UNIVERSITY OF MAURITIUS RESEARCH JOURNAL – Volume 16 – 2010
University of Mauritius, Réduit, Mauritius

Research Week 2009/2010

Climate Change Effects On Agricultural Pests: The Response of *Liriomyza Huidobrensis* to Drought-Stressed Potato Plants

S. Facknath
Faculty of Agriculture,
University of Mauritius
Email: sunif@uom.ac.mu

D.J Wright
Faculty of Natural Science,
Imperial College London

Paper Accepted on 16 April 2010

Abstract

Climate change is predicted to bring about major changes in pest and disease incidence, resulting from the effects of global warming and changes in rainfall patterns. While increasing temperatures are expected to influence growth and development, and duration of pest life cycles, changes in precipitation, relative humidity and availability of water can have an effect on pest-host relationships.

This study reports the results of one such study conducted in Mauritius and UK, on an important insect pest, *Liriomyza huidobrensis*, attacking major crops such as potato, beans, onion, egg plant, etc. in Mauritius.

Drought stress changed the response of *L. huidobrensis* adults to its potato host. In olfactometer studies, the insects exhibited distinct preference for greener and more succulent potato leaves, irrespective of their intraplant distribution, as opposed to the normal preference of adults to the older leaves of the lower canopy. This was inspite of the fact that biochemical studies of drought stressed and non stressed potato plants showed that the drought stressed plants had a better nutritional profile for the insect larvae than the non-stressed ones.

On the other hand, metabolomic studies correlated the changed host preference demonstrated by the pest to variations in the metabolite profiles of drought stressed and non-stressed plants, which indicates that adult host preference is not always positively related to offspring performance.

Key Words

Metabolomics, nutrient levels, insect behaviour, host preference, host performance.

1.0 Introduction

Global environmental change is expected to have a significant impact on food systems worldwide, through its influence on plants as well as on the other biotic and abiotic components of agroecosystems. Agriculture of any kind is strongly influenced by the availability of water. Climate change will modify the amount of water available for agricultural production through its effects on rainfall, evaporation, runoff, and soil moisture storage. The situation will be further exacerbated by global warming, with higher temperatures increasing plant demand for water. Increased evaporation from the soil and accelerated transpiration in the plants themselves may lead to drought stress in the plants.

The effects of climate change are already being felt in Mauritius. Rainfall patterns are becoming erratic with long spells of drought where annual rainfall is close to or below 1,500 mm (Met Office, 2004), and strong rainfall events (above 2,500 mm) leading to flash floods with associated infrastructural damage and, on one occasion in March 2008, even loss of human lives. Overall, estimates over the past 50 years suggest a general reduction of about 100 mm in the annual rainfall. Mauritius has already been classified as a water-scarce country according to the criteria used by the United Nations Environment Programme. Studies by the Meteorological Office (Met Office, 2008) have shown that over the years there has been an increase in annual mean sea levels as well as in the mean annual temperatures recorded over the island.

Global warming and changes in rainfall patterns will have significant impact on pest and disease ecology and incidence. While increasing temperatures are expected to influence growth and development, as well as the duration of pest life cycles, changes in precipitation, relative humidity and availability of water can have an effect on pest-host relationships.

Understanding climate change is vital for ensuring resilience of food security systems. Increased knowledge and comprehension of the interactions between and among climatic changes, plant physiology and pest ecology will help in the development of adaptation strategies for climate change.

Liriomyza huidobrensis (Blanchard) (Diptera:Agromyzidae) is a polyphagous insect, with leafmining larvae. Adult *Liriomyza* spp. females cause damage to plants by puncturing leaves with their ovipositor for feeding and oviposition. Eggs are laid inside the host leaf, and larvae form white, irregular mines as they feed. *L. huidobrensis* larvae feed on the chloroplast-containing spongy lower mesophyll (Weintraub & Horowitz, 1995; Heinz & Chaney, 1995). Fully developed larvae come out of the leaf mines, fall to the ground and pupate. Adults cut their way out of the pupal case, after a pupation period varying from 8-15 days, depending on temperature (Parrella, 1987). The larvae complete their entire development in one leaf, and the choice of larval habitat and food is made by the ovipositing female, who uses the physical, physiological and biochemical characteristics of the leaf to make her choice.

Within-plant dispersion of *Liriomyza* spp. is characterised by a significantly higher number of punctures and larval mines on the lower plant stratum as compared to the middle and upper strata of their host plants (Hammad & Nemer, 2000). Since *Liriomyza* larvae are internal feeders and complete their entire development in one leaf, the choice of larval habitat and food is made by the ovipositing female. Normally, oviposition, larval and pupal survival are significantly higher in the larger lower leaves, followed by the middle leaves and least on the smaller upper leaves (Facknath, 2005).

However, changes in water regime can have variable effects on insect incidence and damage, either due to the direct effect of water stress on the insects themselves, or indirectly through the effect on their host plant physiology. This study reports the effects of drought stress on *L. huidobrensis* preference and performance on potato plants.

2.0 Methodology

Potato cv, Mondial were grown in plastic pots (12 cm base diameter, 18 cm top diameter, and 14 cm height) in four kg of soil (type Low Humic Latosol; USDA Classification: Tropeptic Haplustox).

Drought stressed and normal plants. Drought stressed potato plants were obtained by stopping all irrigation when plants were 3 weeks old. Plants were subjected to 2 levels of drought stress, as follows:

Mildly drought-stressed plants: no watering for 1 week, and
Strongly drought-stressed plants: no watering for 2 weeks.
Preliminary work had shown that plants unirrigated for 15 ± 3 days revived and continued growth normally when later irrigated. Plants left unirrigated for more than an average of 2 weeks and later irrigated had impaired growth and were smaller in size when fully grown, than were plants provided with recommended amounts of water regularly.

Normal plants were irrigated with 500 ml of water daily.

2.1 Insect preference studies

Potato leaves in the lower 1/3rd of the plant ('lower plant canopy') are older and larger, while the leaves in the uppermost 1/3rd of the plant ('upper plant canopy') are younger and smaller. Behavioural response of *L. huidobrensis* to excised leaves of different ages from normal and drought-stressed plants was studied using a 4-arm perspex olfactometer, as described by Facknath *et al.* (2009). Two day old, mated females were released one at a time in the central arena and the odour arm it entered was noted after a period of 10 minutes. 50 such olfactometer runs were performed, using a different naive insect each time.

The feeding and oviposition preference was studied in a choice test, with three potted potato plants (1 normal, 1 mildly drought stressed and 1 strongly drought stressed) placed in a wooden cage. Ten mated females were released in the cage for 48 hours, after which they were removed. The number of punctures was counted on leaflets from the upper, lower and middle canopies. After 4 days, the number of mines were counted and used as an indication of the number of eggs laid per leaflet. The surface area of the leaflets was measured using a portable leaf area meter.

2.2 Plant data

Water, carbohydrates, total sugars, lipids, nitrogen, protein, phosphorus, potassium, calcium and magnesium were estimated in normal, mildly drought stressed and strongly drought stressed potted potato plants (uninfested). Total carbohydrates were estimated colorimetrically using the phenol - sulfuric acid method (Mikkelson & Corton, 2004). Absorbance of the yellow orange colour was read at 420 nm and total carbohydrates estimated from a calibration curve. Total sugars were estimated colorimetrically using anthrone reagent under acidic conditions. The absorbance of the blue-green colour was read at 620 nm and total sugars estimated from a calibration curve. Soxhlet extraction with petroleum ether was used to estimate total lipids. Nitrogen was determined by the Kjeldahl method described by Sillanpaa (1990). Protein was estimated using a factor of 6.25. The phosphorus in the solution was determined by spectrophotometry using ammonium molybdate solution and ascorbic acid (Sillanpaa, 1990). Measurements were made at the visible wavelength of 880 nm, using a standard calibration curve. Potassium was determined in this solution using flame emission photometry (Sillanpaa, 1990). Calcium and Mg were determined using atomic absorption spectrophotometry (Sillanpaa, 1990).

Each analysis was performed thrice.

2.3 Volatile entrainment and analysis

Volatiles were entrained from leaves and stems in intact potted potato cv. Anya plants. Only the stems and leaves of the potted plant were enclosed in a PET bag, and secured at the base of the plant above soil level by a piece of gardening wire. Care was taken to avoid bruising the plant tissue which can lead to release of induced volatiles (Agelopoulos *et al.* 1999). Air filtered and cleaned by passing through activated-charcoal filter (6-8 mesh size), and regulated by an air pump, was pushed in at the bottom end of the bag, while air was pulled out through an opening at the top. Volatiles were trapped on Tenax TA held in injector Optic liners for 4 h (Stewart-Jones & Poppy, 2006). Each entrainment was replicated 4 times. Samples on Tenax were desorbed using an Optic 2 programmable injector, and initially analysed on a HP Mass Selective Detector with non-polar fused capillary column coated with HP-1MS. Samples were injected in splitless mode and data was captured and analysed by Enhanced ChemStation software. Spectral searches were conducted using Wiley275 and NIST98 databases.

Samples in solution were also injected onto the Tenax TA packed in Optic injector liners and these were analysed after thermal desorption on the GC as above. Identifications were based on spectral library searches and Kovats Indices. Where possible, identities were confirmed with authenticated standards, which were run on both the GC after thermal desorption and the GC-MS.

2.4 Data Analysis

The olfactometer data were checked for normality and subjected to a Generalised Linear Model (GLM) to identify factors of significance, using the SAS software. Replicates and non-responders were considered as blocks. Metabolomic data was subjected to paired t-test.

3.0 Results and Discussion

In normal plants, females exhibited the usual preference for the larger older leaves, followed by the middle leaves, and then the smaller younger leaves (Facknath, 2005). However, in drought stressed plants, this preference was lost, and females were attracted in equal degrees to all leaves, irrespective of age. The insects not being in physical or visual contact with the host leaves, this change is in all probability due to quantitative differences in the metabolite profiles of drought stressed and non-stressed plants. The ability to detect and respond to quantitative changes in individual volatiles or volatile blends can provide the insect with essential information on the physiological condition of a given host plant, for the purpose of host selection and host colonisation.

Table 1: Behavioural response of *Liriomyza* adults to leaves of different ages excised from normal and drought-stressed potato plants

	Frequency of choice exhibited by *L. huidobrensis* adults (%)		
Potato plants	**Leaves excised from**		
	Lower canopy	**Middle canopy**	**Upper canopy**
Normal	55 a	35 b	10 c
Mildly drought stressed	41 a	39 a	20 b
Strongly drought stressed	34 a	34 a	32 a

The greater preference exhibited by *L. huidobrensis* females to non stressed leaves in an olfactometer study, where the insects are not in physical or visual contact with the host leaves, indicates that the choice is a consequence of the changed metabolite profiles of drought stressed and non-stressed plants (Table 1). On the other hand, physical and/or visual contact with the host plant resulted in a preference for non-stressed plants compared to drought stressed ones (Tables 2 and 3), in spite of the higher levels of nutrients in the stressed leaves (Table 5).

Table 2: Preference of *L. huidobrensis* for feeding on different aged leaves of normal and drought-stressed potato plants

No. of feeding punctures/cm^2 leaf area by *L. huidobrensis* females				
Potato plants	**Lower canopy**	**Middle canopy**	**Upper canopy**	**Total**
Normal	3.8 ± 0.12 aA	3.0 ± 0.13 aB	2.40 ± 0.12 aC	3.07 a
Mildly drought stressed	2.1 ± 0.13 bA	1.9 ± 0.09 bA	1.3 ± 0.08 bB	1.77 b
Strongly drought stressed	0.69 ± 0.04 cA	0.64 ± 0.03 c A	0.60 ± 0.01 cA	0.64 c

* Means ± SD followed by the same small letter down a column are not significant at P=5%.

Means ± SD followed by the same capital letter across a row are not significant at P=5%.

Table 3: Preference of *L. huidobrensis* for oviposition on different aged leaves of normal and drought-stressed potato plants

No. of viable eggs laid /cm^2 leaf area by *L. huidobrensis* females				
Potato plants	**Lower canopy**	**Middle canopy**	**Upper canopy**	**Total**
Normal	0.31 ± 0.10 aA	0.27 ± 0.11 aB	0.15 ± 0.11 aC	0.24 a
Mildly drought stressed	0.14 ± 0.12 bA	0.12 ± 0.01 bB	0.06 ± 0.001 bC	0.11 b
Strongly drought stressed	0.07 ± 0.001 cA	0.06 ± 0.009 cA	0.06 ± 0.001 bA	0.06 c

* Means ± SD followed by the same small letter down a column are not significant at P=5%.
Means ± SD followed by the same capital letter across a row are not significant at P=5%.

In normal plants, females probed leaves of the lower canopy to a greater extent for feeding, and laid a larger number of eggs, than in the middle canopy leaves. Leaves of the upper canopy are smaller, thinner and have a greater number of trichomes while older leaves are larger, thicker and have fewer trichomes (Facknath, 2005). Leaves of the upper canopy were least probed and had fewer eggs laid in them. But in drought stressed plants this preference was disturbed, and females probed leaves of all ages almost equally. However, overall, females preferred normal plants to drought stressed ones, for feeding and oviposition, in spite of the higher levels of nutrients in the stressed leaves (Table 5).

Table 4: Performance of *L. huidobrensis* on normal and drought-stressed potato plants

	Normal	**Mildly drought-stressed**	**Strongly drought-stressed**
Larval mines/leaflet	14.06 ± 1.06 a	1.18 ± 0.07 b	0.06 ± 0.002 b
Pupae formed	11.5 ± 1.43 a	0.66 ± 0.041 b	0.01 ± 0.002 b
Adults emerged	10.5 ± 1.01 a	0.60 ± 0.034 b	0.008 ± 0.004 b

Pupal length (mm)	1.9 ± 0.44 a	1.6 ± 0.19 b	1.5 ± 0.28 b
Pupal weight (mg)	0.21 ± 0.01 a	0.18 ± 0.02 a	0.18 ± 0.012 b
Adult length (mm)	2.0 ± 0.20 a	1.9 ± 0.16 a	1.7 ± 0.23 b
Adult weight (mg)	0.50 ± 0.037 a	0.48 ± 0.01 a	0.44 ± 0.009 b
Prepupal + pupal duration (days)	11.0 ± 1.08 a	10.7 ± 1.05 a	10.8 ± 1.07 a
Adult longevity (days)	15.4 ± 1.20 a	13.9 ± 2.07 b	13.3 ± 2.04 b
Female fecundity (days)	176.7 ± 10.9 a	120.7 ± 12.6 b	124.3 ± 10.4 b

* Means ± SD followed by same letter within a row are not significant at P=5%.

Larval development was adversely affected in the stressed leaves with only about 70% of *L. huidobrensis* larvae reaching L_3 stage (Table 4), as opposed to 90% in normal leaves. While overall larval and pupal survival did not appear to be affected (based on number of pupae and adults formed in each case), there were significant differences in the size and weight of pupae formed, and the size and weight of the subsequent adults.

Reduction in the amount of water in the plant tissues, i.e. in the larval food, may have led to reduced feeding, and resulted in the smaller size and reduced weight of pupae and adults obtained from stressed plants. Reduced level of water in the plant tissues may also be responsible for concentrating plant allelochemicals, some of which could have had a feeding deterrent effect on the larvae.

Table 5: Biochemical composition of leaves from normal and drought-stressed potato plants

	Normal leaves	**Mildly drought-stressed leaves**	**Strongly drought-stressed leaves**
Water (%)	82.4 ± 3.53 a*	68.3 ± 7.41 b	49.8 ± 6.29 c
Protein (%)	27.5 ± 0.97 a	29.4 ± 0.89 ab	32.5 ± 0.80 b
Carbohydrates (%)	18.9 ± 0.12 a	21.6 ± ab	23.4 ± b
Sugars (%)	8.2 ± a	10.4 ± b	13.7 ± c
Lipids	33.3 ± a	33.2 ± a	31.4 ± a
N (%)	4.4 ± a	4.7 ± ab	5.2 ± b
P (%)	0.26 ± a	0.34 ± ab	0.37 ± b
K (%)	3.5 ± a	3.8 ± ab	4.1 ± b
Ca (%)	1.7 ± a	1.9 ± a	1.9 ± a
Mg (%)	0.6 ± a	0.7 ± a	0.7 ± a

* Means ± SD followed by same letter within a row are not significant at P=5%.

Drought stress resulted in physiologically stressed potato plants that exhibited water deficit and wilting. The increase in levels of carbohydrate and sugar following water deficit (Table 5) has also been reported in other plant species (Bray *et al.* 2000; Pinheiro *et al.* 2001). In many plants, drought inhibits growth more than it does photosynthesis (Pinheiro *et al.* 2001), leading to an increase in soluble sugars, with an increased partitioning of fixed carbon to sucrose (Quick *et al.* 1992). Sugars often serve as phagostimulants, and their absence or shortage can lead to a reduction in insect feeding rates. However, very high sucrose concentrations have also been shown to lower feeding rates in insects (Mittler & Meikle, 1991).

The increase in protein (Table 5) could be the result of an increase in nitrogen content and/or amino acid concentration of leaf tissue, which often occur under conditions of drought stress (Volaire *et al.* 1998), and can be expected to increase insect preference and improve performance. On the other hand, the tougher foliage that often results from drought stress can reduce nitrogen availability and adversely affect insect performance (McMillin & Wagner, 1995).

Reduced level of water in the plant tissues also had an effect on production and emission of allelochemicals (Table 6), which could influence on insect behaviour and feeding/oviposition preference. Inbar *et al.* (2001) attributed the poor performance of caterpillars on drought-stressed plants to both reduced water content and elevated allelochemistry.

Table 6: Metabolomic profiles of normal and drought-stressed potato plants

Volatile	**Normal (NM)**	**Mildly drought-stressed (MD)**	**NM/ MD**	**Normal (NS)**	**Strongly drought-stressed (SD)**	**NS/SD**
3 methyl 1 butanol	518.0	159.6	3.25*	62.7	37.5	1.67
(Z) 3 hexenal	769.2	666.4	1.15	65.6	39.6	1.66*
Hexenal	407.4	318.5	1.28	79.5	44.9	1.77*
(E) 2 hexenal	5400.5	4013.4	1.35	2237.0	621.0	3.60*
(E) 2 hexen-1-ol	342.5	307.9	1.11	128.3	36.1	3.55*
Limonene	16.4	111.2	0.15	16.7	4.8	3.49*
Benzyl alcohol	1688.1	725.9	2.33*	554.2	273.9	2.02*
Benzeneacetaldehyde	6036.7	2523.4	2.39*	1146.4	269.5	4.25*

γ- terpinene	1.7	123.8	0.01*	10.8	1.4	7.83*
Methyl benzoate	17.1	33.6	0.51	284.1	81.4	3.49*
DMNT	0.0	4.1	0.00*	73.7	112.3	0.66*
Benzeneethanol	1951.8	479.7	4.07*	16.6	137.8	0.12*
Methyl salicylate	1987.2	2889.7	0.69*	1901.6	2880.8	0.66*
α copaene	742.9	172.7	4.30*	303.0	74.4	4.07*
ß funebrene	333.1	325.2	1.02	93.0	229.6	0.41*
(E) (Z) α alpha farnesene	6690.8	8037.6	0.83*	1843.8	5867.7	0.31*
Germacrene D	9318.3	12698.5	0.73*	3164.8	10588.7	0.30*
α zingerberene	4709.8	5978.8	0.79	1931.4	4669.5	0.41*
ß sesquiphellandrene	1975.7	2381.4	0.83	1306.9	2502.7	0.52*
(E) nerolidol	118.8	52.2	2.28	54.4	140.8	0.39*
Germacrene D-4-ol	1220.3	1835.0	0.66	642.2	3246.0	0.20*
Caryophyllene oxide	600.7	311.8	1.93	132.7	28.4	4.68*
Ledol	1361.9	1632.3	0.83	487.1	2563.4	0.19*

* significantly different at P=5% with paired t-test.

Metabolomic profiles showed that there was no qualitative difference in the volatiles released by normal potato plants and drought-stressed plants (Table 6). The difference was mainly quantitative, with some volatiles being emitted at higher amounts by stressed plants and others by normal well-watered ones. The drought-stressed plants produced lower levels of all the general green leaf volatiles (GGLVs). Leafminer adults are attracted to the GGLVs, which could explain the higher number of adults moving towards the normal leaves/plants in the behavioural studies. On the other hand, the other volatiles were affected to differing degree by the drought stress, e.g. there was a reduction in volatile emissions from drought stressed plants compared to normal plants with respect to methyl butanol, benzyl alcohol, benzene acetaldehyde, α copaene, caryophyllene oxide, while emissions of DMNT, methyl salicylate, (E) (Z) α alpha farnesene, germacrene D, α zingerberene, ß sesquiphellandrene, germacrene D-4-ol, and ledol were higher in drought stressed plants compared to normal plants. Takabayashi et al. (1994) demonstrated that lima bean plants released higher levels of volatiles when water stressed than when adequate water was supplied. The leafminer adults may have been attracted by any one or more of these volatiles or to a blend of certain of the volatiles.

Emissions of γ-terpinene, methyl benzoate and limonene were reduced in mildly drought-stressed plants but increased to levels higher than that for normal plans in strongly drought-stressed plants. Limonene, even when acting alone, has been shown to be a kairomone for leafminer adults (Zhao & Kang, 2002). On the other hand, emissions of benzene ethanol and ß funebrene were less affected by mild drought stress but much more significantly affected by strong drought stress.

DMNT and methyl salicylate are known to be normally wound and/or herbivory induced, i.e. biotic stresses. Their lowered levels in drought stressed plants in the present study indicate that abiotic stresses can also influence production and/or emission of these volatiles. While some insects are attracted to DMNT and methyl salicylate released by plants damaged by herbivory (Wei and Kang, 2006), others are repelled.

Results of the study indicate that the drier conditions resulting from climate change will bring about changes in volatile emissions as well as the concentration of nutrients in potato plants, and that *L. huidobrensis* will respond to the variation in the metabolite profiles as well as to the nutrient status of its host. Changes in the profile of secondary metabolites in the potato plant may serve to attract more *L. huidobrensis* adults to the potato plant, which will attack leaves of all ages equally. Drought-stressed plants were apparently nutritionally superior to normal plants, demonstrated by both an increase in primary nutrients as well as micronutrients. However, this increase in nutritional superiority did not translate into increased preference nor improved performance of the leafminers. In actual fact, insect performance was lower on drought stressed plants, the reduction being proportional to the degree of the stress.

4.0 Conclusion

Drought stress resulted in the breakdown of the preference for older leaves normally exhibited by *L. huidobrensis*, both for adult feeding (and consequently adult fitness) as well as for laying eggs. However, overall, healthy plants were more attacked than were drought stressed ones, with strongly stressed plants being least preferred, in spite of the fact that drought stressed plants had a better nutritional profile for the larvae than the non-stressed ones. This increase in nutritional superiority did not translate into increased preference nor improved performance of the leafminers.

The drier conditions resulting from climate change will bring about changes in volatile metabolite emissions and concentration of nutrients in potato plants. *L. huidobrensis* will respond to the variations in the metabolite profiles and to the changed nutrient status of its host. Changes in the profile of secondary metabolites in the potato plant may serve to attract more *L. huidobrensis* adults to the potato plant, which will attack leaves of all ages equally. Changes in leaf nutrients will contribute to change in larval, pupal and adult performance, and eventually to pest incidence and damage.

5.0 Acknowledgement

The authors gratefully acknowledge the contribution of Prof. Guy Poppy and Dr. Alex Stewart-Jones of the University of Southampton, UK for the GC-MS work, and Dr. Keith Waters, CSL, York, UK for providing *L. huidobrensis* insects. All work on *L. huidobrensis* in UK was done under MAFF licence PHL 189A/5100(04/2005).

6.0 References

- AGELOPOULOS, N. G., HOOPER, A. M., MANIAR, S. P., PICKETT, J. A. & WADHAMS, L.J. (1999). A novel approach for isolation of volatile chemicals released by individual leaves of a plant in situ. *Journal of Chemical Ecology*, **25**(6), 1411-1425.

- BRAY, E. A., BAILEY-SERRES, J. & WERETILNYK, E. (2000). Responses to abiotic stress. In *Biochemistry and Molecular Biology of Plants,* pp 1158– 1203. (Eds B. B. BUCHANAN, W. GRUISSEM & R. L. JONES). American Society of Plant Biologists, Rockville, Maryland.

- FACKNATH, S., STEWART-JONES, A. & WRIGHT, D.J. (2009). Neem chemicals disturb the behavioral response of *Liriomyza huidobrensis* to conspecific-induced potato volatiles. *Pure & Applied Chemistry* **81** (1). 85-95.

- FACKNATH, S. (2005). Leaf age and life history variables of a leafminer: the case of *Liriomyza trifolii* on potato leaves. *Entomologia Experimentalis et Applicata,* **115**, 79-87.

- HAMMAD, E.M.A.F. & NEMER, N.M. (2000). Population densities, spatial pattern and development of the pea leafminer (Diptera: Agromyzidae) on cucumber, Swiss chard and bean. *Journal of Agricultural Science,* **134**, 61-68.

- HEINZ, K.M. & CHANEY, W.E. (1995). Sampling for *Liriomyza huidobrensis* (Diptera: Agromyzidae) larvae and damage in celery. *Environmental Entomology*, **24**, 204-211.

- INBAR, M., DOOSTDAR H. & MAYER, R.T. (2001). Suitability of stressed and vigorous plants to various insect herbivores. *Oikos,* **94**, 228–235.

- MCMILLIN, J. D. & M. R. WAGNER. (1995). Season and intensity of water stress: host-plant effects on larval survival and fecundity of *Neodiprion gillettei* (Hymenoptera: Diprionidae). *Environmental Entomology*, **24**, 1251–1257.

- MET OFFICE (2004). Report of the Meteorological Office. Republic of Mauritius.

- MET OFFICE (2008). Report of the Meteorological Office. Republic of Mauritius.

- MIKKELSEN, S.R. AND CORTON, E. 2004. *Bianalytical Chemistry*. John Wiley & Sons.

- MITTLER, T.E. & MEIKLE, T. (1991). Effects of dietary sucrose concentration on aphid honeydew carbohydrate levels and rates of excretion. *Entomologia Experimentalis et Applicata,* **59**, 1–7.

- PARRELLA, M.P. (1987). Biology of *Liriomyza. Annual Revue of Entomology,* 32, 201-224.

- PINHEIRO, C., CHAVEZ, M.M. & RICARDO, C.P. (2001). Alterations in carbon and nitrogen metabolism induced by water deficit in the stems and leaves of *Lupinus albus* L. *Journal of Experimental Botany,* **52**, 1063 - 1070.

- QUICK, W.P., CHAVEZ, M.M., WENDLER, R., DAVID, D., RODRIGUES, M.L., PASSARINHO, J.A., PEREIRA, J.S., ADCOCK, M.D., LEEGOOD, R.C. & STITT, M. (1992). The effect of water stress on photosynthetic under field conditions. *Plant, Cell and Environment,* **15**, 25-35.

- SILLANPAA, M. (1990). Micronutrient assessment at the country level: an international study. *FAO Soils Bulletin No. 63.* FAO, Rome.

- STEWART-JONES, A. & POPPY, G. M. (2006). Comparison of glass vessels and plastic bags for enclosing living plant parts for headspace analysis. *Journal of Chemical Ecology,* **32**, 845-864.

- TAKABAYASHI, J., DICKE, M. TAKAHASHI, S. POSTHUMUS, M.A. & VAN BEEK, T.A. (1994). Leaf age affects composition of herbivore-induced synomones and attraction of predatory mites. *Journal of Chemical Ecology* **20**, 373-386.

- VOLAIRE, F., THOMAS, H. & LELIEVRE, F. (1998). Survival and recovery of perennial forage grasses under prolonged Mediterranean drought. II. Water status, solute accumulation, abscisic acid concentration, and accumulation of dehydrin transcripts in bases of immature leaves. *New Phytologist*, **140**, 451–460.

- WEI, J.N. & KANG, J.Z. (2006). Volatiles released from bean plants in response to agromyzid flies. *Planta,* **224**, 279-287.

- WEINTRAUB, P.G. & HOROWITZ, A.R. (1995). The newest leafminer pest in Israel, *Liriomyza huidobrensis*. *Phytoparasitica,* **23**, 177– 84.

- ZHAO, Y.X. & KANG, L. (2002). Role of plant volatiles in host plant location of the leafminer, *Liriomyza sativae* (Diptera: Agromyzidae). *Physiological Entomology,* **27**, 103-111.