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Citrus Leaf Miner (*Phyllocnistis citrella* Stainton, Lepidptera: Gracillariidae): Biolology and Management: A Review.

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Review Article

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ABSTRACT

The citrus leaf miner (CLM), Phyllocnistis citrella Stainton (Lepidptera: Gracillariidae), is a potentially serious pest of citrus (oranges, mandarins, lemons, limes, grapefruit and other varieties) and related Rutaceae (kumquat and calamondin) and ornamental plants almost worldwide. Several other hosts (Leguminaceae, Lauraceae, Oleaceae etc.) have been reported for CLM, however larvae do not complete their life cycle on these incompatible hosts. CLM is a widespread Asian species, first described from Calcutta, India. It has been a widely distributed major pest in citrus-growing regions of Asia for many years. In the last 20 years, leaf miners have invaded most of the citrus-producing regions of the world, including the Mediterranean Basin and North, Central, and South America. The citrus leaf miner larvae only infest the younger, flushing foliage causing damage in nurseries and new plantings because of which the growth of young trees is retarded. The pest damage has shown a 50% increase in citrus canker in orchards infested with CLM. The total generation period of CLM fluctuates between 13-52 days with 2-10 days for egg hatching, 5-20 days of larval period and 6-22 days of pupal development and a temperature of 30°C is optimal for CLM development. Depending on foliage flushing cycles and weather conditions 6 to 13 generations per year can be expected. Peak pest activity was noticed during September to November months. In the management of this pest chemical control and biological control are the two key tools. The complex of natural enemies attacking CLM include ants, spiders, small parasitic wasps and predators such as lacewings causing up to 90 percent mortality of larvae and pupae. Three of the most effective wasps are Ageniaspis citricola and Cirrospilus quadristriatus and Semiolacher petiolatus. The parasitisation rate was lower in June and July, being higher from August onwards and highest during September month. Unfortunately, the "best" natural enemy may not be found until all natural enemies and their biologies are known. Effective chemical control of CLM is difficult because the larva is protected by leaf cuticle and the pupa is protected by rolled leaf margins. However many pesticides belonging to different chemical groups were tested and found effective in its management. Several reports indicated that, the foliar application provides control for two weeks. Several bio-rational pesticides were tested and found useful in CLM management. The biology and management methods were reviewed in detail in this paper.

INTRODUCTION

The citrus leafminer (CLM), *Phyllocnistis citrella* Stainton (Lepidptera: Gracillariidae), is a potentially serious pest of citrus and related Rutaceae and ornamental plants almost worldwide ^[1,2,3]. *Phyllocnistis citrella*, originally described from India ^[4], was confirmed by Don Davis, a specialist in the Gracillariidae family, at the Smithsonian Institution (USNM). In most parts of southern Asia, Australia, and East Africa, where it is present, it is considered one of the major citrus pests. In late May 1993 the citrus leafminer was discovered in southern Florida, the first record of this pest for Florida, the continental United States, and the New World ^[5]. Similarly its first record from southern and northern Iran, with a dramatic increase and widespread dispersal, was noted in 1961 and 1994, respectively ^[6].

The CLM mines leaves, surface tissue of young shoots and stems, and less frequently the fruit [7]. The pest populations build up is more on new flush. Larval mines are found in greatest number in October through December, but can be seen throughout the year on new flush. Nurseries and newly planted trees will have reduced growth ^[8]. Almost all the citrus orchards were mostly found affected by the pest attack to a greater or lesser extent. Sometimes the indirect damage of CLM is very important. Mining of immature foliage by the larvae can lead to reduced growth rates, yield and mined surfaces serve as foci for the establishment of diseases such as citrus canker. Xanthomonas citri. In the absence of citrus canker, citrus leafminer is a serious pest of rapidly growing immature or pruned trees. But in presence of citrus canker, it is a major pest of both immature and mature trees [9]. Therefore, it is important to select less toxic chemicals against the natural enemies in order to expect both the activity of natural enemies and control effect of insecticides for suppressing the infestation of CLM. Citrus leafminer rarely attacks fruits. Biological control is a promising pest management option against CLM. Recent records indicate that 80 species of parasitoids have been reared from the leafminer worldwide ^[10]. Monitoring is another important tool available to help. Many pheromone traps are available to capture male moths. This is not a good way to monitor for CLM density, but it does reveal the insect's presence or absence from an orchard. Traps should be hung at shoulder height just inside the foliage of the citrus trees, the traps needs to be put out when citrus trees are pushing new growth. Do not prune out CLM damaged leaves because most leaves are still being sources for the tree, and pruning pushes new leaves creating new susceptible growth. Keeping in view the importance of this pest here an attempt is made to review the historical distribution, biology, seasonality and various management methods, which can be suitably adopted in integrated pest management programme.

Origin and Distribution

CLM is a widespread Asian species ^[11] first described from Calcutta, India ^[4]. It has been a widely distributed pest in citrus-growing regions of Asia for many years. In the last two decades, leafminers have invaded most of the citrus-producing regions of the world, including the Mediterranean Basin ^[12] and North, Central, and South America ^[13]. It is known from East Africa - Sudan to Yemen ^[14], through southern Asia -Saudi Arabia to India ^[15] and Indonesia ^[1], north to Hong Kong and China, Philippines ^[16], Taiwan ^[17,18], and southern Japan ^[19]. It is also found in New Guinea and nearby Pacific Islands. It was introduced into Australia before 1940, and by 1995 had spread across the continent ^[20]. CLM also occurs in South Africa and in parts of West Africa (CAB). CLM was first discovered in Florida in May 1993 in several citrus nurseries in Homestead, Florida, other parts of Dade County and spread to all Florida citrus counties. By 1994 it has spread to Alabama, Louisiana and Texas ^[21]. By 1995, the citrus leafminer was discovered in Central America, western Mexico, and several Caribbean islands ^[22]. In 2000, it arrived in southern California from Mexico ^[23], and was first detected in Hawaii on Oahu, spreading to Kauai and Maui in 2001 and Molokai and Hawaii (the Big Island) in 2002 ^[21]. It was first reported from Australia in 1918 and South Africa as early as 1908, from southern and northern Iran in 1961 and 1994, respectively ^[6], in the east Mediterranean region of Turkey in June 1994 ^[24].

Economic Impact

Citrus leafminer larvae feed by creating shallow tunnels, referred to as mines, in young leaves. It is most commonly found on citrus (oranges, mandarins, lemons, limes, grapefruit and other varieties) and closely related plants (kumquat and calamondin) The larvae mine the lower or upper surface of the leaves causing them to curl and look distorted. The CLM larvae only infest the younger, flushing foliage. Adults lay their eggs on both the upper and lower surface of the leaves which are less than 1/2 inch in length. Mature citrus trees (more than 4 years old) generally tolerate leaf damage without any effect on tree growth or fruit yield. Citrus leafminer is likely to cause damage in nurseries and new plantings because the growth of young trees is retarded by CLM infestations. However, even when infestations of CLM are heavy on young

trees, trees are unlikely to die, affect production on mature trees, but this has been demonstrated only on limes ^[22]. Only rarely will CLM form mines on fruit. Another small moth species, called the citrus peelminer, *Marmara gulosa* is more typically found mining within the fruit peal ^[9]. The CLM damage typically occurs on leaves and can be observed on young, tender shoots as well and the total leaf damage was ranged from 12-85% ^[25] and 2-55% ^[26] in citrus and up to 90% on about 200 acres of Persian limes ^[5]. Reports from Australia indicate infestations of one to three mines per leaf, while wetter conditions in other areas, such as Florida, may support more miners per leaf ^[22].

Economic losses due to the citrus leafminer include 1) increased costs for protecting nursery trees and young non-bearing citrus, 2) reduced sales to home gardeners, and 3) increased orchard production costs, either directly, through the use of pesticides (largely ineffective), or indirectly, through treatments that disrupt biological control and Integrated Pest Management programs. Except on limes, the loss of yield on mature trees because of foliage damage has not been demonstrated. leafminer damage increases incidence and intensity of citrus canker lesions in Florida^[9] and in India it has shown a 50% increase in citrus canker in orchards infested with CLM ^[27]. Leaf damage by CLM was negatively correlated with net photosynthesis of trees in an orchard and that leaf area damage and reductions in net photosynthesis due to CLM are related to the number of larvae per leaf as well as to mining duration ^[28]. In case of heavy attack, the growth can be slowed down in young trees, and yield is reduced in mature trees ^[29].

Host Plants

CLM is common on species of citrus and related Rutaceae within its range ^[1]. It is most commonly found on leaves of all citrus, including orange, lemon, lime, tangerine, etc. Other Rutaceae recorded as hosts include:

- Aegle marmelos (L.) Corr. Serv. in India [15],
- Atalantia sp. in the Philippines [16],
- Murraya paniculata (L.) Jack. in India.
- Poncirus trifoliata (L.) Raf. in India [11], and
- Various native Rutaceae in Indonesia [1].

Other reported hosts include

- Jasminum sambac (L.) Aiton (Oleaceae) in India [15],
- mistletoes on citrus (Loranthus sp., Loranthaceae) in the Philippines,
- Pongamia pinnata Pierre (Leguminosae) in India, and
- Alseodaphne semecarpifolia Nees (Lauraceae) in India [30].

Several other hosts have been reported for CLM, but larvae do not complete their life cycle on these incompatible hosts

- Murraya koenigii L. Sprengel (Rutaceae) in India [15],
- Jasminum sp. and Jasminum cinnamomum Kobuski (Oleaceae) in India,
- Dalbergia sissoo Roxb. ex DC (Leguminosae) in India (Latif and Yunus 1951),
- Salix sp. (Salicaceae) in India,
- Grewia asiatica L. (Tiliaceae) in India [30].

Biology and Seasonality

The biology of CLM has been reported by a number of researchers, including Badawy ^[14], Beattie ^[2], Clausen ^[19], Fletcher ^[15], Kalshoven ^[1], and Latif and Yunus ^[30].

Adults of the CLM are too minute to be easily noticed, and are active diurnally and in the evenings. Adults are minute moths with a 4 mm wingspread. It has white and silvery iridescent scales on the forewings, with several black and tan markings, plus a black spot on each wingtip. The hind wings and body are white, with long fringe scales extending from the hind wing margins. In resting pose with wings folded, the moth is much smaller in appearance (about 2.4 mm). The head is very smooth-scaled and white and the haustellum has no basal scales. CLM is most easily detected by its meandering serpentine larval mine, usually on the ventral side of the leaf. Eggs of CLM are laid singly on the underside of host leaves.

Egg eclosion occurs within 2 to 10 days, whereupon larvae immediately enter the leaf and begin feeding. Larvae make serpentine mines on young leaves (sometimes also young shoots), resulting in leaf curling and serious injury. Larvae are minute (1-3 mm), translucent greenish-yellow, protected within the leaf mine during their feeding cycle having four instars and development takes from 5 to 20 days. Pupation is within the mine in a special pupal cell at the leaf margin, under a slight curl of the leaf. Pupal development takes 6 to 22 days. Adults emerge about dawn and are active in the morning. Adults live for only a few days. In Florida, a new generation is produced about every three weeks is at dusk or night. Females lay eggs evenings and at night ^[2,14].

Leaf miners have a short developmental time. The total generation period of CLM fluctuates between 13-52 days ^[31] with 2-10 days for egg hatching, 5-20 days of larval period and 6-22 days of pupal development ^[32]. The length of the life cycle varies with temperature, averaging about 17 days at 25°C (Marjorie and Nguyen, 1997). Generally the developmental time (egg to adult) of CLM decreased with increasing temperatures, ranging from 51.7 days at 15°C to 10.1 days at 35°C. The immature mortality was highest at 15°C and lowest at 30°C. No eggs were deposited at 15°C. Net reproductive rate (Ra) was the highest at 30°C with 50.19 female/ female and a temperature of 30°C is optimal for CLM development ^[33].

Infestation levels of 1-3 mines per leaf occur in Australia. In Florida, which has a much wetter climate, infestations of 15-20 miners per leaf are common. Generations per year appear to be nearly continuous: 6 in southern Japan ^[11], 9 to 13 in north central India ^[34] 10 in southern India ^[31]. Depending on foliage flushing cycles and weather conditions as many as 6 to 13 can be expected ^[22]. The pest has about 5-9 generations in a year, with peak periods in early summer and early autumn ^[6].

Population establishment does not depend exclusively on the existence of resources, but also on suitable climatic conditions. This was evidenced by the absence of attacks on the first shooting, which began in late winter. Meteorological factors and resource availability as a whole explain about 64% and 53% of the observed variation in the population size of CLM, respectively in the organic and conventional orchards ^[35]. CLM populations increased during spring and summer, declined during fall and disappeared in the winter [36]. Three population peaks were observed, First in April (spring flush), small second peak in July and third peak in September (autumn flush). These peaks so obtained coincided with the availability of new flush and the percentage infestation was correlated significantly and positively with maximum and minimum temperatures and average rainfall in Jammu region ^[37]. CLM populations were highest during the warmer months (April to September) and lowest during the cooler months (November to March). Populations peaked during June in Florida (Charles, 2007). CLM was active throughout the year especially on fresh growth ^[38]. The heaviest CLM infestation 55% and 49% was recorded at Charbagh and Palai areas of Pakistan in October ^[26]. In Mexico peak pest activity was noticed during July and October months with on an average of 20% parasitisation ^[39]. Similarly Rehman and Yunus ^[40] reported that peak periods of infestation of CLM occurred from March-May and again from September to November in the former western Punjab and during the end of February on acid lime in Andhra Pradesh^[38]. Pandey and Pandey^[31] Khanna and Pandey^[41] observed increased activity of leaf miner from August to November. CLM mines are found in greatest numbers in October through December, but can be found throughout the year on new flush growth.

Management

Cultural Practices

CLM populations could be partially suppressed if tree flushing patterns could be modified so that long intervals occurred without flushes. Unfortunately, it is impossible to achieve this by altering irrigation and fertilization practices under subtropical climatic conditions because summer rainfall is abundant. In irrigated citrus production regions, this tactic may be feasible. Other management options, such as host plant resistance, were not feasible over the short term and there is no clear evidence that any varieties are intrinsically resistant to attack by the CLM. Do not prune CLM damaged leaves because this causes off-season new flush growth that provides sit for CLM oviposition and remove water shoots because these shoots produce young leaf growth on which CLM can produce. The next alternative is the use of pheromone technology. There are several advantages of using pheromone-based mating disruption to control CLM. 1) It has no negative impact on biological control, 2) it has no negative impact on the environment, and 3) it prevents mating, thus controlling the pest before it damages the leaf. A pheromone to attract males of CLM was developed in Japan by Ando et al ^[42], called (7Z, 11Z)-7, 11-hexadecadienal.

Traps containing the pheromone are available and should be used to determine when the moths are flying ^[43]. Monitoring should begin when 50% of the trees are actively flushing, usually February through May and September through October. A recent study also reported on the utility of traps baited with a commercially produced synthetic sex pheromone lure of *P. citrella* for monitoring the pest in Florida^[44].

Biological Control

Biological control of the CLM is one of the key tools in controlling it. Natural enemies of CLM include small parasitic wasps and predators such as lacewings. The predators are generally associated with heavy infestations. Three of the most effective wasps are Ageniaspis citricola and Cirrospilus quadristriatus (both introduced from South-East Asia in 1990-92) and Semiolacher petiolatus, a native species. Parasitism by other wasps native to Australia (Cirrospilus near ingenuus, Symplesis sp. and Zaommomentedon brevipetiolatus) has also been observed [5,20] recorded about 39 species of Asian parasitoids of P. citrella. Ujiye [62] identified 13 parasitoids of the pest in Honshu (Japan), 11 of which belonged to the family Eulophidae and the others to the families Braconidae and Elasmidae. Browning et al. [45] recorded 13 parasitoids in Florida in 1993. Liotta [46] recorded 11 parasitoids belonging to the family Eulophidae in Sicily and Quilici et al. ^[47] found 4 parasitoid species in France. Schauff et al. ^[10] recorded over 80 species of chalcidoid parasitoids reared from P. citrella throughout the world. Cirrospilus phyllocnistoides is the most abundant parasitoid of CLM. First and second instars of P. citrella were most subject to ant predation. Ageniaspis citricola was another important parasitoid of P. citrella and caused 8.2-28.6% mortality compared to 9.6-14.7% from indigenous parasitoids [48]. In Argentina Ageniaspis citricola is causing about 29.5% parasitism and is the most prevalent species in the fall ^[36] and is causing 86% parasitism in Texas and Mexico ^[39]. Pnigalio sp. was the most common parasitoid found attacking CLM in citrus orchards of China ^[49]. Similar observations were reported by Chen and Lou ^[50,51,52] in the Fuzhou region [73] recorded 67.6% parasitism of Tetrastichus phyllocnistoides (synonym of C. phyllocnistoides; Sheng, pers. comm.). Yingfang Xiao [53] reported Phytoseiulus persimilis, Galendromus occidentalis, and Neoseiulus californicus as potential biological control agents of CLM in Alabama and Zagrammosoma multilineatum is the most important parasitoid in Mexico causing nearly 70% parasitisation ^[39] and up to 60% in Florida. Biological control and applications of oil are suitable methods to help reduce populations of CLM in Florida. The complex of natural enemies attacking CLM in Florida, including ants and spiders, can cause up to 90 percent mortality of CLM larvae and pupae [9]. CLM can be effectively controlled by the parasitoid, Eulophids Cirrosphilus spp. The predators attacking the CLM include green lacewing larvae (Chrysoperla rufilabris Burmeister), ants (including the red imported fire ant, Solenopsis invicta Buren), thrips and spiders (Araneae) [54]. However, the economic importance of this pest is rising these days probably due to disturbance of the ecological balance [55].

Four spider species were found feeding on larvae and pre-pupae, *Chiracantium inclusum*, *hibana velox*, *Trachelas volutes and Hentzia palmarum* in Florida indicating that spiders are the major mortality factors of CLM ^[54]. Predation by spiders was the single most important mortality element, which accounted for 50–70% of all deaths. Predation by ants was second, accounting for 10–19% of all deaths. Predation by predatory insect larvae accounted for 3–27% of all mortalities, while parasitism contributed the least (0–10%) to CLM mortality in Alabama ^[53]. The parasitism levels achieved by native parasitoids varied, ranging up to 60%. Parasitism levels were lowest in late winter and early spring in Florida ^[54]. The parasitisation rate was lower in June and July, being higher from August onwards. The maximum parasitisation rate was observed in September, being 38% in 1997, 63% in 1998, 54% in 1999, 74% in 2000, and 72% in 2001 in Turkey ^[33]. The total parasitism from November to March was high at about 70% in Texas ^[39].

Historical analyses indicate that natural enemies that are known to be host-specific and effective at low host densities are more closely synchronized in their habits and better attuned in their nutritional needs, reproductive potential and searching behaviour than generalists. When they can be identified, they are likely to be effective and reliable biological control agents. Unfortunately, the "best" natural enemy may not be found until all natural enemies and their biologies are known. Furthermore, the "best" natural enemy species may differ throughout the geographic range of the target pest due to differences in climate or to interactions with other species such as competition or hyper parasitism ^[54].

Chemical control

The goal of cultural, chemical and other control tactics is to protect the main growth flushes. Chemical control is an inappropriate management strategy for the CLM over the long term due to high costs, concerns about the development of resistance to pesticides by the CLM and other pests, disruption of biological control agents of other citrus pests, concerns about pesticide residues on food and in the ground water, negative effects on worker safety, and effects on non-target organisms in the environment. Resistance to pesticides has developed in CLM populations in China ^[57].

Much work has been done using chemical control, especially in India. As a result, treating with insecticides may affect parasite populations and orchardists should consult with state experts on the timing and frequency of such treatments ^[23]. Various spray regimes, time of growth flushes, and promotion of biological control are recommended in Australia ^[20]. Effective chemical control of CLM is difficult because the larva is protected by leaf cuticle and the pupa is protected by rolled leaf margins. For several authors, the foliar application provides control for only two weeks ^[58,59,60]. Insecticide efficacy is diluted as the young flush expands ^[61]. CLM has also a long history of resistance to many insecticides ^[57]. Effective timing of pesticide application is critical to optimize leafminer management. To maximize kill of CLM larvae with foliar larvicides, applications should be made during a window when CLM larvae hatch and begin feeding. Applications can be timed relative to budbreak of new flush. In general, the earliest applications should be made around 13 days after budbreak, and the very latest applications should be approximately 30 days after budbreak.

Reddy et al., ^[62] reported Monochrotophos causing 100% larval mortality till 12 days after spraving and [74] reported effective CLM control with fenpropathrin, fluvalinate, monochrotophos, dimethoate, methyl-o-demeton, endosulfan etc. Similarly, Zheng and Huang reported fenvalerate, methomyl, cartap, cascade and azadirachtin as effective pesticides against CLM. Mansoor et al., [63] also reported cascade, match, pirate, hostathion and methyl parathion as effective pesticides in CLM management. The combination treatment viz., neem seed kernel extract 4%+ cypermethrin 0.5 ml/L (full dose) was found to be the best combination to manage CLM infestation with minimum leaf damage, spinosad, azadirachtin. and abamectin are capable of suppressing CLM well below economic injury levels (one larva per leaf per growing tip) [64]. Foliar application of Dynamec (vertimec) 1.8% EC (936 ml/ha), virate 23% EC (624 ml/ha), bythroid EC 05 (500 ml/ha) or Karate 5% EC (624 ml/ha) gave better control of CLM, Phyllocnistis citrella ^[55]. Reldan, Runner, Tracer, Sirenol, Palizin and oil are active against CLM, demonstrating that dips of these pesticides penetrate into leaf mines and the adjuvant ingredient of Reldan, Runner, Tracer, Sirenol, Palizin and mineral oil might reduce the infestation by acting as an oviposition deterrent in the field [75]. Abamectin + PO (petroleum oil) caused the highest reduction (87.2%), thiamethoxam (0.05%) provided good control (74.0%) ^[8,49]. Annual applications of Temik or Metasystox-R were effective in leafminer control ^[75]. Imidacloprid (0.005%), fenvalerate(0.005%) and thiodicarb (0.075%) are effective treatments against CLM in acid lime and sweet orange respectively [65,66,67,68], Raut et al., also reported thiomethoxam 0.2ml + DDVP 0.5ml/L as effective treatment against leaf miner in acid lime upto 21 days. The same was supported by Raga et al [8] reported that thiomethoxam @ 5g a.i./100 L of water providing 74% larval control upto 7 days in Tangor Murcott in Brazil. Biorational insecticides abamectin, novaluron and spinosad provided good control of CLM up to 14 days after spraying in acid lime [69].

Among the commercial formulations of *Bt* and *Bt* plus MO against the CLM, it was observed that the CLM larval mortality was higher suggesting that the oil reduced the infestation by acting as an oviposition deterrent ^[70]. However Azadirachtin (Neemix) + oil, diflubenzuron (Micromite) + oil, fenoxycarb (Eclipse) + oil, and oil alone (FC 435-66) were classified as IPM-compatible insecticides and safe to the parasitoid *Ageniaspis citricola* ^[71]. Extracts and the two biorationals, neem oil and abamectin, decreased the larval population significantly to lower numbers than that of the control at 10 days after spray. The biorationals abamectin and spinosad can be effectively used in CLM management and are relatively no-toxic to beneficial insects and mites ^[36]. *Melia azadirachta* extracts clearly had adverse effects on the CLM by decreasing the number of live larvae ^[72]. Among the organic products, foliar spray with biodigester (digested extracts of plants having insecticidal properties, dung and urine) @ 1:3, and digested solution of dung and urine @ 1:1 are effective treatments against CLM recording minimum damage (1.7%) in acid lime.

CONCLUSION

Citrus leaf miner is a widely distributed major citrus pest throughout the world. This is a serious menace to young nurseries and causes nearly 85% leaf damage and indirectly favouring the citrus canker disease increase up to 50%. Hence its management has become inevitable. The pest control should be integrated rather than depending only on chemical control as most of the farmers do. Therefore pests should be monitored regularly to know their abundance and time of activity. This aids in making timely decisions and maintaining farmer's economic and environmental balances. Biological control is the best option for long-term control, however the effective control of CLM is complicated by its high migration

ability from orchards, high fertility as well. CLM has a history of developing resistance to insecticides making it difficult to achieve sustainable control. More attention should be given to the knowledge of the biology and ecology of parasitoid species, both to better use them in biological control programs and to enhance natural bio-control. Bio-pesticides, including botanicals, can offer a safe and effective alternative to conventional insecticides for controlling the pest within an integrated pest management programme. The future research should be more concentrated on development of pheromone technology because it is easy and economical to adopt and also environmental friendly.

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