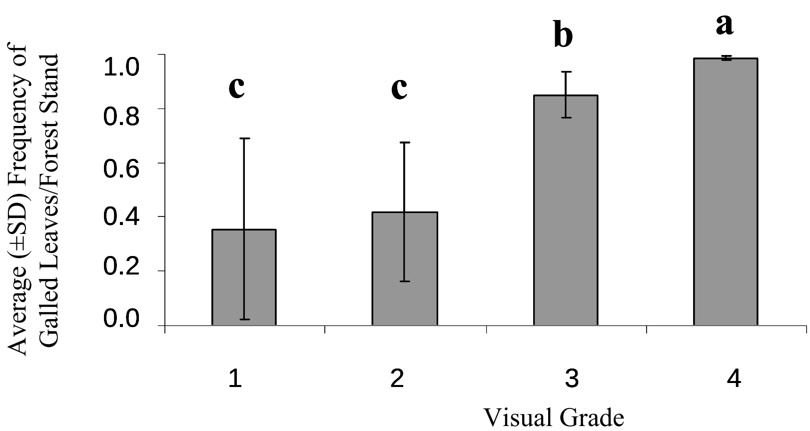

Figure 7: The association between Visual Grade and the average frequency of galled leaves in sample branches from trees in the lower canopies of eucalyptus trees. Medians with different letters are significantly different (P < 0.01).

Table 3: Forest stand and regional injury assessments of January 2006 and January 2007; Visual Grade and Injury Index assessments.

Region	Forest Stand Name	Visual Grade (V.G)		Frequency of Galled Leaves (F.G)		Proportion of Galled Leaf Surface (P.G.)		Injury Index (I.I)	
		2006	2007	2006	2007	2006	2007	2006	2007
North Golan	1. Alonei Habashan	1	1	0.07	0.54	0.09	0.04	0.006	0.022
South Golan	2. Keshet	1	1	0.05	0.21	0.04	0.02	0.002	0.004
	3. Nov	1	1	n/a*	0.11	n/a*	0.01	n/a*	0.001
	Average	***1***	***1***		***0.002***	***0.002***			
Hula Valley	4. Hula Valley	1	1	0.87	0.8	0.27	0.04	0.20	0.032
Galilee	5. Alon Hagalil	3	2	0.9	0.25	0.21	0.01	0.189	0.003
	6. El Roi Junction	3	3	0.99	0.74	0.27	0.08	0.267	0.059
	7. Golani Junction	2	2	0.57	0.62	0.32	0.09	0.182	0.056
	Average	***2.7***	***2.3***		***0.21***	***0.03***			
Coastal Plain	8. Hadera	4	3	0.98	0.83	0.31	0.05	0.304	0.042
	9. Ilanot	4	3	0.99	0.8	0.48	0.12	0.475	0.096
	Average	***4***	***3***					***0.38***	***0.069***
Western Negev	12. Eitan	2	1	0.15	0.18	0.03	0.02	0.005	0.004
	13. Gvaram	3	2	0.84	0.84	0.61	0.04	0.512	0.034
	14. Nahal Phora	2	n/a*	0.09	n/a*	0.05	n/a*	0.005	n/a*
	15. Nahal Snaiim	2	2	0.34	0.49	0.13	0.03	0.044	0.015
	Average	***2.5***	***1.6***					***0.14***	***0.017***

I.I. was derived from the average frequency of galled leaves and the average proportion of galled leaf per forest stand. *n/a these sites were inaccessible during the period of sampling. The stand injury assessment data is also geographically displayed in four maps; Appendix, Map II: Visual Injury Assessment of stands and regions for 2006, Map III: Injury Index of stands and regions for 2006, Map IV: Visual Injury Assessment of stands and regions for 2007, and Map V: Injury Index of stand and regions for 2007 (these maps were produced using ArcView GIS software (ArcView GIS, California, USA)).

B) Stand and region injury assessment using V.G. and I.I.:

In order to characterize the infestation levels of different stands and the different regions throughout the country, the Visual Grading (V.G.) and Injury Index (I.I.) indexes were used. The definition of I.I. as related to V.G. was derived from their relationship as described in the previous section (IA). The I.I. range obtained was compared to each V.G. Since a separation was needed between V.G. of 1 and 2, we decided that the V.G. of 1, which is characterized as "healthy", will be matched with an I.I. of "0". Thus, the ranges used to characterize the level of injury in the study stands are summarized in Table 2 and are based on the results of the previous section (including the variability). The observed Visual Grading (V.G.) and the derived Injury Index (I.I.) for years 2006 and 2007 for all the inspected stands and averages per region are presented in Table 3. Table 3 also presents the frequency of galling (F.G.) and the proportion of leaf area galled (P.G.) for all the investigated stands and for the two assessed years.

Table 2: The ranges used to characterize the categories of the level of injury for the Visual Grade and Injury Index.

Level of Injury	Visual Grade (V.G.)	Injury Index (I.I.)
Healthy	1	0
Low	2	0.001 - 0.025
Intermediate	3	0.026 - 0.209
Heavy	4	0.210 - 0.380
Heaviest	5	> 0.389

In general, observed V.G scores matched with calculated I.I. scores for forest stands and years (Table 3). However, some discrepancies were found; in 2006, the Hula Valley gave a V.G. of 1, representing a healthy forest stand, while the I.I.= 0.2, suggesting an intermediate injury. Similarly, differences between the two assessment systems were detected in 2007. In the Coastal Plain where the I.I. scored very low (0.069), while the V.G.= 3 suggesting intermediate infestations (Table 3). V.G. and I.I. scores for 2006 were significantly higher than 2007 (for example, I.I. between years $T = 5$, n = 11, $P < 0.02$). In general, the most infested region throughout the two years was the Coastal Plain, which also showed a dramatic drop in both V.G. and I.I. from 2006 to 2007 (Table 3). The least injured region throughout the two years was the Southern Golan (Table 3). It is interesting to note that F.G. for all the stands between 2006 and 2007 was statistically similar ($T = 27$, n = 11, $P > 0.05$) (Table 3). In contrast, differences between the two years in P.G. were significantly different ($T = 0$, n = 11, $P < 0.01$), suggesting that the drastic drop in I.I. between years was mainly affected by the proportion of leaf area covered with galls and not by the frequency of leaves galled.

II. Spectral signatures of galled and non-galled leaves and saplings

A) Standardization of measuring technique at the leaf level:

1) Spectral signature of leaves as affected by general infestation condition:

Initial preliminary measurements were taken from galled and non-galled leaves of saplings in order to understand the spectral signature system before embarking on further, more extensive experiments. This data was not used further and is presented here to describe the mean spectral reflectance of several random galled and non-galled leaves and their average λ_{re} was observed throughout the spectra. The characteristic reflectance spectra for both situations can be seen in Figure 8. The spectra are characterized by a peak that is found at the start of near infrared (NIR) (700-900 nm) region. A reflectance plateau is then seen at 750nm ("NIR Plateau") (Figure 8A). Galled leaves were characterized by a shift in reflectance towards the red region of the spectra (Figure 8B). Using the first derivative of the reflectance, it can be seen that the average λ_{re} was observed at 715 nm, while in the reference "healthy leaves" the average λ_{re} 725 nm (Figure 8B).

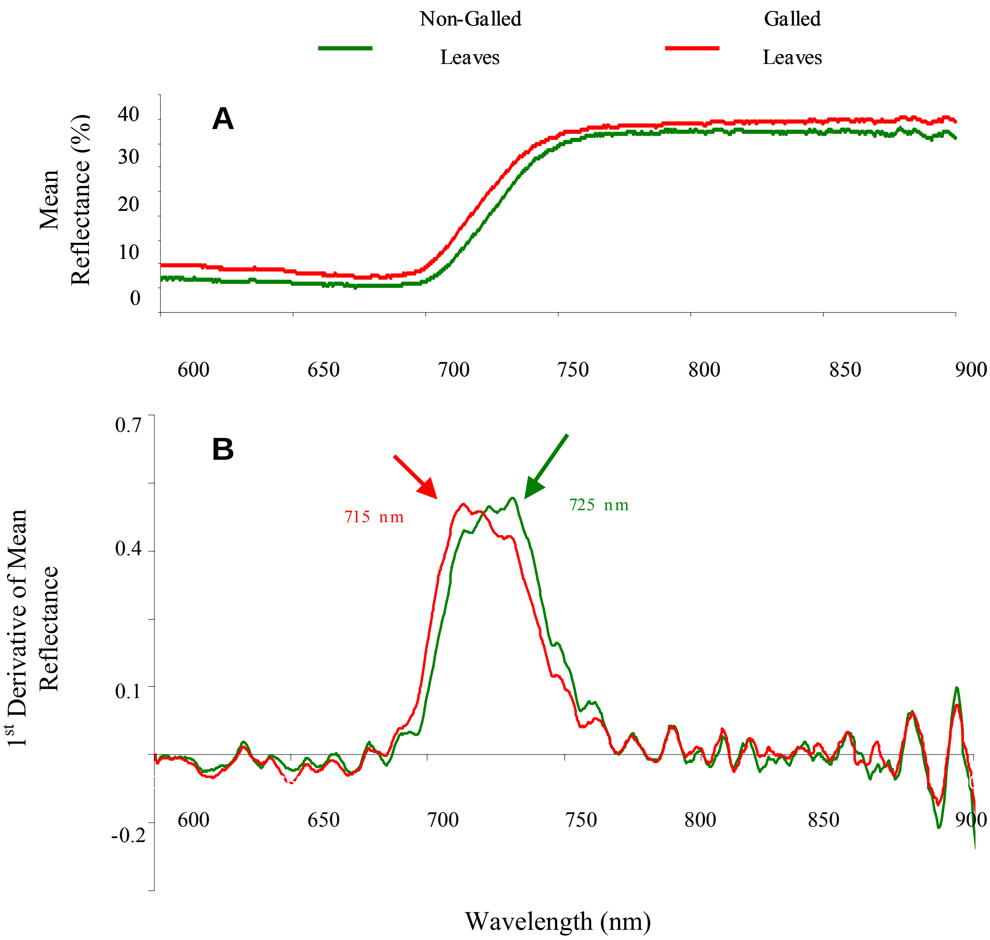

Figure 8: The effect of galling and non-galling on the spectral signatures of random leaves. A: The mean reflectance of galled and non-galled leaves and B: 1st Derivative of mean reflectance of leaves, galled and non-galled. 715 nm is the average λ_{re} of the galled leaves and 725 nm is the average λ_{re} of the non-galled leaves.

2) Leaf section:

The reflectance of different leaf sections in both healthy and galled leaves was investigated in order to standardize the spectrometer measuring methods for this study. Different sections of leaves were measured and Table 4 provides the average λ_{re} in each leaf section. No significant differences in wavelengths were detected between leaf-sections in non-galled leaves (F = 0.39; df = 4,35; P > 0.05) and in galled leaves (F = 0.22; df = 4,115, P > 0.05). As a result, the central section of the leaf was used for spectral measurement throughout the study.

Table 4: The average λ_{re} (wavelength where the maximal value of the 1st Derivative curve is in the red edge region) as related to the location on leaf for galled and non-galled leaves. Mixed saplings bore galled and non-galled leaves.

Position (part of leaf)	**Average λ_{re} (± SD)**	
	Healthy Leaves on Healthy Saplings	**Galled Leaves on Mixed Saplings**
Proximal	726.8 ± 4.5	720.8 ± 6.8
Proximal Center	725.3 ± 7.8	721.5 ± 6.4
Center	726.4 ± 6.6	722.2 ± 8.2
Distal Center	726.9 ± 5.2	720.1 ±10.9
Distal	728.8 ± 3.2	721.3 ± 8.3
	F = 0.39 P > 0.05	F = 0.22 P > 0.05

3) Establishment of reference spectrum:

The present experiment intended to establish which category of healthy leaves (from completely healthy or from mixed saplings) to use as reference. In non-galled saplings, the average λ_{re} was 726.8 nm ± 5.5 compared to 721.7 nm ± 5.1 found on mixed saplings bearing healthy leaves and galled leaves. The wavelengths of non-galled (i.e. healthy) leaves in completely healthy saplings or in mixed saplings were similar. A slight shift to the red region of the spectrum was found in healthy leaves originating from mixed saplings. Since the shift was relatively low (~5 nm), it was decided to pool all the "healthy" leaves and use their average as a reference to investigate the spectra of galling and related factors (age, etc.).

B) Spectral signature variability:

1) Spectral signature of leaves as affected by gall color:

This experiment tested the spectral signatures of different colored galled leaves. For the green galled leaves measured, the average λ_{re} was 712.6 nm ± 4 compared to pink galled leaves 716 nm ± 9. There were no significant differences between the average λ_{re} for pink and green colored galled leaves ($t = 1.04$, $P > 0.05$).

2) Spectral signature of leaves as affected by gall development:

The effect of gall age and stage of development on the reflectance spectrum was investigated in this experiment. Table 5 shows the average λ_{re} in galled leaves of different stages of development. The average λ_{re} was observed in each of these age categories was compared with the spectra of non-galled leaves. The average λ_{re} observed in galled leaves one, two and three months old did not significantly differ from their reference leaves (Table 5). The only situation where the spectra of galled leaves significantly differentiated from reference leaves was in galling older than three months (up to six months). In this case, the average λ_{re} of galled leaves was observed at 708 nm, in contrast to 720 nm of reference leaves (Table 5).

Table 5: The effect of gall development on the average λ_{re} (wavelength where the maximal value of the 1st Derivative curve is in the red edge region).

Age of Galling (month)	**Average λ_{re} (± SD)**		**Statistics**		
	Reference Leaves	**Galled Leaves**	***t***	**df**	***P***
1	724.7 ± 2.25	723.76 ± 2.41	1.33	43	> 0.05
2	720.16 ± 4.35	719.82 ± 4.17	0.30	57	> 0.05
3	722.67 ± 5.65	723.96 ±17.86	0.42	45	> 0.05
3 - 6	720.01 ± 7.34	708.30 ± 2.21	5.8	13	< 0.01

3a) Spectral signature of leaves as affected by gall density:

The effect of galling density of leaves (in leaves not showing any sign of necrosis) upon the reflectance spectrum was investigated in this experiment. Figure 9 is a scatterplot showing the relationship between the average λ_{re} observed and the level of galling densities on leaf surfaces. The figure shows that the larger the surface of leaf covered with galled tissue, the lower the average λ_{re} The association between these two parameters was significant, as inferred from a Pearson correlation analysis (r = - 0.66, $P < 0.05$).

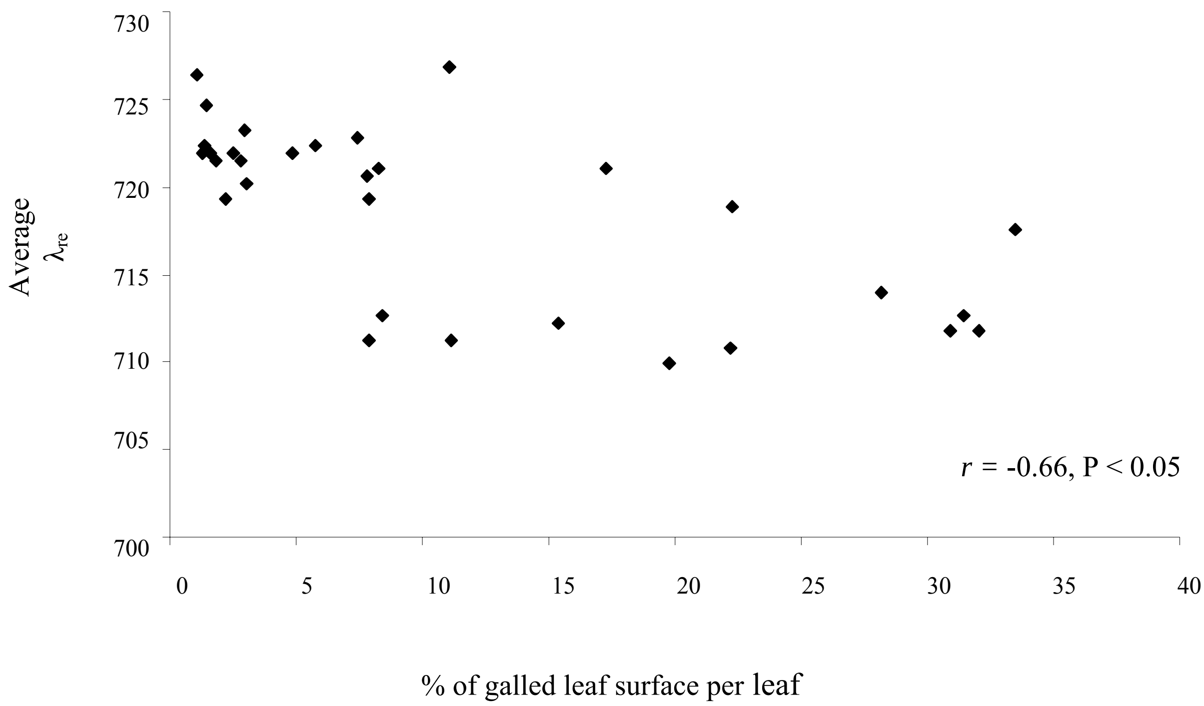

Figure 9: The effect of galling density (without necrosis) on the average λ_{re} (wavelengths where the maximal value of the 1st Derivative curve is in the red edge region).

3b) Spectral signature of leaves as affected by gall density and leaf necrosis:

Since necrosis may have an important impact upon the reflectance spectrum of leaves, we investigated how the degree of leaf necrosis combined with the degree of galling affects the average λ_{re}. Figure 10 shows the average λ_{re} of different degrees of necrosis and various densities of galling per leaf. It can be seen that there isn't a wavelength response (e.g. a flat surface response) in relation to an increase in both necrotic and galled areas. The average λ_{re} of the majority of measurements of galled leaves with necrosis, were between 705–715 nm.

The multiple regression analysis performed to the data suggests that the combined degrees of necrosis and galling did not change the average λ_{re}; $F_{2,\ 24} = 0.14$, $P = 0.86$. However, the average λ_{re} in necrotic-galled leaves (pooled data) significantly differed from the wavelengths of reference non-galled, non-necrotic leaves ($t = 15.45$, df = 65, $P < 0.01$): The average λ_{re} for necrotic leaves was 709.31 nm while that of the reference was 726.84 nm.

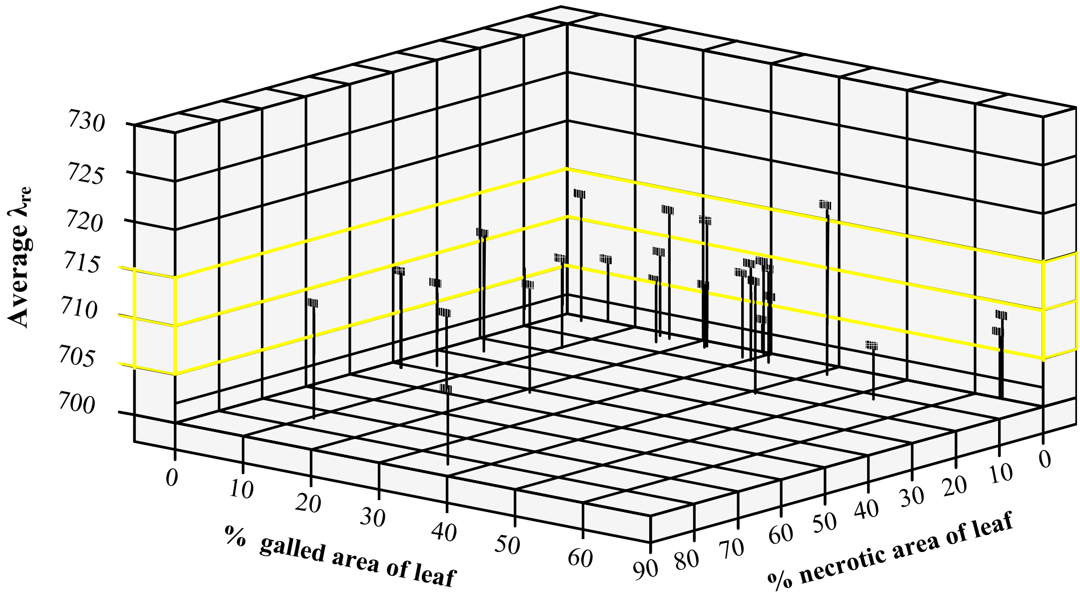

Figure 10: The effect of leaf necrosis and degree of galling on average λ_{re} (wavelength where the maximal value of the 1st Derivative curve is in the red edge region). The average λ_{re} of the majority of measurements of galled leaves with necrosis, was between 705–715 nm.

A step further: spectral signatures of whole galled and non-galled saplings:

Figure 11 shows the results of the average λ_{re} (several saplings and measurements) that were recorded in galled and non-galled saplings. A significant difference in the average λ_{re} between galled and non-galled saplings was found (t = 3.7, df =18, $P < 0.01$) (Figure 11A). A displacement of ~ 11 nm of the red edge peak maxima towards shorter wavelengths was recorded for galled saplings (Figure 11B).

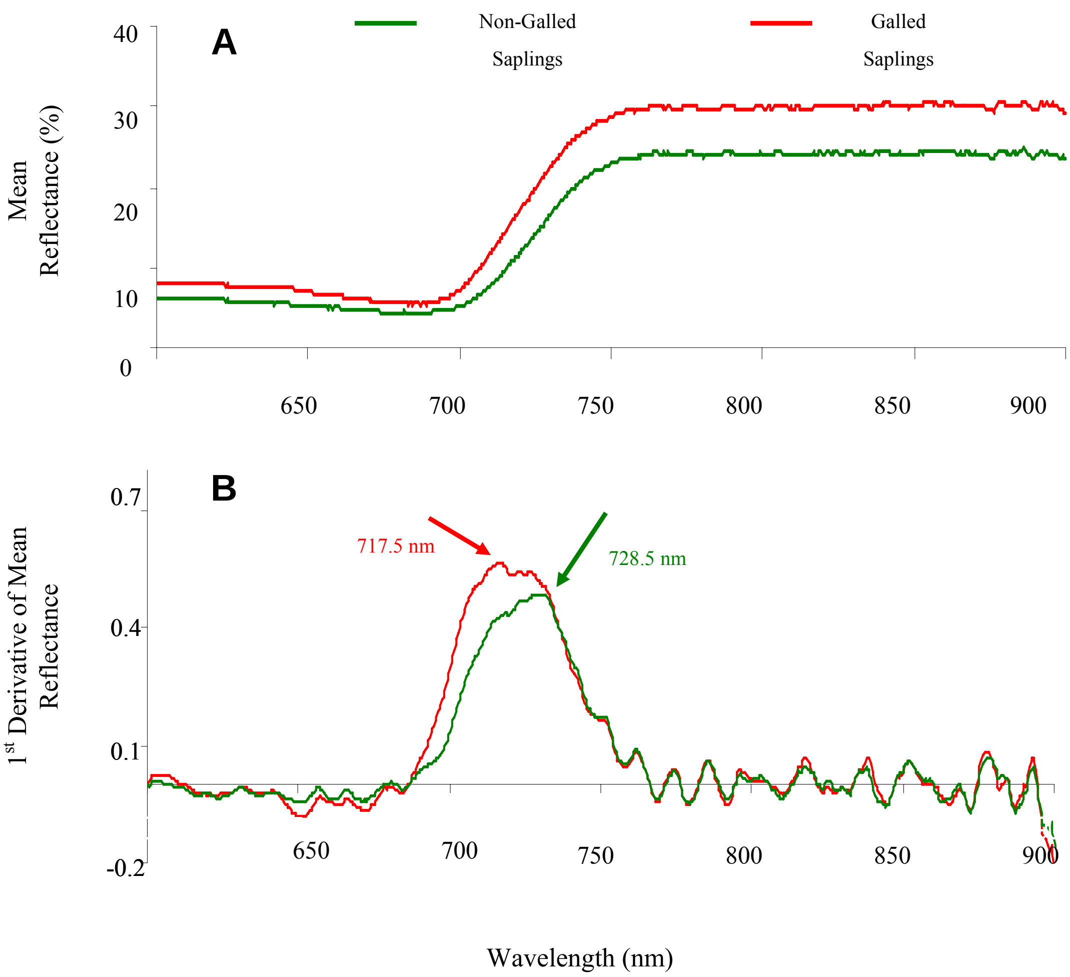

Figure 11: The effect of galling and non-galling on the spectral signatures of whole saplings. A: The mean reflectance of galled and non-galled whole saplings and B: 1st Derivative of mean reflectance of whole saplings, galled and non-galled. 717.5 nm is the average λ_{re} of the galled saplings and 728.5 nm is the average λ_{re} of the non-galled saplings.

III. Assessment of adult wasp emergence trends

A) Determination of seasonal wasp emergence:

The data collected from the green sticky traps from January 2006 to December 2006 revealed that adult wasps emerged from their over wintering galls in the spring between March 1st and April 15th (Figure 12). The time of adult emergence in the different regions seems to be driven by degree-days. Early adult emergence (between March 1st and March 15th) was detected in the Western Negev and Coastal Plain (Figure 12). Accumulation of degree-days (DD) in these areas was faster than in other regions (inset in Figure 12). Adult wasps in the Jerusalem area, the Galilee and the Golan emerged a month later (Figure 12). In these areas, the accumulation of DD was slower than in the other regions, especially in the Jerusalem area where wasps emerged the latest (inset of Figure 12). The first peak of emergence in all areas was detected around April - May (Figure 12). A second peak was detected in mid-summer, June - July. Accumulated DD from January 1st until the emergence in all regions ranged from 310 to 509 (inset of Figure 12). The average number of accumulated DD (from January 1st) required by the wasp to emerge from the pupae was calculated at 388.82 ± 85.21.

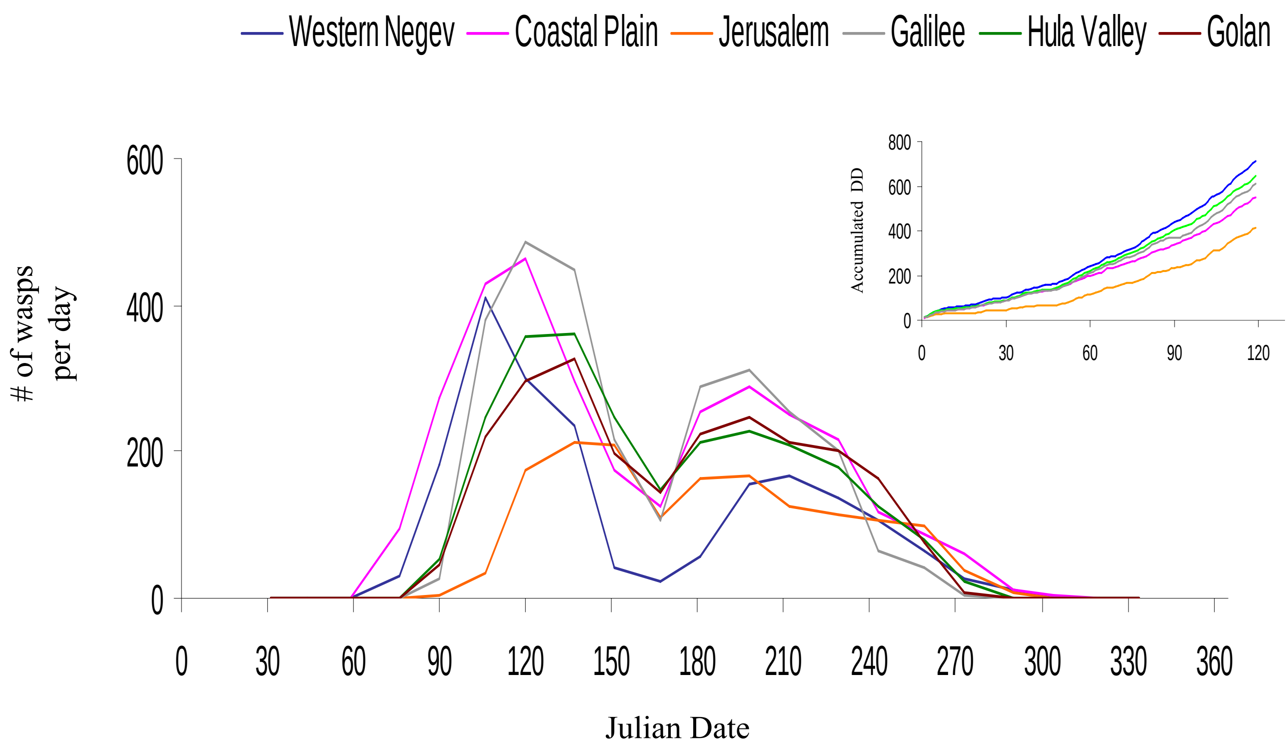

Figure 12: Seasonal wasp emergence trends by region. Inset: Accumulated degree-days for adult wasp emergence by region.

DISCUSSION

I. Forest injury assessments

Assessing forest health is a growing concern for government, industry and communities across the globe. Economically and sociologically, forests provide a multiplicity of uses and benefits to people (Waters and Stark, 1980). Damaging forest processes include fire, weeds, vertebrate and insect pests, pathogens and abiotic factors such as drought, salinization, soil acidification, air pollution and land clearing (National Forest Inventory, 2003). Phytophagous insects are integral components of forest ecosystems and normally are present at a relatively low density, causing little damage, and having negligible impact on tree growth and vigor. However, sporadically, in time or space, some species may grow rapidly to damaging numbers, developing outbreaks that may persist for a variable length of time before subsiding. Such large populations may have adverse effects on many aspects of forests such as tree growth and survival, yield and quality of wood and non-wood products, wildlife habitat, recreation, aesthetics, and cultural value (Waters and Stark, 1980).

In addition to the sporadic outbreaks of native herbivores, a recent growing area of concern in forest pest management is the rise of invasive forest insects, also known as alien species. Invasive species occur outside their normal distribution range and establish in natural or semi-natural ecosystems or habitats. They act as agents of change and threaten native biological diversity. Invasive alien species can reduce the uniqueness of regional fauna and flora, and breaks down geographical barriers that maintain global biodiversity (Xu et al. 2006). The risks associated with the invasion of alien species are increasing with rapid international exchange and the massive movement of people. Invasive alien species expedite the loss of species and genetic biodiversity, destroy the structure and functions of ecosystems and cause huge economic losses. For instance, invasive forest species have caused annual losses worth 4 billion USD to the United States and 720 million dollars in Canada (Otten, 2005). Although quantitative estimates of the economic impacts are not readily available for other countries, invasive species, no doubt, significantly impact forest productivity, leading to economic losses especially in regions heavily dependent on forest products. Although not yet evaluated and fully understood, the accidental introduction of the invasive gall-forming wasp, *O. maskelli* into countries in the Middle East, North Africa and the Mediterranean Basin, is expected to create detrimental effects on eucalyptus forests and plantations.

There are two generic approaches to the assessment of insect related damage associated with trees in forests; ground based assessments and airborne assessments (i.e. aerial sketch-mapping and remote sensing). Both assessments are considered important and interrelated (Stone and Coops, 2004). Evaluating forest pest injury should be based on indicators that are quantitative or qualitative variables which can be measured or described and which demonstrate trends when observed periodically (Montreal Process, 1995). The central objective of this study was to evaluate and develop practical injury assessment tools for injury sustained to eucalyptus trees in Israel by the invasive galling pest, *O. maskelli*.

The existing subjective forest injury assessment based on visual grading (V.G.) was compared to an objective protocol that was based on an Injury Index (I.I.) derived from sampled eucalyptus leaves throughout the country. The viable application of remote sensing as an additional assessment tool of forest galling has also been examined in this study. We based our investigations on previous studies that demonstrated significant progress in the application of digital, remotely sensed imagery to detect and classify damaged forest canopies (Lillesand and Keifer, 2001, Stone et al. 2003, Wulder et al. 2005). The success of this approach depends, in part, on sound understandings of the progression of symptoms at the leaf, tree crown and stand scale, especially those symptoms that influence reflectance behavior (Stone and Coops, 2004). Therefore, in this study we created the preliminary foundation for implementing remote sensing as an injury assessment tool by exploring, measuring and analyzing spectral reflectance curves at the leaf and sapling levels.

It is known that forest assessment protocols should be objective, repeatable and cost effective thus enabling authorities to better manage their infested forests (Stone and Haywood, 2006). However, visual assessments, involving incidence and severity estimations, are adopted by assessors of forests in most countries because they are currently the most time and cost effective methods available to survey large areas (Smith et al. 2005). Most efforts to evaluate forest pest problems are made using an ordinal scale from zero (no incidence) to four (complete defoliation/desiccation) (Smith et al. 2005). Although these programs provide information from vast areas, the accuracy of injury levels is not often available (Smith et al. 2005). The visual assessment used in this study to evaluate injury levels of *O. maskelli* in eucalyptus forest stands in Israel was based on an ordinal scale of one to five. This visual assessment was chosen as an evaluation tool because of its cost and time effectiveness. The comparison between the I.I., the developed objective index based on quantitative sampled data, and the subjective V.G., based on the valuation of a subjective observer, revealed overall correspondence between the two injury assessment methods. Significant differences between the two assessment methods, however, were found when eucalyptus injury levels were low.

This inability to accurately determine damage at low injury levels by the V.G. system highlights some of the limitations of the subjective assessment. Certain circumstances may change the cost/effectiveness of subjective injury assessments. An example of this is when early detections and management may significantly change the economic outcome of a late detection. Another drawback of the V.G. system is implicit in the subjectivity of the method and the evaluating subject. In a similar study conducted in Australia, the accuracy and precision of assessing damage at a tree level were determined to ensure the reliability, objectivity and repeatability of the Crown Damage Index (CDI) method used by forest officials (Stone et al. 2003a,b). This last study compared visual assessments by varying the qualifications and training levels of "assessors" (e.g. human subjects used to assess damage) to a CDI standardized index for assessing crown damage of young eucalypts.

The index was developed in order to statistically summarize results for reporting obligations at regional, state and national levels. It took into account the incidence and severity of missing, necrotic or discolored foliage at a whole tree level (Stone et al. 2003a,b).

The major findings of this study were that visual perception varies between individuals and it often influences how forest officials score damaged forests and affects their capacity to be trained. It was shown that experienced forest entomologists scored more accurate results and provided the most repeatable estimates compared to less experienced assessors (Smith et al. 2005). Although our study did not include the evaluation of injury by non-expert foresters, we may expect similar results: in the present study the person ranking the eucalyptus forest health situation as affected by *O. maskelli* in both assessment years was a trained forest entomologist with a number of years experience. An additional finding in the Australian study was that assessors were able to predict the Crown Damage Index (a combined index of all damage classes) to within 12% of measured damage levels (Smith et al. 2005), indicating that visual inspections can be an important way of determining injury levels.

It is important to mention that other factors may have influenced the differences found between the visual observations and the quantitative sampling in this study. All of the sampling (for the I.I.) of eucalyptus branches was taken from trees found within each forest stand while the visual inspections were performed from outside the forest, 3 m distance from the forest stand. These differences may have effected the interpretation of the galling situation in each forest.

Both assessments, the V.G. and I.I., illustrated improvements in overall forest health between the sampling period of 2006 and 2007. These drastic changes in the galling situation across Israel's various geo-climatic regions can be partially explained by the release of a natural enemy for biological control of *O. maskelli* in the winter of 2006. A total of about 12,000 adult *Closterocerus chamaeleon* (Girault), (Hymenoptera: Eulophidae) were liberated in six sites in three regions; the Coastal Plain, the Bet Shean Valley and the Golan (Protasov et al. 2007). Preliminary findings suggest that this parasitoid seems to be an efficient agent and has already lowered the population density of its host galler, *O. maskelli* in some locations after less than a year since its release (Protasov et al. 2007). The fact that the two assessment systems showed the decline in damage between 2006 and 2007 suggest that within certain limits, both the V.G. and I.I. systems are good indicators of forest health. Another interesting outcome of the release of the parasitoids that was noticed during the course of this study was that there were changes in gall density that were found on leaf samples between the sampling years 2006 to 2007. The percentage of galled leaf surface was generally lower in 2007, while the frequency rate of galling remained relatively the same between the years.

The increasing demands of environmental stewardship and profitability placed on managers and authorities of commercial and non-commercial forests are changing the levels of precision and resolution of information required on the status and conditions of their forests. Technologically advanced tools, such as remote sensing, monitor forest health trends with higher accuracy, efficiency and objectivity than field surveys (Stone, 1998). Airborne instruments are optical sensors that measure the amount of light reflected from vegetation within specific regions of the electromagnetic spectrum.

Certain regions of the spectra are tested because they contain wavelengths sensitive to changes in the biochemical constituents, principally leaf pigments (the chlorophylls, carotenoids and anthocyanins), the internal cellular structure and water content of leaves and leaf surface properties (Stone and Coops, 2004) (Appendix, Figure I).

As far as we are concerned, this seems to be the first study that investigates the spectral signatures of eucalyptus leaves galled by the Eulophid wasp, *O. maskelli*. Comprehensive assessments of the spectral signatures of various situations and morphological conditions of the galled and non-galled leaves in this study have been an attempt to understand the complex relationship between galling and its impact on leaf physiology. At the leaf level, deriving a basic understanding of physiological characteristics on spectral properties has been of great interest and is the foundation for the development of remote sensing sampling strategies and data interpretation methods. It is known that leaf optical properties are governed by surface and internal structure properties as well as by the concentration and distribution of biochemical components (Stone et al. 2001). Thus, remote analysis can be used to assess both the structure and the physiological status of trees assisting in efficient and accurate forest pest management.

In this study the reflectance spectra of leaf and sapling samples (both galled and non-galled) showed spectral features similar to that of most green vegetation (Appendix, Figure II). These spectral signatures reveal well known physiological aspects of green vegetation and have been explained in more detail by Datt (1999) in his study of visible and near infrared reflectance and chlorophyll content in eucalyptus leaves. The blue spectral region from 400 to 500 nm is typically characterized by a low reflectance due to the strong absorption by chlorophyll *b* and the carotenoids. In the green wavelength region there is a broad reflectance peak near 550 nm. A reflectance minimum corresponding to the main chlorophyll *a* absorption is centered in the red wavelength region near 680 nm (Appendix, Figure I). Between 680 nm and 750 nm there is a steep rise in reflectance which results from the chlorophylls ceasing to absorb at wavelengths beyond 700 nm and an increase in scattering by the leaf internal structure. From 750 to 900 nm the reflectance is at a maximum (Datt, 1999). It is known that the absorption efficiency of chlorophyll decreases in stressed vegetation (increasing red reflectance), while the near infrared (NIR) reflectance decreases due to changes in the cell structure of the leaf. In stressed vegetation, change in near infrared reflectance of leaves is often detectable before changes in the visible spectrum become apparent. Remote sensing is therefore a useful tool for detecting vegetation stress caused by conditions as varied as disease, insect damage and drought (Carter and Knapp, 2001). It has been demonstrated in this study that galled and non-galled leaves and saplings display different spectral signatures in the NIR region in certain circumstances revealing that galling is indeed inducing a certain degree of stress to its host leaves. Previous studies of the effects of galling on chlorophyll content of leaves relay contrasting accounts: either it has been shown that there is a reduction of photosynthesis activity (Buss, 2003) or conversely galls increase photosynthesis rates of their host leaves (Larson, 1998). This discrepancy between Buss and Larson may be related to the galling stage and will be discussed further.

An examination of the damage caused by *O. eucalypti*, a gall wasp causing similar galling to *O. maskelli* reports similar uncertainty stating that whether it is the presence of the galls disrupting photoproductivity of the leaves, or the premature abscission of heavily-galled leaves, the hosts of *O. eucalypti* (Transversaria) in New Zealand (primarily *Eucalyptus saligna* and *E. botryoides*) are significantly affected in terms of health and growth (Withers and Raman, 2003).

The differences that have been detected in this study have been revealed through analysis that emphasized the comparison of average λ_{re}. The two major findings in this study are that, compared to non-galled leaves, galling of three to six months decreased the reflectance in the NIR region (700-1000 nm), and that galling induces a shift in the red edge (680-750 nm) towards shorter wavelengths. This pattern of reflectance has been consistent with finding of other studies of leaf damage by stress inflicting agents, such as insects and pathogens (i.e. fungi). As an example, Wilson et al. (1998) investigated the spectral reflectance characteristics of Dutch elm disease, a fungal disease spread by bark beetles (Scolytidae), and found an associated drop in the NIR reflectance for damaged elm leaves. Furthermore, results from spectroradiometry readings of individual cotton leaves revealed that percent reflectance patterns were distinctive for beet army worm, *Spodoptera exigua* (Hubner), damaged leaves compared to healthy cotton leaves: damaged leaves had lower NIR values than the healthy leaves (Sudbrink et al. 2003).

The position of the red edge is consistent among different tree species and generally ranges from 680 to 750 nm. Stress on vegetation has been shown to cause a shift of the red edge inflection point, (REIP), to shorter wavelengths, a so called *red edge region*. In the present study leaves and saplings that had three to six month old galling demonstrated such a shift. A possible explanation of this phenomenon is a change in chlorophyll levels, caused by galling. A rise in reflectance as a result of stress-induced reduction in chlorophyll content is detected first in regions where chlorophyll absorption is weak, and as a result a "shift" of the red edge of the reflectance curve is indicative of chlorophyll content (Carter and Miller, 1994). Other possibilities include changes in the chlorophyll absorption features. The shift of the red edge to shorter wavelengths during senescence or stress-induced chlorosis has been demonstrated by many others (e.g. Rock et al.1988; Datt, 2000; Carter and Knapp, 2001) and has been related to a reduction in the depth and breadth of the chlorophyll absorption feature. Vogelmann et al. (1993) showed such a shift in the laboratory with declining chlorophyll content from insect damage in *Acer saccharum* leaves.

Although not clear at the present time, this may also be the case with galled eucalyptus, in which the wavelength of the maximum slope of the red/near infrared boundary has been shown to be a good indicator of chlorophyll content in eucalypts (Datt, 1999). These stress factors are detected in the NIR region of the spectral curves and therefore remote sensing could serve as a potential tool for detection of galled trees in eucalyptus forests. Insights into how different situations of galling effect spectral signatures are important in order to maximize forest pest management using remote sensing. Gall color did not significantly influence the spectral curves of both green and pink galls in the NIR However, a stronger shift into the red region was found with red coloration.

These findings correspond with previous observations where measured reflectance of shaded and sun exposed leaves of *Acer saccharum* showed similar patterns: shaded leaves had slightly lower reflectance in the visible wavelengths and NIR than sun-exposed foliage (Mohammed et al. 2000). These results suggest that besides the gall effect upon reflectance, color (as expressed by the production of pigments) may also slightly influence the reflectance spectra.

Leaves with galls at a certain age of development, the degree of galling density and level of necrosis on the leaf surface all effected the expression of the spectral curves leading to the conclusion that galling is a stress factor for eucalyptus leaves. The reasoning behind measuring the reflectance curves of different periods of gall development was to understand how the physiology of the leaf changes with the life cycle of the wasp, therefore, suggesting the stage of wasp development where remote sensing could best serve as a detection tool for leaf galling. The spectral characteristics of light reflected from vegetation has long been relied upon as an indicator of plant stress. Increased reflectance in the far-red and near infrared part of the spectrum (690–720 nm) is a particularly generic response, providing an earlier or more consistent indication of stress than reflectance in other regions of the electromagnetic spectrum (Carter and Knapp, 1999). Stress to vegetation is typically explained by the tendency of stressed leaves to lose chlorophyll. Although the effects of galling on leaf photosynthesis is varied (Larson, 1998 and Buss, 2003), perhaps the age of gall determines when there are spectral signature changes in leaf reflectance.

The results of this study indicate that there weren't significant differences in leaf reflectance between the first three months of development of galled and non-galled leaves. It takes approximately 90-100 days in the warm season for the gall to reach the maximum width and for the wasp to complete its development (Mendel et al. 2004), and for the first two months after wasp infestation, gall swelling is not visual on the leaf surface. It is has been shown in this study that only in later stages of development (three months and older) significant differences in the average λ_{re} and by this stage, visual galls, represented by swelled leaf tissue is present. The fact that differences between galled and non-galled vegetation can only be detected once the wasp has reached a certain level of development inside the leaf gall, suggests that probably only advance stages of galling detrimentally affect the physiology of the leaf. This point requires further research. However, the fact that the spectra did not shift before clear symptoms appeared on the leaf surface of the leaf makes the ability of remote sensing weaker for the early detection of infestations.

The two injury symptoms tested as indicators for changes of the average λ_{re} were the percentage of gall density and percentage of necrosis on leaf surfaces. It was shown in this study that as gall density increases on the leaf surface, the average λ_{re} decreases. This phenomenon in which a progressive shift in the average λ_{re} occurs with larger degrees of leaf injury has been studied in eucalyptus leaves exhibiting symptoms of crown dieback associated with various sap sucking and leaf mining insects in Australia (Stone et al. 2001). The extent and severity of damage varied from localized small spots to larger proportions of leaf tissue.

Insects significantly influenced leaf reflectance depending on the species composition and density of insects present (Stone et al. 2001). In another laboratory study examining the reflectance curves of pine needles (*Pinus radiata*) infested with the fungus, Dothistroma needle blight, caused by *Dothistroma septosporum* (Dorog), a progressive change in the reflectance spectra with increasing severity of the *D. septosporum* disease on the pine needles can be seen and the key feature of the first derivative reflectance curves was a progressive decline of the curve peaks at the red edge corresponding to the significant flattening of the red edge slope with increasing *D. septosporum* infection (Stone et al. 2003). The authors suggest that this may be because *D. septosporum* is a fungal pathogen that produces a complex series of damage symptoms: initial chlorosis, production of red and brown metabolites, rapid loss of cellular integrity, cell necrosis, and eventual desiccation.

In addition to the evidence found at the leaf level that galling injury can be detected from reflectance in the red edge region, measurements taken at the plant level showed similar results. The success of scaling up and applying spectral features identified at the leaf scale to tree canopies has been mixed largely due to significant variability in canopy reflectance compared with leaf samples. The reflectance of canopies is not only influenced by leaf properties but also tree-crown density, leaf shape and orientation, background canopy reflectance and measurement geometry. Nevertheless, diagnostic features identified in the leaf spectra have the potential to form the basis of future spectral indices or algorithms of canopy health (Stone et al. 2001).

The use of spectral properties in the detection of physiological strains on eucalyptus leaves and saplings caused by a gall wasp galling appears promising and encourages efforts to develop monitoring tools at remote scales to aid sustainable forest management of the gall forming wasp, *O. maskelli* in eucalyptus forests around the world.

II. <u>Assessment of adult wasp emergence trends</u>

In addition to forest injury assessments, understanding of the natural history of insect pest is fundamental to the success of any forest pest management program. The temporal and spatial dynamics of forest pest populations are a basic component of pest management systems because these details are needed in order to develop predictive models and the development of treatment agendas (Waters and Stark, 1980). Predicting the timing of particular stages in the life cycle of pest insects is important in studies of their population dynamics and for forecasting pest insect attacks (Nylin, 2001). In this study, it was revealed from trapping adult wasps by sticky traps, that adult emergence in the spring occurs between March 1^{st} and April $15^{t.h}$. Early adult emergence (between March 1^{st} and March 15^{th}) was detected in the Western Negev and Coastal Plain. Adult wasps in the Jerusalem area, the Galilee and the Golan emerged a month later. The time of adult emergence in the studied regions seems to be driven by the accumulation of temperatures and therefore a range of accumulated degree-days for *O. maskelli* were determined in different regions in Israel in this study. Physiological time is often expressed and approximated in degree-days (DD).

Each species requires a defined number of degree-days to complete its development (Santolamazza-Carbone, 2006). The accumulated degree-days from a starting point can help predict when a developmental stage will be reached and therefore lead to effective management (Santolamazza-Carbone, 2006).

Systemic insecticides have been experimented with in the past against *O. maskelli* and another eucalyptus gall wasp in Israel, *Leptocybe invasa* (Hymenoptera: Eulophidae). These chemicals were selected for two reasons: the difficulty of accessing the developing larvae inside the gall, surface spraying usually losses its potency within a couple of days and both wasps tend to gall new, young leaves. The galls are known to be strong sinks on the leaf and therefore it can be assumed that the pesticide will be concentrated in the gall (Mendel et. al. 2004). Seasonal timing of these experiments was particularly important in order to coincide with different stage of the developing wasps and the growing season of the eucalyptus trees. Tests were initiated in the spring and end of the summer. Forecasting biological events (namely, emergence) of wasp populations may reduce the use of pesticides by accurately predicting the timing of a susceptible stage (Nestel et al. 1995).

The average number of accumulated DD (from January 1^{st}) required by the wasp to emerge from the pupae was calculated at 388.82 ± 85.21. Accumulation of DD was faster in the Western Negev and Coastal Plain compared to other regions. Accumulation of DD was slower in other regions, especially in the Jerusalem area where the wasp emerged the latest. These findings suggest a clear effect of geographic location (latitude and elevation) upon the thermal units required to complete an insect generation, or to attain a certain phenological stage. The preliminary results of this study indicate that management activities (principally insecticide spraying) of adult wasps could be timed with emergence periods. This estimation had an approximate variation of 20%. Depending on the region, this level of uncertainty corresponds to a possibility of error that ranges between five to ten days to mistakenly determine the time of adult emergence. The implication of this forecasting error will depend on the management and pest control technique applied against the pest.

The concept of using degree-days as a management strategy of galling insects is applied in the United States, for the control of the gall wasp, *Callirhytis cornigera* (Osten Sacken) (Eliason and Potter, 2000). This is a cynipid wasp with alternating generations that produce large, woody stem galls and tiny blister-like leaf galls on pin oak, *Quercus palustris* Muenchhausen. Attempts were made to disrupt the life cycle by targeting two different life stages: the 1st brood of adult gall wasps during their emergence from stem galls, and larvae developing in leaf galls. Trees at the study site were monitored daily, beginning in mid-March, to determine when adult *C. cornigera* began emerging from woody stem galls. Treatments were applied on the 29^{th} of March 1998, two days after the first wasps were observed, at an accumulation of 86.78 degree-days at base $10^{o}C$ from January first. Canopy sprays applied at bud burst, coinciding with emergence of the asexual wasps from twig galls, had the intended effect, reducing leaf gall density by 66% to 91% (Eliason and Potter, 2000). It has been concluded in this study that poorly timed insecticide applications might even be counterproductive (Eliason and Potter, 2000).

CONCLUSIONS

Attempts to assess the injury caused by this wasp have been reported in this study. While visual forest injury assessments may be cost effective and less time consuming, there is an accuracy that is lacking with this technique. It has been found that the Injury Index, the quantitative technique of assessing injury, is more articulate at defining lower levels of injury, while higher levels of injury are similarly categorized using both techniques.

Remote sensing in the near infrared region, with particular attention to the red edge region, may also be a significant technique of assessing the injury caused by this wasp. This laboratory study has shown that there are spectral differences between galled and non-galled leaves and saplings in the red edge region, signifying that galling is causing stress to host tissue. Sensors that are sensitive to the red edge region should be used when assessing the injury caused by the gall wasp. It is to be expected that the differences between healthy and galled growth will only be seen when the gall is at the final stages of maturation, when there is high density and necrosis on the leaves. These differences should lead to further examination of the impact galling has on the physiology of the leaf.

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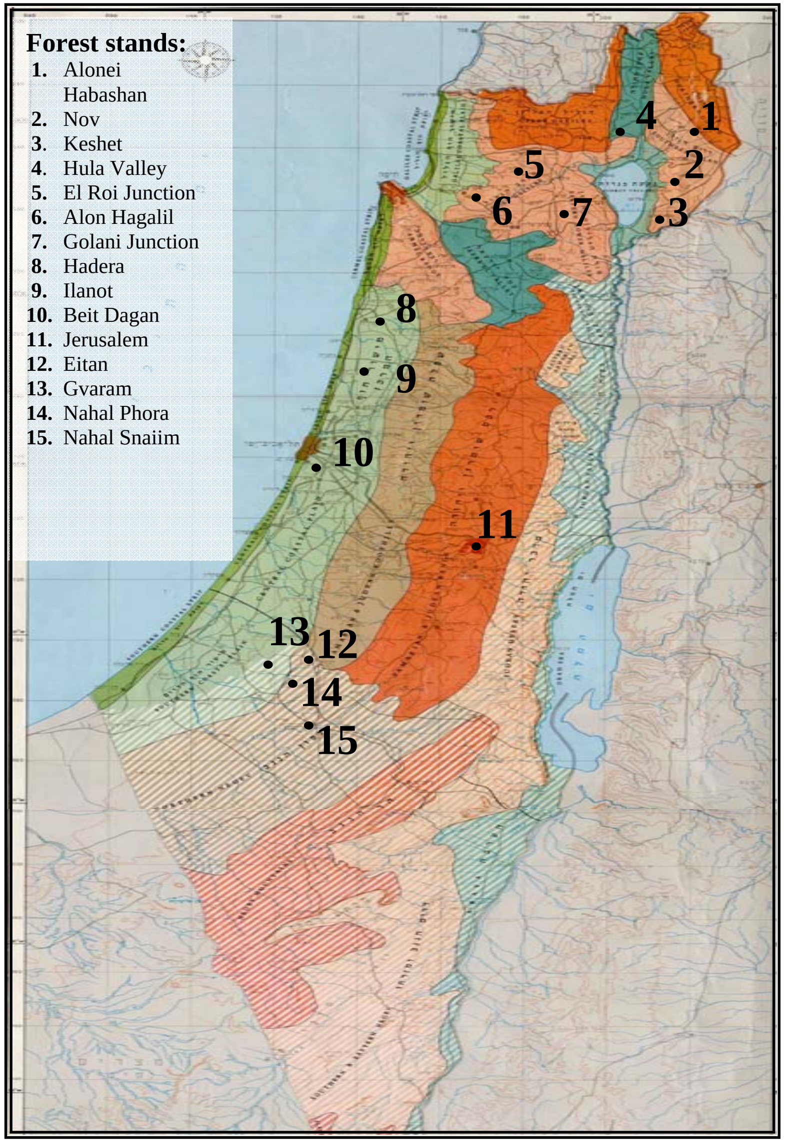
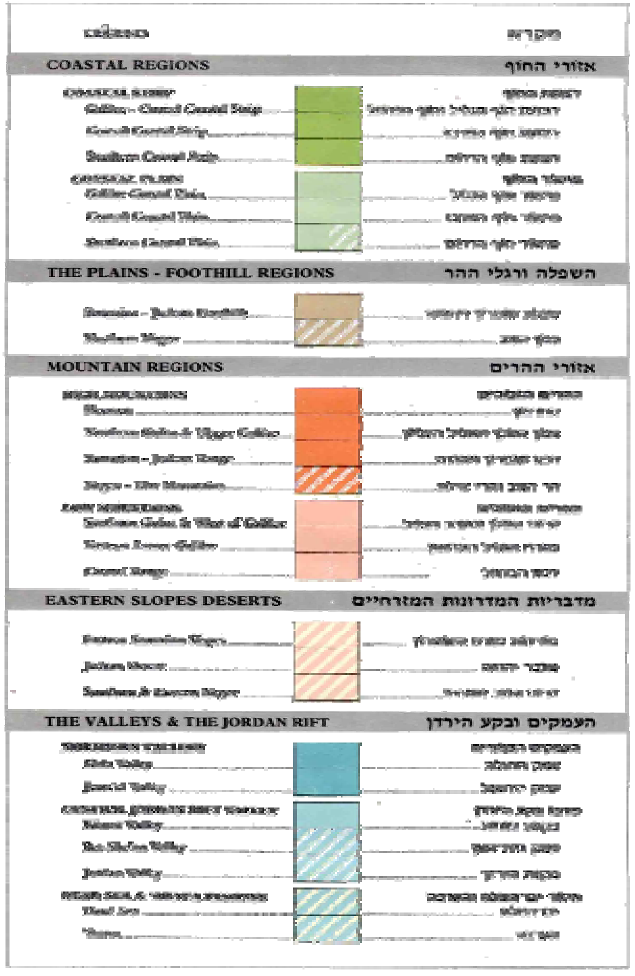
APPENDIX

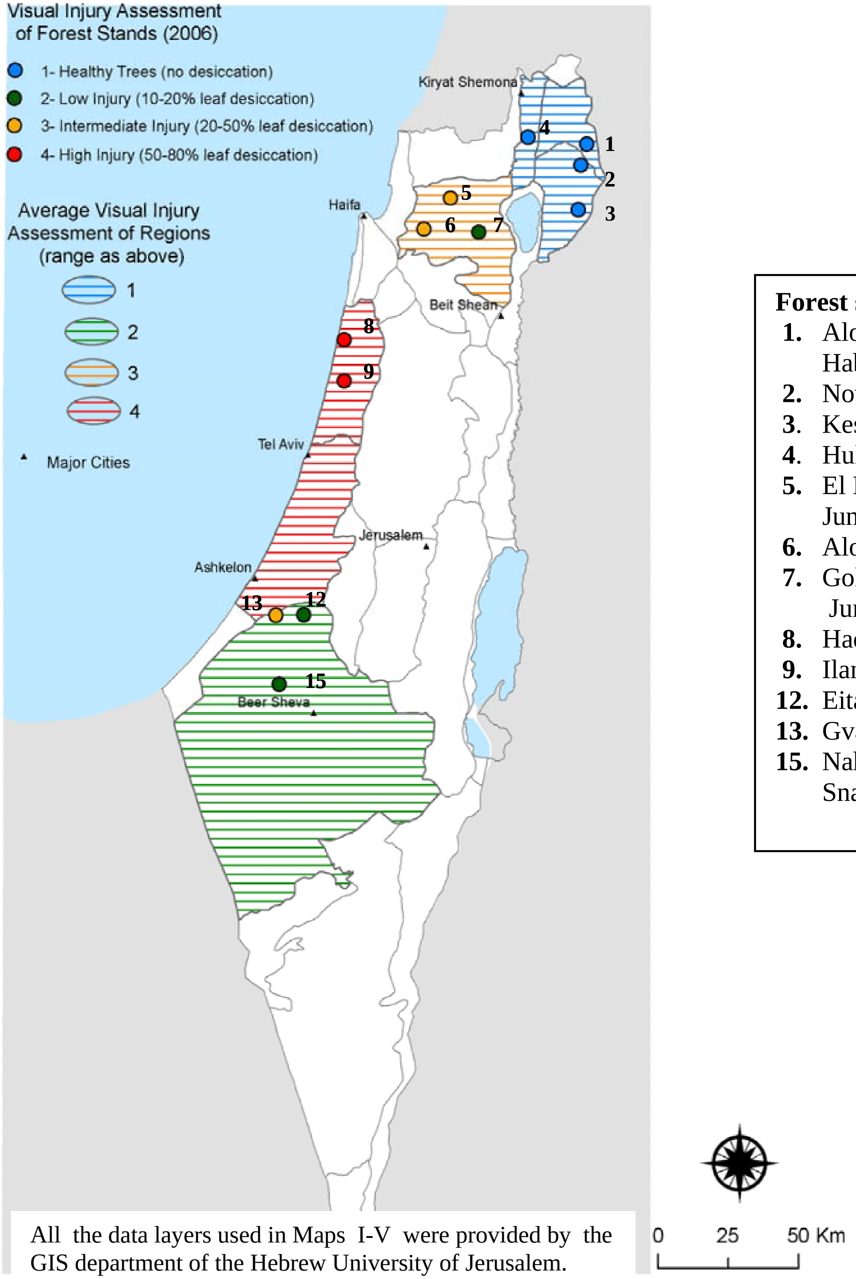
Map I: *Eucalyptus camaldulensis* forest stands and geo-climatic regions of Israel.





Map produced by: Israel Meteorological Service, 1992
This map is divided into regions according to the climatic regime based on the weighing of the following data: temperature, humidity, precipitation, wind, etc. .Each regime is characterized by a uniform macroclimatic regime, but may include certain anomalies.

Map II: Visual Injury Assessment of *E. camaldulensis* forest stands and studied regions for Winter 2006.

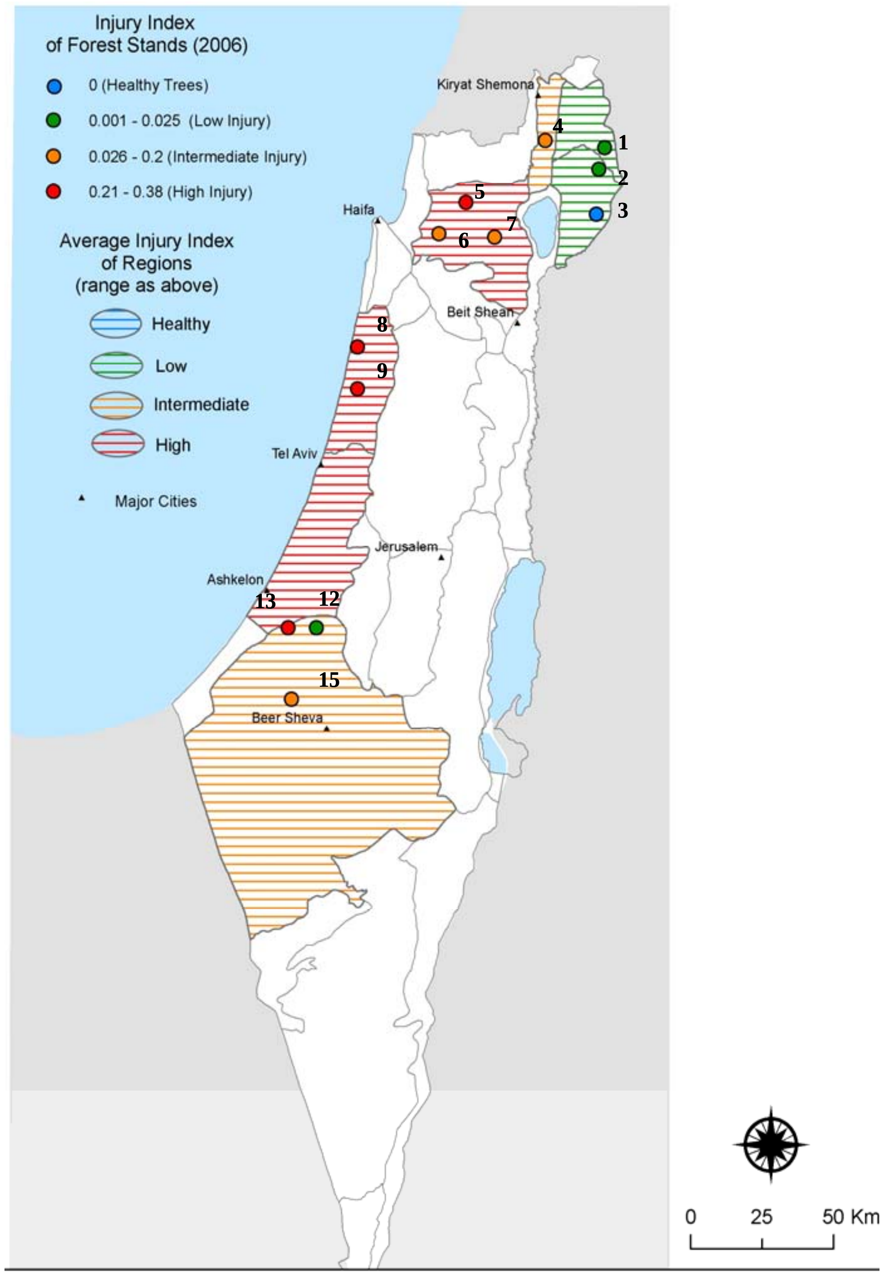


All the data layers used in Maps I-V were provided by the GIS department of the Hebrew University of Jerusalem.

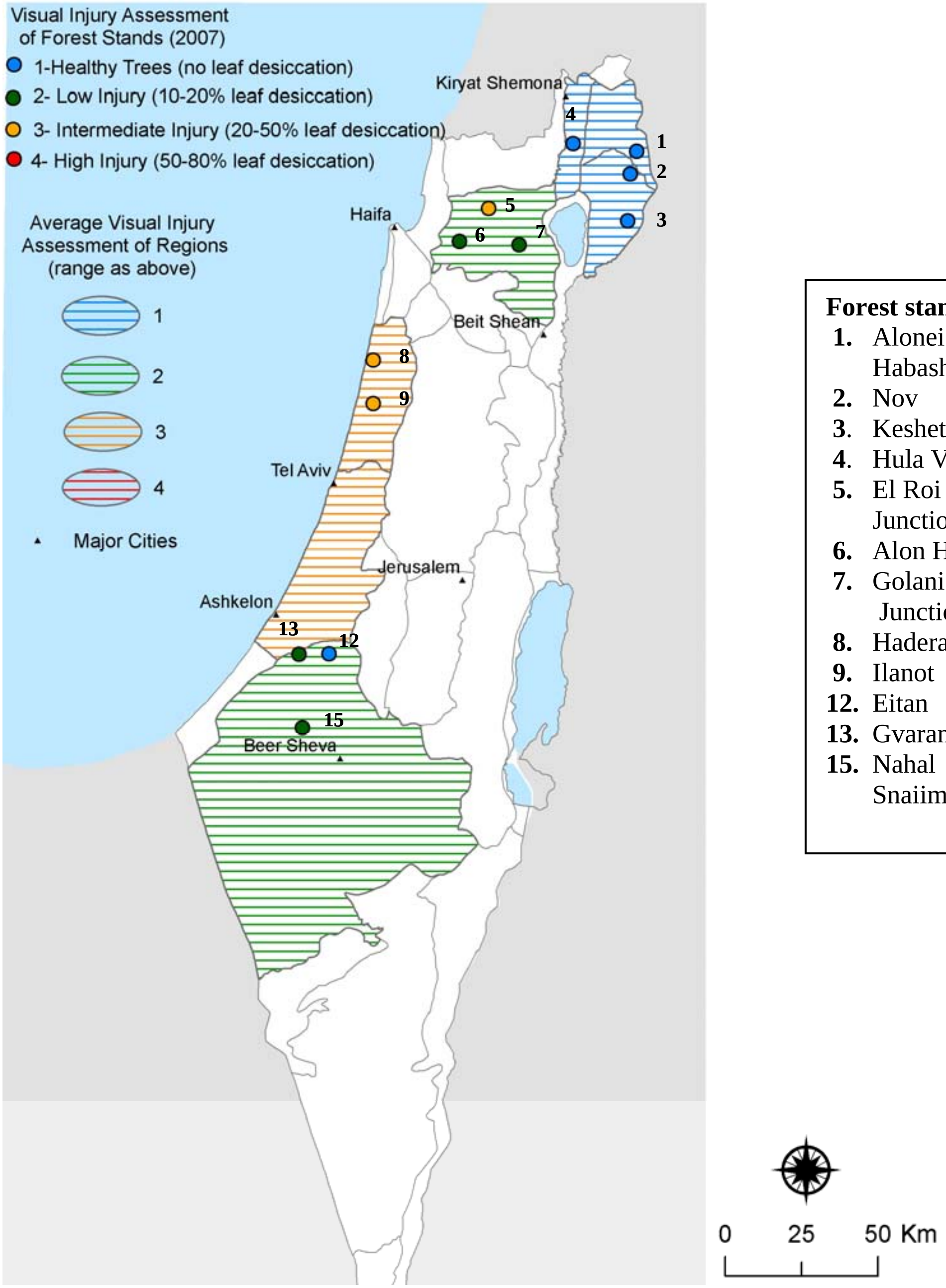
Forest stands:

1. Alonei Habashan
2. Nov
3. Keshet
4. Hula Valley
5. El Roi Junction
6. Alon Hagalil
7. Golani Junction
8. Hadera
9. Ilanot
12. Eitan
13. Gvaram
15. Nahal Snaiim

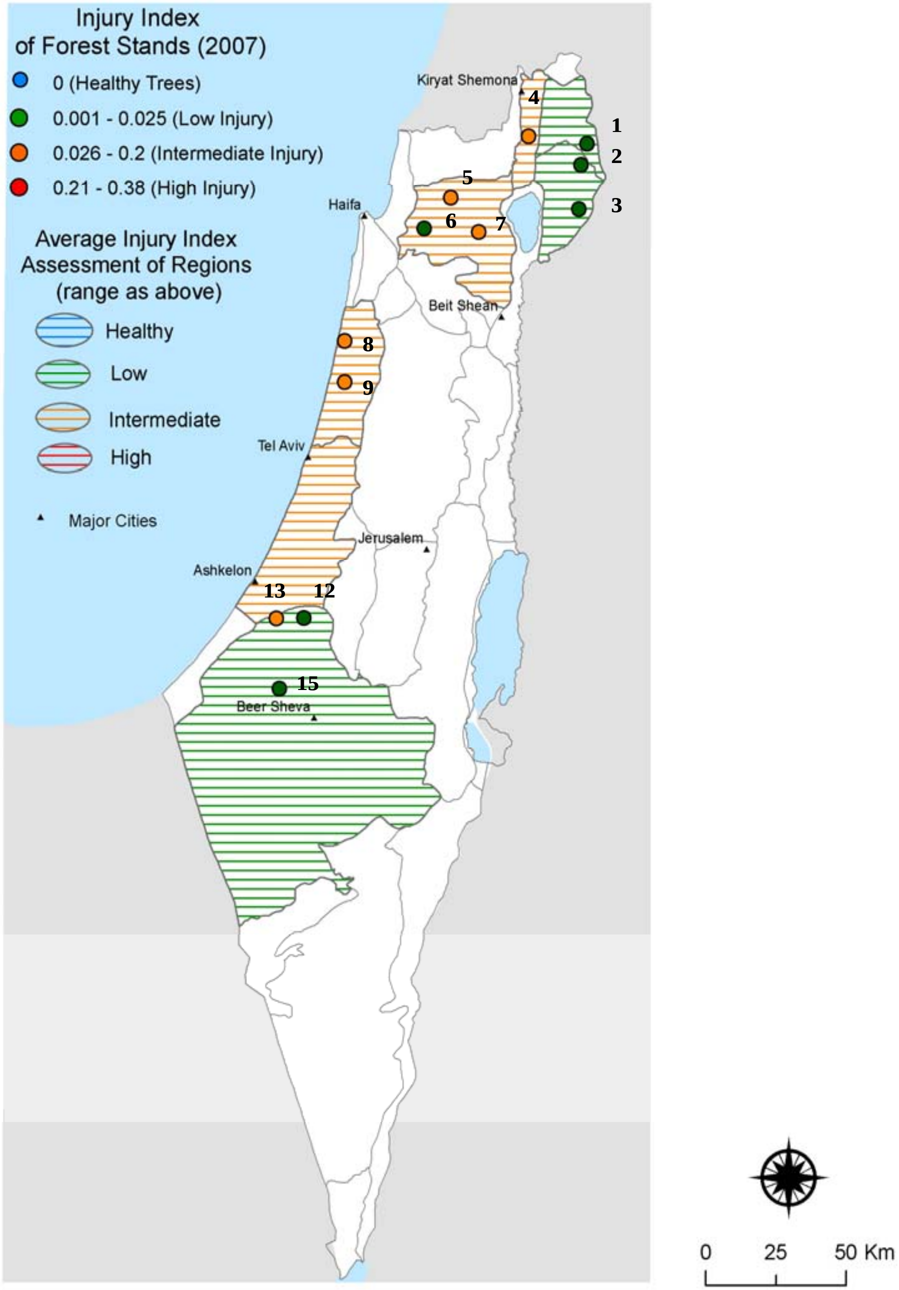
Map III: Injury Index Assessment of *E. camaldulensis* forest stands and studied regions for Winter 2006.



Map IV: Visual Injury Assessment of *E. camaldulensis* forest stands and studied regions for Winter 2007



Map V: Injury Index Assessment of *E. camaldulensis* forest stands and studied regions for Winter 2007



Forest stands:

1. Alonei Habashan
2. Nov
3. Keshet
4. Hula Valley
5. El Roi Junction
6. Alon Hagalil
7. Golani Junction
8. Hadera
9. Ilanot
12. Eitan
13. Gvaram
15. Nahal Snaiim

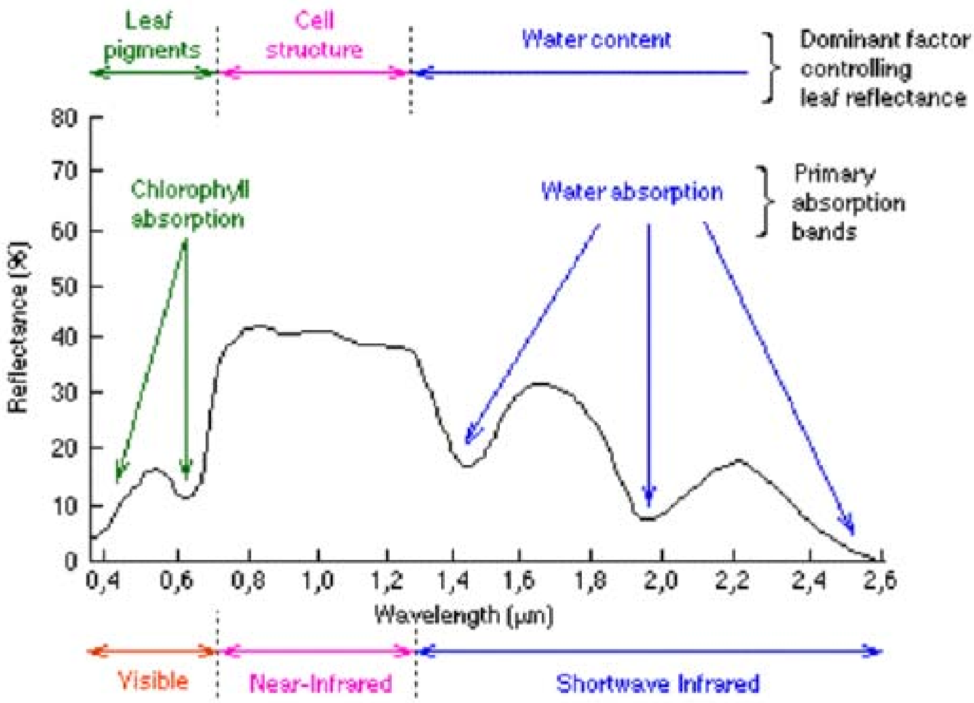


Figure 1: Spectral signature of green vegetation, with emphasis on physiological characteristics expressed throughout the electromagnetic spectrum (Sanderson, 2000).

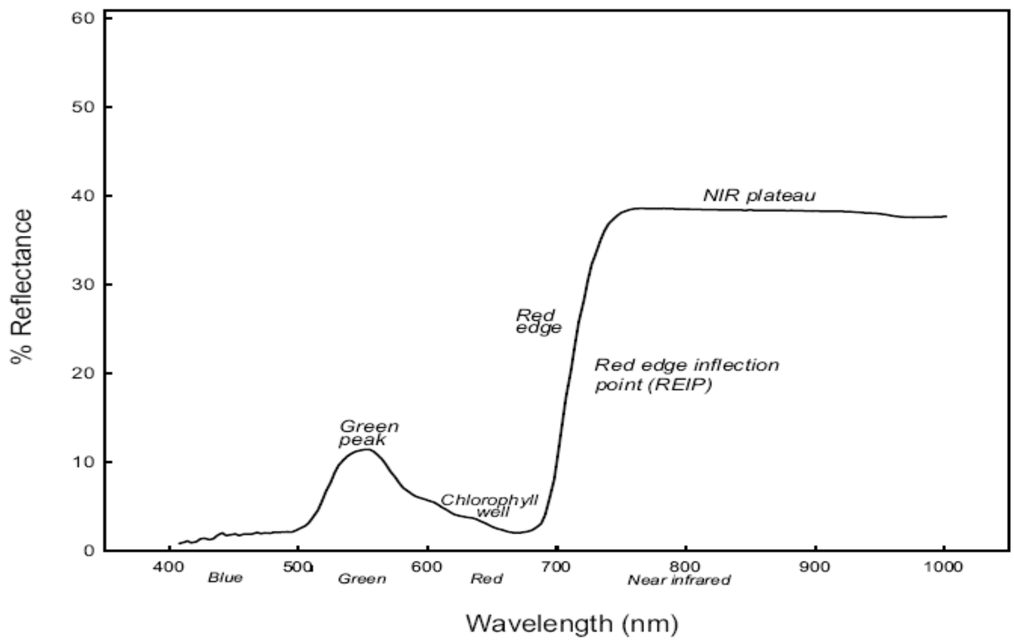


Figure II: Typical spectral signature of green vegetation, note the red edge region (Sanderson, 2000).

1

Healthy
tree

2

1- 20%
desiccation
of tree

3

20%- 50%
desiccation
of tree

4

50%- 80%
desiccation of
tree

5

80%-100%
desiccation of
tree

Figure 1: Gradient of injury levels (one to five) of individual *Eucalyptus camaldulensis* trees used for the Visual Grading of forest stands. The grades were determined by the overall % of leaf desiccation of trees for each forest stand.

תקציר

בשנים האחרונות צרעת עפצי החטטים, *Ophelimus maskelli* (Ashmead) (ולהלן הצרעה) (Hymenoptera: Eulophidae) חדרה לאזור אגן הים התיכון. הצרעה התפשטה תוך זמן קצר בסביבתה החדשה. בישראל אובחנה לראשונה ב-2003. מאז, תפוצתה בארץ גדלה במהירות, ועוררה צורך בהכנת תכנית פעולה לעצירתה. הצרעה מחוללת עפצים על מספר מיני איקליפטוס ובעיקר על איקליפטוס המקור, *Eucalyptus camadulensis*. העפץ מאופיין בהתעבות מקומית מעוגלת הבולטת משני צדדי העלה. הווצרות העפצים יכולה להוביל לנזק כבד לעצים על ידי ייבוש צמרות העצים ונשירת העלים.

המטרה של עבודה זאת היא לבחון שיטות הערכה שונות אשר יכולות לעזור בהערכת עוצמת הפגיעה הנגרמת ע״י הצרעה. בחנו את הערכת רמת הנגיעות בעפצים על ידי הסתכלות (V.G) (מיון ויזואלי) בשיטה זאת נקבע סולם נזק מ-1 עד 5. השוואנו בין המיון בהסתכלות לבין רמת הנזק (I.I) המתקבלת מדגימות עלווה ללא עפצים ועלים מעופצים מ-13 מקומות בישראל. בהערכת נזק נבדקו שני גורמים. אחד כלל קביעת צפיפות העפצים לעלה (P.G) והשני כלל את שכיחות העלים הנגועים (F.G) בעפצים בדגימה. ההשוואה בין רמת הנזק שהתבוססת על דגימה כמותית לזו של מיון ויזואלי (סובייקטיבי על ידי צופה) מגלה התאמה בין שתי שיטות ההערכה. הבדלים מובהקים בין שיטות ההערכה נמצאו כאשר רמת הנזק על העץ היתה נמוכה והיה קושי בהערכה לפי הסולם בהסתכלות. ממצאים אחרים הראו שהשכיחות במספר העלים הנגועים ביערות שנבדקו ב-2006 ו-2007 לא נבדלו מבחינה סטטיסטית. מצד שני נמצא הבדל מובהק בכמות העפצים לעלה. מכאן ניתן להסיקבהפחתה בנזק משנה אחת לשנייה (כנראה בשל שחרור צרעות טפיליות ב-2006) מתבטאת בעיקר בירידה בשטח היחסי המכוסה עפצים בעלה ולא במספר העלים המעופצים.

בחנו גם את השימוש בכלי חישה מרחוק לנטור של הפגיעה של הצרעה. שיעור ההחזר של קרינה ספקטראלית מעלים נגועים ולא נגועים בעפצים, בדגימות, נמדד באמצעות ספקטרופוטומטר ידני. בדיקה זאת ותוצאותיה הצביעו על כך שהשיטה עשויה לשמש ככלי חישה מרחוק להערכת רמת נזק ביער. בדיקת אפיונים ספקטרליים של העלה הציגה מידע אודות התכונות הפיזיולוגיות של העלה ובסיס לפיתוח תכנית דיגום ורישום לחישה מרחוק. עבודה זאת כוללת גם אפיון ספקטרום ההחזר הנוצר בשל העיפוץ. הספקטרום מושפע מגיל העפצים, מצפיפות העפצים לעלה מורמת נקרוזה של העלה. הבדיקה התמקדה באורכי גל בתחום ״הקצה אדום״ (680-780 nm) אזור אשר משמש לרוב לחישה מרחוק למטרת זיהוי עקות בצומח.

תוצאות המחקר מצביעות על הבדלים מובהקים בין אורכי גל שמחזירים עלים בעלי עפצים לעלים ללא עפצים בתחום ״הקצה אדום״. ההבדלים הללו התבטאו במיקום אורך הגל שמתקבל ערך מקסימאלי מעקומת הנגזרת בתחום ״הקצה אדום״ (λ_{re}). אורכי גל אלה נטו להיות נמוכים (נטייה לכיוון האדום) יותר בעלים ושתילים בעלי עפצים בהשוואה לעלים ושתילים ללא עפצים. בהשוואה ביו עלים נושאי עפצים בני חודש עד חודשיים לעלים ללא עפצים לא נמצאו הבדלים ב- λ_r. הבדלים מובהקים באורך גל נמצאו בעלים הנושאים עפצים בני יותר שלושה חודשים, כלומר, לאחר סיום התפתחות העפץ. נמצאה קורלאציה מובהקת בין החזר גלי הקרינה לבין צפיפות העפצים על העלה. עלייה בצפיפות העפצים בעלה הביאה לאורכי גל קצרים יותר בהחזר המרבי.

לאור ממצאים אלו, חישה מרחוק כנראה איננה כלי מתאים בזיהוי מוקדם של פגיעת צרעת עפצי החטטים (בהתחשב בצורך בסף נזק וגיל) בתנאים שונים (רמת צפיפות גבוהה, והתפתחות עפצים) אך נראה שיש לכלי זה שימוש אפשרי בהבחנה בין עצים נגועים לעצים לא נגועים ביער. חשיבות מיוחדת לעובדה שייבוש עלים ברמה נמוכה משרה לכיוון אורכי הגל האדומים, בדומה לממצא עם עפצים ישנים וצפופים. השינוי בגלי הקרינה קשור צפיפות העפצים לעלה. תוצאה זאת מצביעה על הקושי להבדיל בין עקה הנגרמת על יד עפצים או מסיבות אחרות. בכל מקרה, התוצאות שהתקבלו מהוות בסיס לשימוש בחישה מרחוק למעקב אחר עקה הנגרמת על ידי צרעות עפצים בעלות אופי פגיעה דומה ביערות איקליפטוס, בהתאם למיקום, המרחב והעלים הניזוקים.

בנוסף להערכת נזק, נושא אחר שנבחן בעבודה זו ,זה לקשור בין מועדי גיחת הצרעות לבתי הגידול. נקבעה בעזרת מלכודות דבק והתבססות על הצטברות ימי מעלות (DD). ניסוי לקשור בין מועדי גיחת הצרעות לבתי הגידול המקומיים, בהתחשב בכך שגל הגיחה הברור והעיקרי הוא באביב, השוני בגיחה באזורים השונים בישראל אכן קשור בהצטברות ימי מעלה. גיחה מוקדמת של בוגרים (בין ראשון לחמישה עשר במרץ) נצפתה בנגב המערבי ובמישור החוף. צרעות בוגרות באזור ירושלים, גליל עליון וגולן הגיחו באיחור של חודש. מועד הגיחה באזורים השונים קשורה למעלות יום מראשון בינואר. תוצאות פרק זה של המחקר הראו שהממוצע של D.D. הנצרך לגיחת צרעה מגולם הוא 85.21 ± 388.82. להערכה זאת עשויה להיות סטייה של 20% בשל תנאי האזור, אשר מראה אפשרות של תווך טעות בין 5-10 יום לגבי זמן הגיחה של הבוגרים.