

## GENETIC RESPONSE OF CITRUS GERMPLASM AGAINST CITRUS LEAF MINER

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### ABSTRACT

Fifteen citrus varieties/ cultivars were evaluated to observe the genetic response against citrus leaf miner (*Phyllocnistis citrella*) for two years at Dept. Plant pathology research area by using Augmented design. *Citrus reticulata* cv. Kinnow, *Citrus sinensis* cv. Jaffa expressed moderately susceptible response towards CLM (17.4 – 18.50%) infestations while seven varieties/ cultivars (*Citrus sinensis* cv. pine apple, *Citrus sinensis* cv. Succari, *Citrus reticulata* cv. mungal singh, *Citrus reticulata* cv. Tangerine, *Citrus reticulata* cv. Malta, *Citrus sinensis* cv. valentia late, *Citrus reticulata* cv. feutral's early) exhibited susceptible response with 20.16 – 29.27 % insect population. *Citrus limonia* cv. china lemon, *Citrus paradise*, *Citrus sinensis* cv. Musambi, *Citrus limettioides*, *Citrus sinensis* cv. blood red, *Citrus limonia* cv. mayer lemon showed high inclination ((30.44 – 38.38%) to *Phyllocnistis citrella*.

**Key words:** *Phyllocnistis citrella*, Citrus leafminer, Genetic response.

### INTRODUCTION

Citrus is one of the foremost fruit of Pakistan in stipulations of area, yield and export. Although citrus crop is kept in great esteem, yet present status is defenseless by a number of factors, which impede the fruit yield and quality (Atiq, 2008) A number of insects attacked on citrus plants. Among these citrus leaf miner (CLM) *Phyllocnistis citrella* (Stainton) causes a huge loss of quality citrus fruits (Khair, 2004). It was exposed in 1993 in Florida and later on broaden all the citrus growing areas of the world (Bermudez *et al.*, 2004; Hoy and Jessey, 2004) It also exacerbates citrus canker disease by exposing leaf mesophyll cells during its feeding and allowing direct penetration of bacterium. (Chagas *et al.*, 2001, Urbaneja *et al.*, 2003; Chagas *et al.*, 2001 and Graham, 2004; Khair, 2004). CLM is found throughout the year but its passion increases during new flushes of the citrus (Barnet *et al.*, 2005). *Phyllocnistis citrella* (Stainton) produces silvery mines on the surface of fruits, leaf and stem and reduces the excellence of fruit and photosynthetic area of the leaf which eventually reduces quantity of the produce (Abdella and Mohamed, 2004; Belasque *et al.*, 2005). The larvae of CLM causes smash up by making zigzag shaped mines in the young leaves between the upper and lower epidermal layers, eating the parenchymatic tissue (Raga *et al.*, 2001). The offended epidermis took the shape of twisted silvery galleries (Legaspi *et al.*, 2001). On the older leaves, brownish patches fashioned, which served as foci of infection for citrus canker. The attacked leaves were twisted or folded over but remained on plants for a long time and the damage gradually spread to fresh leaves (Belasque *et al.*, 2005). Heavily attacked plants could be observed from a distance and young nurseries are most

severely affected. Mining can reduce the plant growth of the young trees and nursery stock (Rogers and Stansly, 2007). CLM can also cause mining of citrus fruit rind but it occurs once in a blue moon (Elzbeth *et al.*, 2008). Impulsive invasion and wide dispersion of *phyllocnists citrilla* in Pakistan creates problem to the formers and researchers. So the present study was conducted to search out the source of resistance among the citrus germplasm because it provides us the best management strategy against citrus leaf miner because different cultivars possessing different rate of tolerance and susceptibility towards CLM (Khair, 2004).

### MATERIALS AND METHODS

Fifteen citrus cultivars Blood red (*Citrus sinensis* cv. blood red), Malta (*Citrus reticulata* cv. Malta), China lemon (*Citrus limonia* cv. china lemon), Mayer lemon (*Citrus limonia* cv. mayer lemon), Sweet lemon (*Citrus limettioides*), Feutral's early (*Citrus reticulata* cv. feutral's early), Jaffa (*Citrus sinensis* cv. Jaffa), Succari (*Citrus sinensis* cv. Succari), Mungal singh (*Citrus reticulata* cv. mungal singh), Grapefruit (*Citrus paradise*), Tangerine (*Citrus reticulata* cv. Tangerine), Musambi (*Citrus sinensis* cv. Succari), Pine apple (*Citrus sinensis* cv. pine apple), Valentia late (*Citrus sinensis* cv. valentia late) and Kinnow (*Citrus reticulata* cv. Kinnow) were collected from Horticulture nursery of University of Agriculture Faisalabad during 2007 for evaluating their resistance or susceptibility towards CLM under natural environmental conditions by using Augmented design. All the recommended agronomic practices were followed to maintain the citrus nursery in good conditions. However, no insecticides

were used in order to develop maximum insect population pressure. Natural inoculum was selected for infection from the fruit plant nursery of the Horticulture Department. Population infestation was recorded by using Lukshman scale (1998). SAS/STAT statistical software was used to perform all the statistical analysis (SAS Institute, 1990). Fisher's protected least significant difference (LSD) was used to separate the means (Steel *et al.*, 1997).

## RESULTS AND DISCUSSION

Citrus leaf miner was active throughout the year and multiplied on young growth. CLM infected young citrus flush including leaves and young stems. Colonization data for citrus CLM on fifteen the varieties of citrus during 2006-07 were presented in Table 1. The CLM population started increasing during first week of September and became maximum in third week of October; the minimum population was observed in the first week of January. Maximum CLM populations was found on *C. limonia* cv. China lemon, *C. paradise*, *C. sinensis* cv. musambi, *C. limettioides*, *C. sinensis* cv. blood red and *C. limonia* cv. mayer lemon, showing infestation severities of 30.44, 31.21, 31.54, 31.77, 33.73 and 34.27, respectively (Table2). The CLM population occurred on *C. reticulata* cv. kinnow and *C. sinensis* cv. Jaffa, with severities 17.40 and 18.50, respectively. Out of fifteen varieties, 7 were at or above economic threshold levels for CLM populations i.e one or two larvae. CLM were found on all citrus varieties in varying degrees. *C. reticulata* cv. kinnow and *C. sinensis* cv. jaffa had the lowest populations whereas *C. paradise*, *C. limonia* cv. China lemon, *C. paradise*, *C. sinensis* cv. musambi, *C. limettioides*, *C. sinensis* cv. blood red and *C. limonia* cv. mayer lemon developed high CLM populations. CLM populations were higher during 2006-07 than 2007-08.

Different citrus varieties expressed varying response toward CLM population. Varieties such as *C. limonia* cv. China lemon, *C. paradise*, *C. sinensis* cv. musambi, *C. limettioides*, *C. sinensis* cv. blood red and *C. limonia* cv. mayer lemon were most susceptible to CLM attack in both years, whereas the least susceptibility was observed on *C. reticulata* cv. kinnow and *C. sinensis* cv. Jaffa.

There was a significant interaction of year x varieties with development of CLM. Varieties responded differently in preference CLM. The most suitable varieties for maximum development of CLM populations were moderately susceptible to highly susceptible varieties to CLM (Table.1&Table 2). *C. sinensis* cv. jaffa that was moderately susceptible during first year became susceptible during second year. Similarly, *C. reticulata* cv. feutral's early, which was susceptible during 2006-07, became highly susceptible during 2007-08. Peak

populations were observed from February to April and August to October during both years. Maximum CLM counts were observed on the variety *C. limonia* cv. mayer lemon. Overall *C. reticulata* cv. kinnow was the most resistant variety tested. and *C. sinensis* cv. blood red, *C. limonia* cv. mayer lemon, *C. limettioides*, *C. paradise* and *C. limonia* cv. China lemon were the most susceptible varieties.

Maximum population infestation was observed during the 2<sup>nd</sup> week of September and third week of April, whereas the minimum population infestation occurred during the third week of January. Population infestation increased from February to April and then decreased and it again increased from the first week of July to the last week of October during both the years. The significant interaction of years x varieties showed that CLM populations varied among citrus varieties during both years. The level of CLM infestation was highest during 2007-08, with a mean population incidence of 38.38, as compared to the highest population incidence of 34.27 in 2006-07.

CLM is the most destructive insect of citrus which reduce the quality and quantity of the citrus fruit (Urbaneja *et al.*, 2003). In the present experiment DMR test showed variable degree of susceptibility among citrus cultivars. Genetic resistance probably is the only durable and long lasting solution to the CLM (Munoz *et al.*, 2008). A possible solution to the insect problem, therefore, is the transfer of resistant genes to most of citrus varieties, which obviously will require long time period (Reference). The short-term solution should be screening of available germplasm (Shevankar *et al.*, 2000) for relative susceptibility, as in this study and to identify low rating variations for breeding manipulation.

Among the citrus varieties tested against CLM, *C. paradise* exhibited high susceptibility while *C. reticulata* cv. kinnow and *C. sinensis* cv. jaffa showed moderately susceptible response towards CLM. Similar results were reported by El-Dessouki (2001) and Xiao *et al.*, (2007). Citrus varieties vary in leaf thickness which is an important criteria for CLM attack (Fahim, 2001 and Khair, 2004). *Citrus limonia* cv. mayer lemon has low value of leaf thickness as compared to other varieties tested which should be the main reason of high infestation rate of CLM. The higher degree of susceptibility among citrus cultivars may be induced due to certain anatomical modifications that can increase or decrease interaction between citrus cultivars and CLM (Hare 1992, Gassmann and Hare 2005, Muller and Riederer 2005, Mathews *et al.*, 2007) or it may be due to certain type of metabolic changes (Smith and Boyko 2007). Certain types of chemical compounds in citrus cultivars may act as repellent or attractant for CLM which induce differential resistance or susceptibility in citrus cultivars for CLM attack. (Isman 2000, Rocchini *et al.*, 2000, and Ode 2006). To elucidate this, further

research on morpho-anatomical and physiological features are necessary.

**Table 1. Screening and grading of 15 citrus varieties/ cultivars against citrus leaf miner in 2006-07**

Varieties/ Cultivars	Population incidence (Mean)	Response
<i>Citrus reticulata</i> cv. kinnow	17.40 <sup>i</sup>	MS
<i>Citrus sinensis</i> cv. jaffa	18.50 <sup>i</sup>	MS
<i>Citrus sinensis</i> cv. pine apple	20.16 <sup>h</sup>	S
<i>Citrus sinensis</i> cv. succari	20.88 <sup>gh</sup>	S
<i>Citrus reticulata</i> cv. mungal singh	21.75 <sup>g</sup>	S
<i>Citrus reticulata</i> cv. tangerine	23.47 <sup>f</sup>	S
<i>Citrus reticulata</i> cv. malta	26.33 <sup>e</sup>	S
<i>Citrus sinensis</i> cv. valentia late	27.99 <sup>d</sup>	S
<i>Citrus reticulata</i> cv. feutral's early	29.27 <sup>cd</sup>	S
<i>Citrus limonia</i> cv. china lemon	30.44 <sup>bc</sup>	HS
<i>Citrus paradise</i>	31.21 <sup>b</sup>	HS
<i>Citrus sinensis</i> cv. musambi	31.54 <sup>b</sup>	HS
<i>Citrus limettioides</i>	31.77 <sup>b</sup>	HS
<i>Citrus sinensis</i> cv. blood red	33.73 <sup>a</sup>	HS
<i>Citrus limonia</i> cv. mayer lemon	34.27 <sup>a</sup>	HS

LSD = 1.39

**Table 2. Screening and grading of 15 citrus varieties/ cultivars against citrus leaf miner in 2007-08**

Varieties/ Cultivars	Population incidence (Mean)	Population incidence (Mean)
<i>Citrus reticulata</i> cv. kinnow	18.93 <sup>i</sup>	MS
<i>Citrus sinensis</i> cv. jaffa	20.86 <sup>h</sup>	S
<i>Citrus sinensis</i> cv. pine apple	22.10 <sup>h</sup>	S
<i>Citrus sinensis</i> cv. succari	22.17 <sup>h</sup>	S
<i>Citrus reticulata</i> cv. mungal singh	24.21 <sup>g</sup>	S
<i>Citrus reticulata</i> cv. tangerine	25.70 <sup>f</sup>	S
<i>Citrus reticulata</i> cv. malta	27.08 <sup>f</sup>	S
<i>Citrus sinensis</i> cv. valentia late	28.86 <sup>e</sup>	S
<i>Citrus reticulata</i> cv. feutral's early	31.65 <sup>d</sup>	HS
<i>Citrus limettioides</i>	32.57 <sup>d</sup>	HS
<i>Citrus paradise</i>	34.35 <sup>c</sup>	HS
<i>Citrus sinensis</i> cv. musambi	35.69 <sup>bc</sup>	HS
<i>Citrus limonia</i> cv. china lemon	36.54 <sup>b</sup>	HS
<i>Citrus sinensis</i> cv. blood red	36.64 <sup>b</sup>	HS
<i>Citrus limonia</i> cv. mayer lemon	38.38 <sup>a</sup>	HS

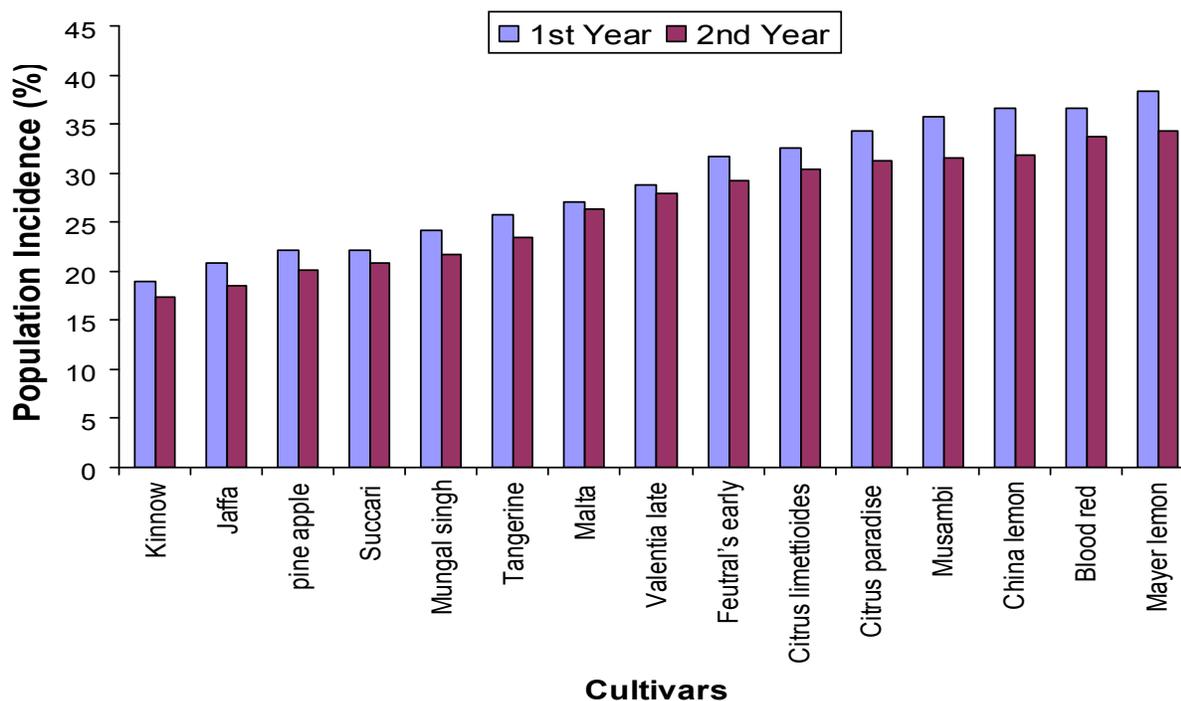
LSD = 1.47

\*Mean values in a column sharing similar letters do not differ significantly as determined by LSD test  $P \leq 0.05$ .

MS = Moderately susceptible S

= Susceptible

HS = Highly susceptible



**Fig. Comparison of genetic response of citrus cultivars against citrus leafminer(CLM)**

## REFERENCES

Abdella, T. E and E. S. I. Mohammed (2004). Guidelines for Testing Insecticides Against the Citrus Leafminer. Proceedings of 70th Pests and

Diseases Committee. Crop Protection Centre. ARC. Wad madani.

Atiq, M. (2007). Prediction of Citrus canker disease and its management. Ph.D. Thesis. Deptt.of Plant Path.Uni.Agriculture Faisalabad.

- Barnet, G. P., C. Margiay, J. Jacas, E. A. Carbonell and M. J. Asins (2005). Genetic analysis of citrus leafminer susceptibility. TAG.110 (8):1393-1400.
- Belaque, J. J., A. L. Parra-Pedrazzoli, J. Rodrigues Neto, P. T. Yamamoto, M. C. M. Chagas, J. R. P. Parra, B. T. Vinyrad, and J. S. Hratung (2005). Adult citrus leafminer (*Phyllocnistis citrella*) are not efficient vectors for *Xanthomonas axonopodis* pv *citri*. Plant Dis. 89: 594-594.
- Bermudez, E. C., N. B. Martinez, J. V. Graziano, H. C. A. Bernal and A. H. Paniagua (2004). *Phyllocnistis citrella* (Lepidoptera: gracillariidae) and its parasitoids in citrus in Ecuador. Florida Entomologist 87:10-17.
- Chagas, M. C. M., J. R. P. Parra, T. Namekata, J. S. Hartung and P. T. Yamamoto, (2001). *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) and its relationship with the citrus canker bacterium *Xanthomonas axonopodis* pv *citri* in Brazil. Neotrop. Entomol. 30:1.
- El-Dessouki, S. A., A. S. El-Khouly, M. W. El-Kordy and I. E. Abdel Rhman (2001). Host preference and seasonal fluctuation of citrus leafminer, *Phyllocnistis citrella* population preferred host in relation to its parasitoids and weather factors. Ann. Agric. Sci., Moshtor.43 (2): 895-901.
- Fahim, S. M. E. M. (2001). Biological and Ecological Studies on The Citrus leafminer *Phyllocnistis citrella* Staint. Thesis for the degree of M.Sc., Deptt. of Plant. Protec. Cairo Uni. Egypt.
- Gassmann, A. J. and J. D. Hare (2005). Indirect cost of a defensive trait: variation in trichome type affects the natural enemies of herbivorous insects on *Datura wrightii*. Oecologia (Berl.) 144: 62-71.
- Graham, J. H., T. R. Gottwald, J. Cubero and D. S. Achor (2004). *Xanthomonas axonopodis* pv *citri*: factors affecting successful eradication of citrus canker. Mol. Plant Pathol. 5: 1-15.
- Hoy, M. A. and C. Jessey (2004). *Ageniapis citricola* (Hymenoptera: Encyrtidae) established in Bermuda. Florida Entomologist 87:229-230.
- Isman, M. B. (2000). Plant essential oils for pest and disease management. Crop. Protect. 19: 603-608.
- Khair, S. M. (2004). Arthropods Pests of Citrus in Sudan. Workshop on Citrus Production in Sudan Present and Future. Ministry of Agriculture and Forests. Khartoum, Sudan.
- Legaspi, J. C., J. V. French, A. G. Zuniga and B. C. Legaspi (2001). Population Dynamics of the Citrus leafminer *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), and Its Natural Enemies in Texas and Mexico. Biological Control 21: 84-90.
- Luckshman., K. M. (1998). Some studies on citrus leafminer. M.Sc.Thesis. Deptt. Of Entomol. Sindh Agri. Uni. Tadojam.
- Mathews, C. R., M. W. Brown and D. G. Bottrell (2007). Leaf extract oral nectaries enhance biological control of a key economic pest *Grapholita molesta* (Lepidoptera: Tortricidae) in peach (Rosales: Rosaceae). Environ. Entomol.36: 383-389.
- Muller, C. and M. Riederer (2005). Plant surface properties in chemical ecology. J. Chem. Ecol. 31: 2621-2651.
- Munaz., S. T, A. H. Garcia, M. J. Perez, J. R. Boyero and E. M. Ferri (2008). Effect of rootstock and flushing on the incidence of three insects on Clementine de Nules Citrus trees. Environ. Entomol. 37(6):1531-1537.
- Raga, A., M. E. Satol, M. F. Souza and R. C. Siloto (2001). Comparison of spray insecticides against citrus leaf miner. Arq. Inst. Biol. 68: 77-82
- Rocchini, L. A., B. S. Lindgren and R. G. Bennett (2000). Effects of resin flow and monoterpene composition on susceptibility of lodgepole pine to attack by Douglas-fir pitch moth, *Synanthedon novaroensis* (Lepidoptera: Sesiidae). J. Appl. Entomol. 124: 87-92.
- Rogers, M. E. and P. A. Stansly (2007). Florida citrus pest management guidelines: Asian citrus psyllid and citrus leafminer. Florida Cooperative Extension Service, Institute of Food and Agriculture Sciences, University of Florida. ENY-734.
- Shevankar., V. J. C. N. Rao and Shyam Singh (2000). Citrus leafminer (*Phyllocnistis citrella*) management. A review. Agric. Rev. 21 (3): 205-210.
- Smith, C. M., and E. V. Boyko (2007). The molecular bases of plant resistance and defense responses to aphid feeding: current status. Entomol. Exp. Appl. 122: 1D16.
- Urbaneja, A., E. Lacer, A. Garrido and J. A. Jacas (2003). Interspecific competition between two ectoparasitoids of *Phyllocnistis citrella* (Lepidoptera: Gracillariidae). *Cirrospilus brevis* and the exotic *Quadrastichus* Sp. Biol. Con., 28: 243-250.
- Xiao, Y., J. A. Qureshi and P. A. Stansly (2007). Contribution of predation and parasitism to mortality of citrus leaf miner *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) Populations in Florida. Biol. Con., 40: 396-404.