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Effect of exclusion net and apple variety on the population density of leaf-mining moths

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Abstract

Leaf-mining moths are known as pests on many fruit trees. With intensifying the chemical control in commercial orchards, their economic importance has increased. Exclusion net proved to be a promising alternative to chemical insecticides in suppressing the population densities of some apple pests. The study aimed to assess the effect of exclusion net on the rate of infestation by leaf-mining moths in the region of Plovdiv and their preference for some new apple varieties. The experiment was set up in two locations (in Plovdiv and the village of Brestnik) each with four rows of apple trees – two rows with exclusion net and two without net. In 2020 on the leaves of 8 apple varieties a total of 5 species of leaf-mining moths were observed: 2 species from the Lyonetiidae family - *Leucoptera malifoliella* and *Lyonetia clerkella*; 2 species from the Gracillariidae family - *Phyllonorycter blancardella* and *Phyllonorycter corylifoliella*, and 1 species from the Nepticulidae family - *Stigmella malella* with *Phyllonorycter blancardella* and *Stigmella malella* being predominant in both sites. Adult moths showed a pronounced preference for laying eggs on the leaves of the Crimson Crisp, Rosella and SuperChief varieties, in the open and under exclusion net. The new varieties Enterprise, Pinova and Fujion were the least preferred. In both sites, the rate of infestation was higher on the apple trees with exclusion net.

Keywords: *exclusion net, Phyllonorycter blancardella, Stigmella malella, apple varieties*

INTRODUCTION

Apple (*Malus domestica* Borkh.) is one of the most important fruit crops in the temperate climatic zones of the world (Zamljen et al., 2021; Kamusiime et al., 2023). The total area of apple orchards is 4.6 million hectares, and the annual yield is about 86.5 million tons of apples (Ismailov et al., 2023). Insects and mites cause significant economic losses to commercial fruit growers (Zhou et al., 2014). Conventional orchards that produce apples are highly managed agricultural ecosystems that heavily rely on pesticides to minimize pest-related losses to fruit quality or yield. Insect net covers are a promising tool, reducing damage on crops by excluding pests (Bastnas & Boini, 2022). Netting systems can increase the sustainability

of apple production, limiting the use of resources, from water to chemical treatments for pest control (Böckmann, 2022). Nets are widely used in crop production as barriers against abiotic and biotic factors that compromise crop productivity and fruit quality (George et al., 2004, Castellano et al., 2008, Sivakumar & Jifon, 2018). Nets also reduce farmer reliance on agrochemicals, making them an environmentally-friendly alternative to chemical pesticides (Briassoulis et al., 2007). Whether or not such netting can also be used to exclude insect pests, protect fruit, and ultimately reduce insecticide use, has been recently investigated by several authors. Over the last 20 years, the suppression effect of exclusion netting used in pear (*Pyrus* sp.) and apple orchards on codling moth reproductive behavior, and their subsequent

ability to infest fruit, has been thoroughly documented (Alaphilippe et al., 2016). Nets for hail protection were shown to reduce the invasion of *Cydia pomonella* and *Adoxophyes orana* into apple orchards (Graf et al., 1999), additionally, Tasin et al. (2008) found that mating behavior was disrupted in *C. pomonella* below these nets. According to Rigden et al. (2008) agricultural nets successfully restrict insect pests, such as *Othreis fullonia* and *Othreis maternal* (Lepidoptera). In Italy codling moth (*Cydia pomonella*) damages were reduced to almost 100% (Kelderer et al., 2010). Another research in Italy showed reduction of pest populations of *Halyomorpha halys* (Stål) and *Drosophila suzukii* (Matsumura) under the net. Damaged fruits by *Halyomorpha halys* were reduced to 78% (Candian et al., 2020, 2021). In Canada exclusion nets reduced damages from *Cydia pomonella*, *Rhagoletis pomonella* and *Lygus lineolaris* (Chouinard et al., 2017). In Minnesota, in 2021 and 2022, a study assessed the efficacy of hail netting by comparing the presence of 3 pest species in netted and open plots - the codling moth, apple maggot, and red-banded leafroller (*Argyrotaenia velutinana* Walker, *Lepidoptera: Tortricidae*) (Nelson et al., 2023).

However, because in most crops, the nets must be removed to manage weeds and to apply fertilizer, a complete exclusion of pests is not always feasible. In addition, development of pests from belowground developmental stages beneath the covered area and infested seedlings set limitations to this kind of protection. In addition, net covers also have an impact on natural enemies. Because natural enemies are also excluded (Dib et al., 2010), invasion of pests under net covers can potentially cause increased population build up. Loyd et al. (2003) observed few problems with using orchard netting. Under the net over 50% of shoot tips were affected by *Grapholita molesta* (*Lepidoptera: Tortricidae*). The possible reasons for this negative effect are: the

parasitoids and predators of this pest are excluded or the life cycle of the moth is shortened due to the warmer conditions under the net.

It has been observed in previous studies that there is potential for outbreaks of secondary pests such as the European red mite, aphids, and leafrollers under netting systems (Marshall & Beer 2021, 2022; Chouinard et al. 2022). Despite the fact that complete exclusion nets are capable of excluding some major pests, Alaphilippe et al. (2016) reported that netting enhanced the development of *Dysaphis plantaginea*, *Eriosoma lanigerum* and *Adoxophyes orana* among others. Aoun (2016) and Aoun et al. (2013) also reported the abundance of *Aphis pomi* and *Dysaphis plantaginea* colonies and damages under single row netting compared to full block netting and control. This may be due to an increase in humidity under nets or to the exclusion of beneficial insects that normally keep the aphids under control through predation.

Therefore, effects of net applications have to be assessed accordingly, depending on the specific crops and pests.

The leaf-mining moths are considered indirect pests in apple orchards as they damage a non-marketable plant organ (Sherwani et al., 2016). Nevertheless, at high population density, they may cause early defoliation of the apple trees, which negatively affects bud differentiation. Fruits are not attacked but heavy leaf loss leads to a reduction in the yield for the next year (Bajec et al., 2009; Liu et al., 2015; Šubić, 2015; Lopez-Vaamonde et al., 2021; Čirjak et al., 2022).

The main objectives of this study were to assess the preference of the leaf-mining moths, common for the region of Plovdiv, to new apple varieties and the effect of exclusion net coverage on their population density.



Figure 1. Experimental plot in Brestnik – exclusion net on metal construction (a) and satellite picture of the apple rows with net and in the open (b)

MATERIALS AND METHODS

Experimental Design

During 2020 and 2021, field trials were conducted at 2 identical apple orchards in the experimental fields of the Agricultural University – Plovdiv. Orchards were located in Plovdiv (42.132953, 24.768614) and in the village of Brestnik (42.060600, 24.783388). The trees were planted with an average spacing of 2 m between the rows and 2 m in-between the trees in the row, a total of 125 trees per ha. In each of the locations, two rows were covered with insect net (with supporting metal structure), and two - without insect net (Fig. 1). The nets were deployed after full bloom until harvest, permitting better insect pollination during the flowering period.

The studies were conducted on 8 apple varieties, with all trees planted in 2019 and at the beginning of fruit-bearing age at the time of this study. Five of the apple varieties were new for Bulgaria: Enterprise, Modi, Gala Resistant (Gemini), Pinova and Fujion, and three – well-established: SuperChief, Rosella and Crimson Crisp.

Insect Monitoring

Visual observations and leaf sampling methods were used for the identification of the leaf-mining moths. For estimating the average

population density, all the leaves of 4 trees of each variety were counted and the number of leaves with mines was recorded. The average population density estimated as the rate of infestation, was calculated based on the number of leaf mines recorded on each examined tree at the end of August 2020. The data were statistically processed by Microsoft Excel 365.

RESULTS AND DISCUSSION

Five species of leaf-mining moths (Table 1) were observed in both apple orchards in the region of Plovdiv and in Brestnik: 2 species from the Lyonetiidae family – *Leucoptera malifoliella* and *Lyonetia clerkella*; 2 species from the Gracillariidae family – *Phyllonorycter blancardella* and *Phyllonorycter corylifoliella*, and 1 species from the Nepticulidae family – *Stigmella malella* (fig. 2).

The results obtained for the rate of infestation and preference to apple varieties in the two experimental orchards were very similar and were analyzed jointly. The leaf-mining moths are considered as secondary pests, a results of intensive insecticide treatments, which kill their natural enemies. Therefore, it is important to underline that in both experimental orchards chemical insecticides were not applied during the observations.

Table 1. Species composition of the leaf-mining moths on apple in Plovdiv and Brestnik in 2020

Species	Family	Order
<i>Leucoptera malifoliella</i> (Costa, 1836)	<i>Lyonetiidae</i>	<i>Lepidoptera</i>
<i>Lyonetia clerkella</i> (Linnaeus, 1758)	<i>Lyonetiidae</i>	<i>Lepidoptera</i>
<i>Phyllonorycter blancardella</i> (Fabricius, 1781)	<i>Gracillariidae</i>	<i>Lepidoptera</i>
<i>Phyllonorycter corylifoliella</i> (Hubner, 1796)	<i>Gracillariidae</i>	<i>Lepidoptera</i>
<i>Stigmella malella</i> (Stainton, 1854)	<i>Nepticulidae</i>	<i>Lepidoptera</i>

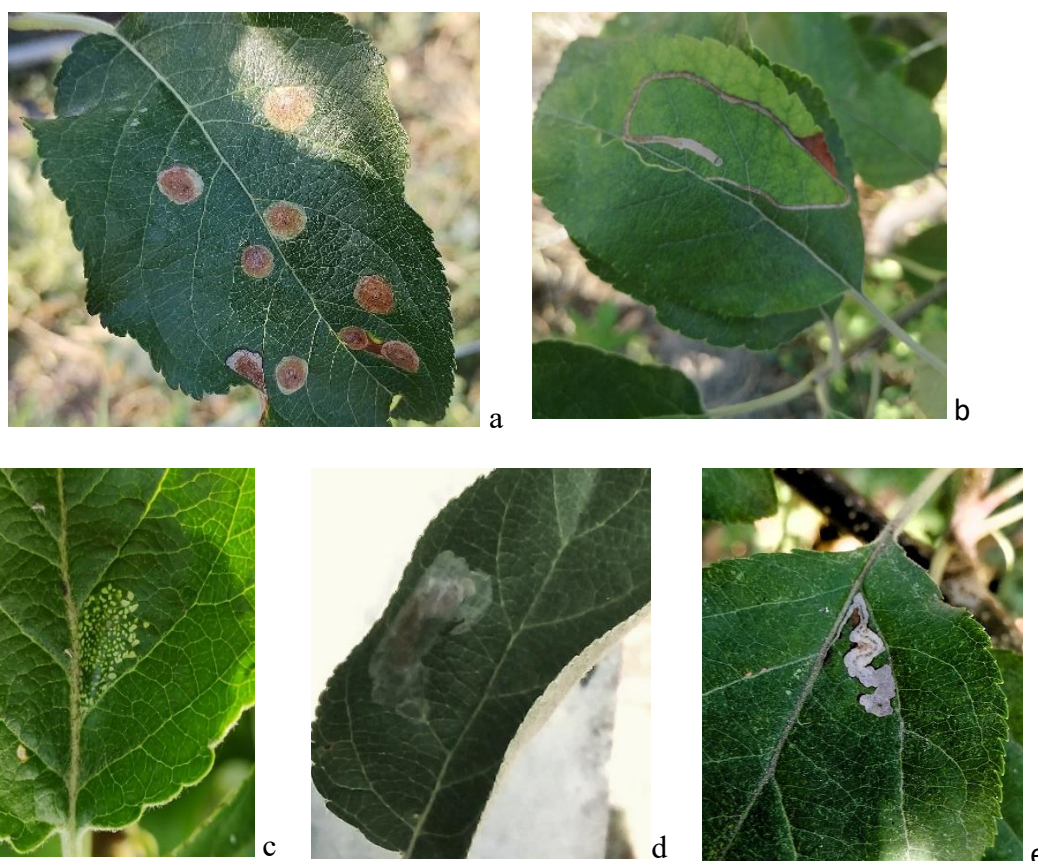


Figure 2. Mines of *Leucoptera malifoliella* (a), *Lyonetia clerkella* (b), *Phyllonorycter blancardella* (c), *Phyllonorycter corylifoliella* (d) and *Stigmella malella* (e)

The average population density for all the species was quite low – from 0.05% of leaves with mines of *L. clerkella* to 3% of leaves with mines of *Ph. blancardella* (Fig. 3). Of the leaf-mining moths, *Ph. blancardella* and *S. malella* were the most abundant with an average rate of infestation (leaves with mines in %) being 3 and 2.59 under exclusion net, and in the open, respectively (Fig. 3). The least numerous were *L. clerkella* and *Ph. corylifoliella*. *Leucoptera malifoliella*, which usually is the predominant

species among the leaf-mining moths in the apple orchards in the region of Plovdiv, was much less numerous with an average density of 0.87% and 0.5% leaf infestation under exclusion net and in the open.

All the leaf-mining species were observed in lower population density on the trees in the open, compared to the trees under exclusion net, except for *Ph. corylifoliella*, the population density of which was slightly higher in the open.

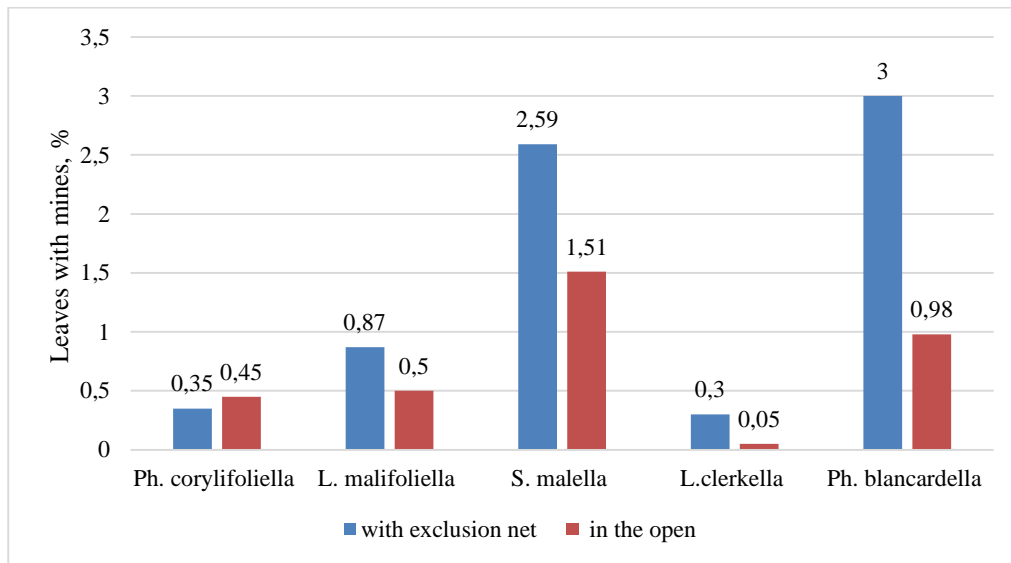


Figure 3. Average population density (% of leaves with mines) of the different species of leaf-mining moths in the region of Plovdiv in 2020, on apple trees with exclusion net and in the open

The data on the rate of infestation on the leaves of the tested apple varieties in 2020 showed a pronounced preference of the leaf-mining moths for laying eggs (respectively development of larvae) on the well-established apple varieties Crimson Crisp, Rosella and SuperChief (Fig. 4). The new for Bulgaria apple varieties Enterprise, Pinova and Fujion were the least preferred, both under the net and in the open. The other two new varieties – Modi and

Gala Resistant occupy an intermediate position in terms of rate of infestation.

On all the varieties, the rate of infestation by leaf-mining moths was higher on the trees under exclusion net, which could be a results of the restricted access of birds, which are one of the largest predators of insects, and limited access of larval parasitoids of leaf-mining moths.

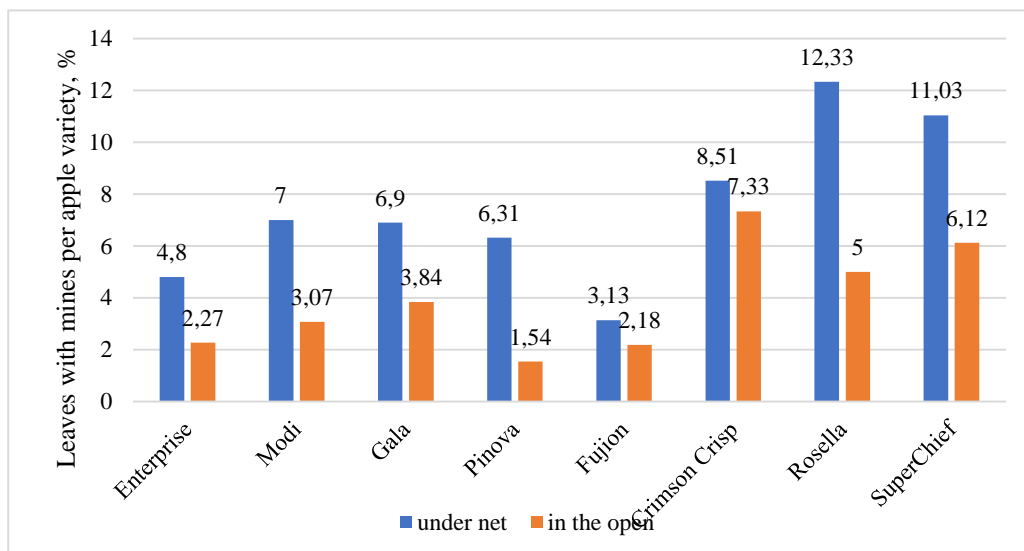


Figure 4. Preference of leaf-mining moths to new and established apple varieties in the region of Plovdiv in 2020, on apple trees with exclusion net and in the open

CONCLUSIONS

Among the leaf-mining moths, damaging leaves of apple in the experimental orchards in the region of Plovdiv, where no chemical insecticides were applied, *Phyllonorycter blancardella* and *Stigmella malella* were the most abundant species, while *Lyonetia clerkella* and *Phyllonorycter corylifoliella* were found only occasionally. The pear leaf blister moth (*Leucoptera malifoliella*) which is economically the most important leaf-mining species in orchards was in moderate population density. The population density of the leaf-mining moths was higher on the apple trees under the exclusion net compared to the trees in the open in both locations – in Plovdiv and Brestnik. The new apple varieties Enterprise, Pinova and Fujion were the least preferred for egg-laying by the leaf-mining moths. A preference for the leaves of the varieties Crimson Crisp, Rosella and SuperChief, was observed both under the net and in the open.

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REFERENCES

- Alaphilippe, A., Capowiez, Y., Severac, G., Simon, S., Saudreau, M., Caruso, S., & Vergnani, S. (2016). Codling moth exclusion netting: an overview of French and Italian experiences. *IOBC-WPRS Bull*, 112, 31-35. <https://doi.org/10.1016/j.biocontrol.2021.104805>
- Amarante, C. D., Steffens, C. A., & Argenta, L. C. (2010). Radiation, yield, and fruit quality of 'Gala' apples grown under white hail protection nets. In *XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010): International Symposium on 934* (pp. 1067-1074). <https://doi.org/10.17660/ActaHortic.2012.934.143>
- Beneficial arthropods, fungal diseases and fruit quality. *Pest management science*, 76(1), 179-187. <https://doi.org/10.1002/ps.5491>
- Bergmeyer, H. U. (1974). Enzymes as biochemical reagents. *Methods of enzymatic analysis*, 1, 473-474.
- Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*, 28(1), 25-30. [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5)
- Briassoulis, D., Mistriotis, A., & Eleftherakis, D. (2007). Mechanical behaviour and properties of agricultural nets – Part I: Testing methods for agricultural nets. *Polymer Testing*, 26(6), 822-832. <https://doi.org/10.1016/j.polymertesting.2007.05.007>
- Brueske, C. H. (1980). Phenylalanine ammonia-lyase activity in tomato roots infected and resistant to the root-knot nematode, *Meloidogyne incognita*. *Physiological Plant Pathology*, 16(3), 409-414. [https://doi.org/10.1016/S0048-4059\(80\)80012-9](https://doi.org/10.1016/S0048-4059(80)80012-9)
- Cai, Q. N., Zhang, Q. W., & Cheo, M. (2004). Contribution of indole alkaloids to *Sitobion avenae* (F.) resistance in wheat. *Journal of Applied Entomology*, 128(8), 517-521. <https://doi.org/10.1111/j.1439-0418.2004.00770.x>
- Candian, V., Pansa, M. G., Santoro, K., Spadaro, D., Tavella, L., & Tedeschi, R.

- (2020). Photosensitive exclusion netting in apple orchards: Effectiveness against pests and impact on beneficial arthropods, fungal diseases and fruit quality. *Pest management science*, 76(1), 179-187. <https://doi.org/10.1002/ps.5491>
- Czerniewicz, P., Sytykiewicz, H., Durak, R., Borowiak-Sobkowiak, B., & Chrzanowski, G. (2017). Role of phenolic compounds during antioxidative responses of winter triticale to aphid and beetle attack. *Plant Physiology and Biochemistry*, 118, 529-540. <https://doi.org/10.1016/j.plaphy.2017.07.024>
- Dixon, R. A., Achnine, L., Kota, P., Liu, C. J., Reddy, M. S., & Wang, L. (2002). The phenylpropanoid pathway and plant defence – a genomics perspective. *Molecular plant pathology*, 3(5), 371-390. <https://doi.org/10.1046/j.1364-3703.2002.00131.x>
- Duan, L., Liu, H., Li, X., Xiao, J., & Wang, S. (2014). Multiple phytohormones and phytoalexins are involved in disease resistance to *Magnaporthe oryzae* invaded from roots in rice. *Physiologia plantarum*, 152(3), 486-500. <https://doi.org/10.1111/ppl.12192>
- Han, Y., Wang, Y., Bi, J. L., Yang, X. Q., Huang, Y., Zhao, X., ... & Cai, Q. N. (2009). Constitutive and induced activities of defense-related enzymes in aphid-resistant and aphid-susceptible cultivars of wheat. *Journal of chemical ecology*, 35, 176-182. <https://doi.org/10.1007/s10886-009-9589-5>
- Imberty, A., Goldberg, R., & Catesson, A. M. (1985). Isolation and characterization of *Populus* isoperoxidases involved in the last step of lignin formation. *Planta*, 164(2), 221-226.
- Koleva-Valkova, L., Piperkova, N., Petrov, V., & Vassilev, A. (2017). Biochemical responses of Peach leaves infected with *Taphrina deformans* Berk./Tul. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 65(3), 871-878. <https://doi.org/10.11118/actaun201765030871>
- Manja, K., & Aoun, M. (2019). The use of nets for tree fruit crops and their impact on the production: A review. *Scientia Horticulturae*, 246, 110-122. <https://doi.org/10.1016/j.scienta.2018.10.050>
- Marshall, A. T., & Beers, E. H. (2021). Efficacy and nontarget effects of net exclusion enclosures on apple pest management. *Journal of Economic Entomology*, 114(4), 1681-1689. <https://doi.org/10.1093/jee/toab094>
- Marshall, A. T., & Beers, E. H. (2022). Exclusion netting affects apple arthropod communities. *Biological control*, 165, 104805. <https://doi.org/10.1016/j.biocontrol.2021.104805>
- Morrison, T. A., & Buxton, D. R. (1993). Activity of phenylalanine ammonia-lyase, tyrosine ammonia-lyase, and cinnamyl alcohol dehydrogenase in the maize stalk. *Crop Science*, 33(6), 1264-1268. <https://doi.org/10.2135/cropsci1993.0011183X003300060030x>
- Sauphanor, B., Severac, G., Maugin, S., Toubon, J. F., & Capowicz, Y. (2012). Exclusion netting may alter reproduction of the codling moth (*Cydia pomonella*) and prevent associated fruit damage to apple orchards. *Entomologia Experimentalis et Applicata*, 145(2), 134-142. <https://doi.org/10.1111/j.1570-7458.2012.01320.x>
- Sha, P. J., Fan, Y. J., Wang, Z. C., & Shi, X. Y. (2015). Response dynamics of three defense related enzymes in cotton leaves to the interactive stress of *Helicoverpa armigera* (Hübner) herbivory and omethoate application. *Journal of*

Integrative Agriculture, 14(2), 355-364.
[https://doi.org/10.1016/S2095-3119\(14\)60793-0](https://doi.org/10.1016/S2095-3119(14)60793-0)

[https://doi.org/10.1016/S0308-8146\(98\)00102-2](https://doi.org/10.1016/S0308-8146(98)00102-2)

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture*, 16(3), 144-158.
<https://doi.org/10.5344/ajev.1965.16.3.144>

Sivakumar, D., & Jifon, J. (2018). Influence of photoselective shade nettings on postharvest quality of vegetables. In *Preharvest modulation of postharvest fruit and vegetable quality* (pp. 121-138). Academic press.

Treder, W., Mika, A., Buler, Z., & Klamkowski, K. (2016). Effects of hail nets on orchard light microclimate, apple tree growth, fruiting and fruit quality. *Acta Scientiarum Polonorum Hortorum Cultus*, 15(3), 17-27.

Wang, R., Wang, G. L., & Ning, Y. (2019). PALs: emerging key players in broad-spectrum disease resistance. *Trends in plant science*, 24(9), 785-787.
<https://doi.org/10.1016/j.tplants.2019.06.012>

Warrier, R. R., Paul, M., & Vineetha, M. V. (2013). Estimation of salicylic acid in *Eucalyptus* leaves using spectrophotometric methods. *Genetics and plant physiology*, 3(1-2), 90-97.

Xu, Y., Guo, H., Geng, G., Zhang, Q., & Zhang, S. (2021). Changes in defense-related enzymes and phenolics in resistant and susceptible common wheat cultivars under aphid stress. *Acta Physiologiae Plantarum*, 43, 1-9.
<https://doi.org/10.1007/s11738-021-03207-3>

Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food chemistry*, 64(4), 555-559.