Coffee leaf miner resistance

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The coffee leaf miner, *Leucoptera coffeella*, is a common pest of coffee plantations in Brazil. Though it is effective, chemical control substantially increases the cost of production and constitutes a significant risk for the environment. Resistant coffee cultivars have been and continue being developed through classic and molecular selection techniques. Basic knowledge about the biology of this insect, the damage it causes to the plants, the identification of sources of resistance, the development of efficient selection methods and knowledge about the genetics of resistance have contributed to the efficiency of the ongoing genetic improvement programs. Recently, coffee genomics studies have also promoted an improvement in the efficiency of the development of cultivars resistant to this insect. We present a short review of each of these aspects of the search for resistance.
Key words: *Coffea*, defense mechanisms, *Leucoptera coffeella*, plant-insect interaction,

Resistência do cafeeiro ao bicho-mineiro: O bicho-mineiro, *Leucoptera coffeella* é praga generalizada da cultura do cafeeiro no Brasil. Apesar de eficiente, o controle químico contribui para a elevação substancial do custo de produção das lavouras e constitui riscos significativo para o ambiente. O desenvolvimento de cultivares resistentes vem sendo realizado a partir de técnicas clássicas e moleculares de seleção. Conhecimentos básicos relacionados à biologia do inseto, ao dano provocado nas plantas, à identificação de fontes de resistência, ao desenvolvimento de métodos eficientes de seleção e ao conhecimento da genética da resistência contribuíram para a eficiência dos programas de melhoramento em andamento. A utilização recente da genômica vem também promovendo um aporte de eficiência no desenvolvimento de cultivares resistentes ao inseto. Uma revisão sucinta sobre cada destes aspectos é apresentada neste trabalho.
Palavras-chave: *Coffea*, *Leucoptera coffeella*, interação planta-inseto, mecanismos de defesa.

INTRODUCTION

Traditionally, coffee production has been one of the pillars of the Brazilian economy. Brazil is the world's largest producer and exporter of coffee, being responsible for about 25 % of the world production. There are an estimated 6 billion coffee bushes, occupying an area of over 2.5 million hectares. Brazilian coffee production is one of the most competitive in the world, and great effort has continually been made to improve even more the efficiency of the coffee production sector, especially as concerns phytosanitary problems.

The coffee plant is the target of a large number of pests, among which the coffee leaf miner, *Leucoptera coffeella* Guérin-Méneville, 1842 (Lepidoptera-Lyonetiidae), is especially important. This insect, which feeds on the palisade parenchyma cells of the leaves (Crowe, 1964; Ramiro et al., 2004), causes considerable damage to the plants (Souza et al., 1998), being considered the principal pest of this crop. The production costs are high in regions wherever there is a high incidence of this insect, mainly because of the lower productivity due to losses incurred because of damage by the leaf miner, and because of the costs of the chemical products, machinery and equipment, and the labor costs involved in control.

Consequently, resistant cultivars are being developed, using genes for resistance to this insect that are present in diploid species of the genus *Coffea*. We discuss aspects of the resistance of the plants to larval and adult coffee leaf miners in this revision.

Description and biology of the insect

In a taxonomic revision made by Mey (1994), 20 species of leaf miners of the genus *Leucoptera* are described as infesting 65 host species, belonging to six families: Betulaceae, Hypericaceas and Salicaceae, Rosaceae, Fabaceae and Aceraceae. Crowe (1964) described four species found on the family Rubiaceae that parasitized coffee plant leaves of the species: *L. coffeella*, *L. meyricki*, *L. coma* and *L. caffeina*. The species found in Brazil, known as the coffee leaf miner, *Leucoptera coffeella*, Guérin-Méneville, 1842 (Lepidoptera-Lyonetiidae), is an introduced pest from Africa (Gallo et al., 1978). The coffee leaf miner was first found in Brazil around 1851, and it was probably introduced on nursery stock imported from the Antilles and Bourbon Island (Von Ihering, 1912). It is considered a monophagous pest, attacking only coffee plants (Reis and Souza, 1986), and it owes its name to the galleries or tunnels formed in the epidermis of the leaf, as a consequence of destruction of the palisade tissue used by the caterpillars for food.

This insect is a micro-Lepidopteran, with a crepescular nocturnal habit. The moths measure 6.5 mm in wingspan, are brownish white, with frilled fore- and hindwings. The caterpillars live within leaf wounds or tunnels that they construct themselves, and when they are completely developed, they are about 3.5 mm long (Souza et al., 1998). This insect has sexual dimorphism, with a 1:1 sex ratio (Speer, 1949; Parra, 1985).

The coffee leaf miner is holometabolous, going through the stages of egg, caterpillar, crysallis (pupa) and adult (Souza et al., 1998). Some relevant aspects of each of these phases are given in this chapter. The biology of *L. coffeella* has been investigated by various authors (Fonseca, 1949; Notley, 1948; Speer, 1949; Katiyar and Ferrer, 1968; Walker and Quintana, 1969; Parra, 1985).

Egg phase: The eggs of *L. coffeella*, deposited by the females on the upper part of the coffee leaves, have a gelatinous aspect and are difficult to see with the naked eye. Temperature and relative humidity of the air have a strong influence on the number of eggs laid per female (Katiyar and Ferrer, 1968). In studies made by Parra (1985), females reared in rearing facilities maintained at 27ºC and nearly saturated relative humidity can lay more than 60 eggs, especially between the 2nd and the 6th day of life of the adults. The duration of the egg phase can be over 20 days (Katiyar and Ferrer, 1968), reduced to four to six days under the optimum conditions established by Parra (1985), after which they eclode into caterpillars.

Catterpillar phase: After eclosion, the *L. coffeella* caterpillars penetrate into the leaf tissue through the lower part of the egg, which is in contact with the leaf epiderm, without contacting the outside. High temperature and relative humidity shorten the larval phase. Between 27ºC and 30ºC, the larval phase on leaves of the *C. arabica* cultivar Mundo Novo varies from 7 to 11 days. At moderate temperatures, around 20ºC, the larval phase varies from 16 to 26 days (Parra, 1985). The coffee leaf miner has four larval instars (Notley, 1948), and one can find various caterpillars of different instars; consequently there is a lack of cannibalism.

Pupal phase: After completing the larval period, the caterpillars are transformed into pupae; they abandon the tunnels and weave a cocoon, normally at the axial part of the leaves. Generally speaking, more pupae are found on the lower third of the plants, the region of the 'skirt' of the coffee bush. In studies conducted in the laboratory, with temperatures varying from 20ºC to 35ºC (Parra, 1985), large variations were found in the duration of the pupal phase. Increases in temperature reduced this duration, with adults emerging after 14 days at 20ºC and 3.6 days at 35ºC. Nevertheless, at this higher temperature, only 25 % reached the adult phase. Temperatures between 27ºC and 30ºC were found to be favorable for development, especially in the pupal phase. Under these conditions, about 95 % reach the adult phase, five days after the caterpillars had transformed into pupae (Parra, 1985). The pupal phase is shorter in females than in males (Notley, 1948; Katiyar and Ferrer, 1968).

Adult phase: Though the damage to the plants is caused by the caterpillars, knowledge about the adult phase is of fundamental importance for the implementation of population-control methods. Characteristics, such as female fecundity, preference for certain hosts and susceptibility to chemicals are intrinsic to this phase of life of this insect. In studies on the biology of *L. coffeela* that had the objective of ecological zoning of this pest, Parra (1985) emphasized the longevity of the adults, considering in the case of the females, two different periods: a) pre-oviposition, and b) oviposition.

Pre-oviposition period – Mating mainly occurs at night (Notley, 1948), though it can also take place in the morning, after the dew dries (Walker and Quintana, 1969). The temperature is inversely related to the length of the period that precedes oviposition, with a mean of 3.6 days for females maintained at 20ºC and 0.5 days for females maintained at 30ºC (Parra, 1985).

Oviposition period – Temperature is the most important factor in this phase; oviposition does not occur below 18ºC

(Speer, 1949; Katiyar and Ferrer, 1968). Under laboratory conditions, food supply can prolong the oviposition period of females maintained at temperatures of from 20ºC to 27ºC, with a reduction in the period at temperatures above 30ºC (Parra, 1985).

Egg-laying capacity – Among the various factors that can influence the egg-laying capacity of the females, food is especially important. In the field, adult coffee leaf miners feed on honeydew, a sugary solution excreted by aphids that suck sap from the plants (Speer, 1949; Crowe, 1964). Under laboratory conditions, Nantes and Parra (1978) tested various sugars and found that a 10 % sucrose solution was the most effective in increasing the number and viability of the eggs. The effects of temperature on the egg-laying capacity of the females are also important, especially when associated with food. According to Parra (1985), females fed with 10 % sucrose solution in an insectary maintained at 27ºC laid 2.1 times more eggs than those that were not fed. At higher temperatures (30ºC), the inverse occurred, probably due to crystallization of the sucrose, making it unavailable. The number of eggs per female is quite variable; on average, females place 75 eggs in 13.4 days on the leaves of susceptible coffee plants (Notley, 1948), with the largest number of eggs laid on day four.

Longevity - Adults maintained in the laboratory at a temperature 25ºC, 70 % relative humidity, and 14h photoperiod, live an average of 13 to 14 days when fed with 10 % sucrose solution (Nantes and Parra, 1978), while unfed adults live only four to five days.

Damage to the plants

Right after eclosion, the caterpillars perforate the upper epiderm of the leaf and penetrate the mesophyll, feeding on palisade parenchyma cells. The lesions that form between the epiderms, also called galleries or tunnels, have irregular margins, are pale yellow in color and later become brownish (Koronnova and De La Vega, 1985).

The necrosed leaf surface reduces photosynthesis (Cibes and Perez, 1957, Walker and Quintana, 1969; Magalhães, 1964), since the flux of water, minerals, and organic matter is impaired (Koronova and De La Vega, 1985). Nevertheless, the loss in production is mainly due to leaf loss (Crowe, 1964), provoked by the increase in the level of ethylene (Souza et al., 1998), principally when the lesions are near the petiole (Notley, 1956).

According to Nantes and Parra (1977b), the area lesioned by a single caterpillar of *L. coffeella* is 1.15cm^2, 1.36cm^2 and 1.03cm^2, respectively, for the *C. arabica* cultivars Catuaí, Mundo Novo and Icatu Vermelho. Third and fourth instar caterpillars are the most voracious, and they do most of the damage to the leaves (Koronnova and De La Vega, 1985). The greater incidence of damage to third and fourth internode leaves (Nantes and Parra, 1977c), accentuates the damage caused by this pest, as photosynthetic activity is highest at these internodes.

The fall of the tunneled leaves is greater in the upper third of the plant canopy, and the production losses are directly related to the intensity of attack and the period in which it occurs. According to Souza et al. (1998), drastic defoliation up till July impedes the formation of floral buds in September and October, and consequently affects fruit production. Drastic defoliation between August and October influence both flowering and fruit formation. Studies conducted by Nantes and Parra (1977a), revealed that reductions of 25, 50 and 75 % of the leaf surface during the dry season resulted in coffee production losses of 9.14 %, 23.53 % and 87.24 %, respectively.

Besides the direct damage, intensive attack by *L. coffeella* provokes weakening of the plants, as evidenced by the data of Koronnova and De La Vega (1985), who found that a loss of 61 % of the leaves caused a 70 % loss in dry matter in the trunk, 60 % in the roots and 50 % of the photosynthetic activity of the remaining leaves. The longevity of the plants attacked by the leaf miner is compromised by the increased energy costs needed to restore the aerial part of the plant (Souza et al., 1998).

Resistance expression

Various studies have been made on the expression and the type of resistance of coffee bushes to coffee leaf miner adults and larvae. The principal characteristics evaluated in plants of various species in this genus are briefly described, as follows.

Leaf anatomy – Anatomical characteristics of the leaf tissue of *C. arabica* and *C. racemosa*, and of resistant and susceptible hybrids from crosses between them, were correlated with the level of resistance to coffee leaf miners in the different populations (Ramiro et al., 2004). Although there were differences in the thickness of the leaf tissues between the parental species *C. arabica* and *C. racemosa*, none of the tissues that were evaluated differed between the resistant and susceptible hybrid plants, suggesting that these anatomical characteristics are not involved in resistance mechanisms in *L. coffeella* (table 1).

Morphology of the leaves – The size and thickness of the leaves and the ploidy level of the plants does not have, according to Medina-Filho et al. (1977a), a relation to resistance to the leaf miner. They observed that the infestation levels of the plants under field conditions were also higher in *C. arabica* cultivars that have small (Monosperma), large (Mundo Novo), or very large (Maragogipe), leaves, as well as in diploid (Monosperma), tetraploid (Mundo Novo and Maragogipe), and octaploid (Bullata) cultivars. Cultivars of *C. arabica* and *C. canephora* with narrow or leathery leaves also had similar levels of infestation. These data were reinforced posteriorly studies by Cardenas (1981). Cardenas also did not find any correlation between resistance to leaf miners and the length, width and thickness of the leaves.

Though *C. stenophylla* and the Mokka variety of *C. arabica* have the same nervation pattern in the leaves, they have distinct reactions to damage caused by *L. coffeella*, as the lesions do not develop in *C. stenophylla*, while they are abundant in Mokka (Cardenas 1981).

Phenological Development - In *C. racemosa, C. arabica*, and in hybrids between these species, the older leaves appear to be more sensitive than the young leaves of the first two pairs (Guerreiro-Filho et al.1999). Similar results were published by Nantes and Parra (1977c), who found that the third and fourth leaf pairs of the cultivars Catuaí, Mundo Novo and Icatu, were more damaged than the first two pairs. The greater larval mortality and the low viability of the eggs and fecundity of the adults, suggest an antibiosis reaction by the plant (Bigger, 1969). Leaf age especially affects caterpillar development and, consequently, the growth of the galleries that have formed. Young leaves of the first and second pair, generally are more resistant than the older leaves of the third, fourth and fifth pairs (Walker and Quintana, 1969). Resistance is expressed through reduced egg laying and increased larval mortality (Bigger, 1969), smaller wounds (Nantes and Parra, 1977a; Cardenas, 1981), or through the finding of fewer leaf miners (Gravena, 1980).

The synthesis of phenolic compounds varies according to the phenological development of the plants, and it is strongly related to their resistance to biotic agents. Studies conducted by Salgado (2004) gave evidence that the level of total phenols did not vary between coffee bushes that produced fruit versus those that did not. Nevertheless, the total phenolic compounds concentration in young leaves was higher than those observed in adult leaves with (25 %) or without (46 %) fruit production. We suggest that the secondary metabolites are shuttled to the younger leaves, which are still only partially lignified, for greater protection against herbivory.

Photochemical compounds - Resistance to the coffee leaf miner could involve substances or chemical compounds that interfere in the development of caterpillars on the species *C. racemosa and C. stenophylla* (Guerreiro-Filho, 1994). In *C. canephora*, the elevated mortality of caterpillars, observed in leaves of the cultivars Conilon (Ferreira et al., 1979) and Robusta (Notley, 1948), is also attributed to the presence of toxic substances in the cell fluids.

The physiological importance of phenolic oxidation in the resistance of coffee plants to attack by *L. coffeella* in the parental species *C. arabica* and *C. racemosa*, and in hybrids between the two with different levels of resistance, were

Table 1. Mean thickness, in μm, of the leaf tissues of the species *Coffea arabica* and *C. racemosa* and of two groups produced by crossing these species (Ramiro, 2003; Ramiro et al., 2004).

Populations*	ADC	ADE	PP	SP	ABE	ABC	PMP (%)	TFL
C. arabica	2.65 b	19.97 c	48.43 a	162.48 a	13.43 c	2.11a	22.99 c	249.06 a
C. racemosa	3.93 a	36.42 a	57.14 a	91.71 c	22.13 a	2.24 a	38.45 a	213.57 bc
C. arabica x *C. racemosa* (R)	2.89 b	23.68 b	47.18 a	109.26 bc	17.79 b	2.11 a	30.10 b	202.91 c
C. arabica x *C. racemosa* (S)	3.35 ab	24.29 b	59.28 a	129.77 b	17.35 b	2.19 a	31.37 b	236.23 ab

** Means followed by the same letter are not significantly different from each other (Tukey 5%). Adaxial cuticle (ADC), adaxial epiderm (ADE), palisade parenchyma (PP), spongy parenchyma (SP), abaxial epiderm (ABE), abaxial cuticle (ABC), percentage of the mesophyll represented by the palisade parenchyma (PMP) and total thickness of the foliar limbo (TFL).*

analyzed by Ramiro (2003) and Ramiro et al. (2006). They indicated that the phenols do not have an important role in the expression of resistance to the coffee leaf miner. Differences were found in the parental species in the total concentration of soluble phenols and in the activities of the oxidative enzymes peroxidase and polyphenol oxidase. However, the resistant and susceptible hybrid plants did not differ for any of these characters. Only in plants of the species *C. racemosa*, parental donator of the genes for resistance to the leaf miner, was significant induction of chlorogenic acid and polyphenol oxidase found. The induction of phenolic activity, and of the enzymes peroxidase and polyphenol oxidase, in response to insect attack, may not be concrete evidence that these substances participate directly in the defense mechanisms.

The concentrations of chlorogenic and caffeic acids, as well as those of caffeine and its derivates, have been related to the development the coffee leaf miner by Magalhães (2005). He found that only the chlorogenic acid concentrations declined with increasing infestation and concluded that these phytochemicals do not have a specific role in the host-pest interaction.

Results published by Guerreiro-Filho and Mazzafera (2000) also gave evidence that the caffeine levels in leaves of *Coffea* species does not correlate with the resistance to leaf miner larvae. The infiltration of various concentrations of alkaloids into leaf disks from *C. arabica* and *C. canephora*, and their hybrids, H14066 C320 and H14136 C304 (susceptible to leaf miners), did not interfere in the normal development of the caterpillars. Also, individual plants with naturally high levels of caffeine in the leaves varied considerably in the severity of the leaf miner wounds.

Nevertheless, when the caffeine level in the coffee plant leaves was correlated with the preferences of the coffee leaf miner females for oviposition, differences were found by Magalhães (2005). He concluded that the caffeine concentration acted as a potential mediator of the host-pest interaction, with this alkaloid stimulating oviposition by the leaf miners.

Magalhães (2005) evaluated the influence of volatile compounds found in coffee leaves on oviposition preference, observing that larger concentrations of p-cymene and lower concentrations of beta cymene were correlated with greater frequency of egg laying. The biological activity of p-cymene was confirmed in an olfactometer, characterizing this substance as a chemical mediator of the interaction between the leaf miner and coffee genotypes.

Types of resistance

Different types and degrees of resistance illustrate the relationship between the coffee plant and the leaf miner. The great variation in the growth of lesions due to coffee leaf miners in leaves of the species *C. racemosa*, *C. setenophylla*, *C. kapakata*, among others (Guerreiro-Filho et al., 1991), is probably due to phytochemicals that interfere in the normal development of the caterpillars and it is a clear example of antibiosis.

In a study of the biology of this insect in *Coffea* species from Madagascar, Guerreiro Filho (1994) observed many other examples of antibiosis, especially when the length of the larval period and of the entire life cycle were compared. While in *C. arabica*, the duration of the larval phase is approximately eight days, in *C. tetragona* it is about 21 days. The entire life cycle varies from 25.4 to 26.7 days in coffee leaf miners that feed on *C. bonnieri*, *C. arabica, C. millotii* and *C. dolichophylla*, and it increases to 35.6 days on *C. tetragona*. Larval mortality is very high in *C. resinosa* and *C. farafaganensis*, and the leaf miners cannot complete their life cycle. Because of the damage provoked by coffee leaf miner caterpillars, *C. perrieri* and *C. vatovavyensis* are considered susceptible to this insect. The viability on these host species was found to be 51.1 and 17.4 %, respectively.

C. canephora seems to be resistant through antixenosis to the coffee leaf miner, since cultivars of this species are less heavily attacked than neighboring plantings of *C. arabica* (Aviles et al., 1983). Accentuated differences in the preference for oviposition on different cultivars were also observed by Matos (2001), revealing the existence of antixenosis-type resistance in *Coffea* spp. In various experiments conducted by this researcher, *C. arabica* was preferred over *C. canephora*, which in turn was preferred over *C. racemosa*. Among cultivars of *C. arabica* and *C. canephora*, the females oviposited preferentially on cultivar Obatã, followed by Catuaí Vermelho and to a lesser extent on cultivar Guarini of *C. canephora*. *C. congensis* was intermediately preferred between cultivars Obatã and Guarini. The biological activity of p-cymene was described by Magalhães (2005) as a mediator of the mechanisms of attraction of the plants for this insect.

Genetics of resistance

The genetic resistance of diploid species of *Coffea* to the leaf miner appears to vary. F_1 hybrids between *C. arabica* and *C. stenophylla* were found to be susceptible to this insect, suggesting that the resistance found in *C. stenophylla* is recessive (Guerreiro-Filho *et al.*, 1987). On the other hand, Medina-Filho et al. (1977b) suggested that resistance through antibiosis to the larval phase found in *C. racemosa* is dominant, and could be determined by one pair of alleles, with modifiers (Medina-Filho et al., 1977a). Reciprocal crosses between some of these species indicate a lack of maternal effects. These results were later confirmed by studies made by Guerreiro-Filho et al. (1999), who concluded that two complementary and dominant genes, denominated *Lm1* and *Lm2*, are responsible for the resistance found in this species. Progenies obtained through autofecundation of heterozygote plants segregate at a proportion of 9 resistant:7 susceptible; in progenies of backcrosses of these heterozygote plants with susceptible parentals, the proportion is 1 resistent:3 susceptible.

Variability for resistance to leaf miners in *coffea*

The coffee germplasm bank of the Instituto Agronômico (IAC) holds 20 of the 80 species described by Bridson and Verdcourt (1988) in the subgenus *Coffea*, in which various studies related to genetic variability for resistance to the principal pests of coffee have been made. The species *C. arabica* was the first to be evaluated in the search for sources of resistance to *L. coffeella* (Medina-Filho et al., 1977a), due to the facility to transfer possible resistance genes to susceptible cultivars of the same species. Approximately 400 introductions, which came from prospective trips made in Ethiopa were evaluated, indicating without exception, susceptibility to the leaf miner (Vilacorta and Sera, 1978). Identical results were found in evaluations made in a coffee plant collection in Yemen (Guerreiro-Filho, unpublished data), a region that harbors considerable diversity in *C. arabica*.

There are controversies about the reaction of plants of the cultivar Mokka to leaf miner attack; it is considered highly susceptible (Cardenas, 1981). No-choice tests made by Guerreiro-Filho (1987) showed that *L. coffella* caterpillars develop normally on leaves of this cultivar, with the leaf area damaged, similar to what is observed for the cultivars Catuaí Vermelho and Mundo Novo, used as controls. However, evaluations made by Medina-Filho et al. (1977a) and Androcioli et al. (1984), revealed that the number of lesions in cultivar Mokka leaves was lower than in the controls, suggesting less preference for oviposition on this cultivar.

In Brasil, chemical control of the leaf miner in plantings made with the cultivar *C. canephora* are normally not necessary (Ferreira et al., 1979), since low leaf miner population levels are frequently observed in the plants. One of the explanations may be related to the high mortality of caterpillars on the leaves of cultivar Conilon, twice that found on leaves of *C. arabica* cultivars (Ferreira et al., 1979). According to these authors, two hypotheses could explain this greater larval mortality, greater adherence between the upper and lower epiderm, which impede the mobility and feeding by the leaf miner larvae, along with the presence of toxic substances in the cellular fluids. The level of infestation in Conilon increases when it is located close to fields of *C. arabica* (Aviles et al., 1983).

The information on infestation in *C. canephora* are contrasting according to Speer (1949); the percentage leaves attacked in the cultivars Conilon and Robusta was found to be similar to that found in leaves of the *C. arabica* cultivars Nacional, Maragogipe and Bourbon. In terms of magnitude of the damage, area of the tunnels, and number of wounds per leaf, Cardenas (1981) did not observe differences between *C. canephora* and the Caturra variety of *C. arabica*. Nevertheless, the leaf miners preferentially oviposited on *C. arabica* leaves because the leaves are darker in the cultivars of this species.

The percentage leaves attacked in the cultivars Robusta and Kouilou (Conilon) in Campinas, respectively 33 % and 34 %, were comparatively lower than that observed in cultivars of *C. arabica*, but much larger than the values found for other diploid species of this genus (Medina-Filho, et al., 1977a).

In tests of germplasms, evaluated in the laboratory for infestation in no-choice tests, using a scale of scores and determining the size of the area damaged by one caterpillar, severe damage was observed in the species *C. canephora* and *C. congensis*, though it was slightly lower than that found in the cultivars Catuaí and Mundo Novo of *C. arabica* (Guerreiro-Filho et al., 1991).

Intraspecific variability in resistance to the coffee leaf miner is low according to studies made by Guerreiro-Filho (1994), who examined three different populations of *C. canephora from Congo, Guinea* and Nana, considered equally susceptible to this insect. However, considerable variability was observed in two populations of *C. congensis*, with susceptible and resistant plants identified.

Different levels of resistance have been reported in diploid (2n=2x=22 chromosomes) species of the genus *Coffea*, in field infestations and under laboratory conditions. The species *C. stenophylla, C. salvatrix, C. liberica* var liberica, *C. brevipes*, *C. sp* ´Moloundou´, *C. jasminoides* and *C. farafaganensis* have been considered resistant, due to the high mortality of the larva, and as a consequence, the reduced leaf area that is lesioned. *Coffea kapakata, C. eugenioides, C. racemosa, C. liberica var. dewevrei, C. humilis, C. tetragona, C. tsirananae,* *C. resinosa, C. millotii, C. bertrandii, C. dolichophylla* and *C. bonnieri* are considered to be moderately resistant, and *C. congensis, C. sessiliflora, P. travancorensis* and *C. perrieri*, moderately susceptible to *L. coffeella* (Sein, 1942; Speer, 1949; Cardenas, 1981; Medina-Filho et al., 1977a,b; Guerreiro-Filho, 1994; Guerreiro-Filho et al., 1991).

Some species are uniformly resistant, others, such as *C. racemosa*, *C. liberica* var. dewevrei and *C. congensis*, have intraspecific variability (Guerreiro-Filho, 1994). Rates of infestation, varying between 8 % and 40 % were observed in the cultivar Laurenti of *C. canephora* in Campinas, SP (Medina-Filho et al., 1977a).

Development of resistant varieties

The development of varieties resistant to insects has been accomplished through the transfer of resistance genes from the species *C. racemosa*, to highly productive and sensitive cultivars of *C. arabica*. Alternative methods, especially the use of molecular biology techniques, have been used for this purpose.

Introgression of genes of C. racemosa: Various species of the genus *Coffea* possess resistance to *L. coffeella*. *Coffea racemosa* was selected as a source of resistance for the genetic improvement of *C. arabica* (Guerreiro-Filho et al., 1990). Besides the resistance to this insect (Medina Filho et al. 1977b), this species was also tolerant of drought and had precocious fruit ripening (Coste, 1954; Levy et al., 1989), characteristics of great agronomic interest. A methodology for precociousness evaluation and for selection of resistant plants is used in the nursery and in controlled infestations in the lab. The method of successive backcrosses, associated with a genealogical method, has been used to develop new cultivars, while selection for resistance and production are made during the various generations.

Selection of transgenic varieties: The gene *cry*1Ac from *Bacillu thuringiensis*, identified as potentially active against *L. coffeella* caterpillars (Guerreiro-Filho et al., 1998), and the development of efficient methodologies for the transformation of plant cells of the species *C. arabica* and *C. canephora* (Van Boxtel et al., 1995; Berthouly et., 1996; Spiral et al., 1993), make it possible to develop transgenic coffee plants that are resistant to leaf miners (Leroy et al., 2000). A large number of plants transformed from clone 126 of *C. canephora* of the cultivar Catimor, and from the F_1 hybrid with *C. arabica*, were obtained and are currently in field trials to evaluate the level and stability of resistance to leaf miners (Perthuis et al., 2005).

Utilization of genomic techniques: The expression of RGAs (resistance gene analogues) in resistant and susceptible plants derived from crosses between *C. arabica* and *C. racemosa* evaluated in infestation tests, through RT-PCR methodology, gave evidence that RGA-type genes are not directly responsible for the resistance of coffee plants to the leaf miner (Maluf and Guerreiro-Filho, 2005). However, analyses of the pattern of segregation of the SSR locus from the ESTs bank of the Coffee Genome, which is currently under development, seem to be still more promising, looking for possible alleles co-segregating with resistance characters in advanced progenies that have been analyzed for response to leaf miner attack (Maluf and Guerreiro-Filho, 2005).

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