Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Monocrotaline presence in the *Crotalaria* (Fabaceae) plant genus and its influence on arthropods in agroecosystems

Presença de monocrotalina em plantas do gênero *Crotalaria* (Fabaceae) e a sua influência sobre artrópodes nos agroecossistemas

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Abstract

Crotalaria (Fabaceae) occurs abundantly in tropical and subtropical regions and has about 600 known species. These plants are widely used in agriculture, mainly as cover plants and green manures, in addition to their use in the management of phytonematodes. A striking feature of these species is the production of pyrrolizidine alkaloids (PAs), secondary allelochemicals involved in plant defense against herbivores. In *Crotalaria* species, monocrotaline is the predominant PA, which has many biological activities reported, including cytotoxicity, tumorigenicity, hepatotoxicity and neurotoxicity, with a wide range of ecological interactions. Thus, studies have sought to elucidate the effects of this compound to promote an increase in flora and fauna (mainly insects and nematodes) associated with agroecosystems, favoring the natural biological control. This review summarizes information about the monocrotaline, showing such effects in these environments, both above and below ground, and their potential use in pest management programs.

Keywords: pyrrolizidine alkaloids, sunn hemp, arthropods, ecological interactions.

Resumo

Crotalaria (Linnaeus, 1753) (Fabaceae) ocorre abundantemente em regiões tropicais e subtropicais e tem cerca de 600 espécies conhecidas. Estas plantas são amplamente utilizadas na agricultura, principalmente como cobertura e adubos verdes, além da sua utilização no manejo de fitonematoides. Uma característica marcante destas espécies é a produção de alcalóides pirrolizidinicos (APs), aleloquímicos secundários envolvidos na defesa das plantas contra os herbívoros. Nas espécies de *Crotalaria*, a monocrotalina é a AP predominante, que tem muitas atividades biológicas relatadas, incluindo citotoxicidade, tumorigenicidade, hepatotoxicidade e neurotoxicidade, além de uma vasta gama de interações ecológicas. Assim, estudos têm procurado elucidar os efeitos desse composto para promover um incremento na flora e fauna (principalmente insetos e nematoides) associados aos agroecossistemas, favorecendo o controle biológico natural. Esta revisão compila informações sobre a monocrotalina, mostrando tais efeitos nesses ambientes, tanto acima como abaixo do solo e a sua potencial utilização em programas de manejo de pragas.

Palavras-chave: alcaloides pirrolizidínicos, crotalária, artrópodes, interações ecológicas.

1. Introduction

Crotalaria is a genus of the Fabaceae family, it covering about 600 species that are divided into sections based on floral morphology (Flores et al., 2009). Representatives of this genus are well distributed in tropical and subtropical regions in which only a limited number of species occur in temperate regions (Palomino and Vázquez, 1991; Flores and Miotto, 2005; Flores et al., 2009).

Crotalaria species are known to contain non-protein amino acids and pyrrolizidine alkaloids (PAs) in their constitution, and the main compound is called monocrotaline (Mattocks, 1986; Hartmann and Witte, 1995; Wink and Mohamed, 2003; Flores et al., 2009; Schramm et al., 2019). Derived from such plants or in isolation, these PAs have biological activities previously reported, including cytotoxicity, tumorigenicity, hepatotoxicity, and neurotoxicity in humans and animals, and also influencing a wide range of ecological interactions (Culvenor et al., 1976; Boppré, 1990; Brown Junior and

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Received: October 2, 2021 – Accepted: December 6, 2021

Trigo, 1995; Hartmann and Witte, 1995; Hartmann, 1999; Trigo, 2000; Nishida, 2002; Trigo, 2011).

The high concentration of PAs in *Crotalaria* is responsible for the low palatability and biosynthesis of sexual pheromones in the *Utetheisa ornatrix* (Linnaeus, 1758) (Lepidoptera: Archiidae) moth, for example (Eisner and Meinwald, 1995; Nishida, 2002; Ferro et al., 2006). Furthermore, *Crotalaria* species have demonstrated biological activity on phytonematodes (Wang et al., 2002), which has enabled these plants to manage populations of these organisms in agricultural production systems (Boppré and Thoden, 2010; Ntalli and Caboni, 2012).

The cultivation of *Crotalaria* is widely used in agriculture, mainly, due to the high biomass production and nutrient cycling, especially nitrogen (Lima Filho et al., 2014). In addition, these plants can be inserted in agroecosystems with greater intensity so that they can play an important role in integrated pest and disease management. Whether this effect by the PA content or by increasing the diversity of the local flora, which provides favorable conditions for the establishment and perpetuation of natural biological control, through the biological balance of the environment.

In this review, we analyze some characteristics of the *Crotalaria* species, especially its chemical composition, relating the influence exerted by such plants on the associated fauna, above and below ground, when inserted in agroecosystems, highlighting their potential use as a cultural management strategy in integrated pest management programs.

2. The Plant Genus *Crotalaria*: Description and Applications

The plant genus *Crotalaria* belongs to the Fabaceae family, Papilionideae subfamily and Crotalarieae tribe (Palomino and Vázquez, 1991), which comprises approximately 600 described species (Flores et al., 2009). Most species are native to India (Pacheco and Silva-López, 2010); however, eastern and southern Africa, Mexico, and Brazil can also be highlighted as they are important diversity hubs for these species (Palomino and Vázquez, 1991; Flores et al., 2009). In Brazil, the genus *Crotalaria* includes 31 native and 11 exotic species, popularly known as "*xique-xique*" or "*guizo de cascavel*" because of the rattling sound when the pod is shaken, similar to the rattle of *Crotalus* sp. (Reptilia: Viperidae) (Tokarnia et al., 2000; Flores et al., 2009; Scupinari et al., 2020).

In general, *Crotalaria* presents annual, erect, bushy, determinate-growing plants, with simple, alternate, lanceolate to obovate leaves, and a slightly hairy surface and a glabrous stem (Tokarnia et al., 2000; Andrade et al., 2008; Flores and Miotto, 2005; Flores et al., 2009). The flowers are usually yellow, sometimes streaked with red, arranged in showy racemes, calyx larger than the corolla, opening from the base to the top in a staggered fashion. They have an attractive scent and a high concentration of floral resources (nectar and pollen), widely available during the entire flower development, essential characteristics for attracting pollinators and natural enemies (Meagher Junior et al., 2019). The pods are cylindrical 4 to 6 cm in length, and propagation is by seeds, which are small and reniform in shape, with a variation in the color of the tegument (Andrade et al., 2008).

Crotalaria species are easily adaptable to sandy soils with low fertility and can provide increases of up to 100% in the crop yield implanted in succession (Wutke et al., 2014). Thus, they can be found in different environmental conditions, such as areas close to rivers, cliffs, restingas, forest edges, fields and savannas (Garcia et al., 2013). Furthermore, *Crotalaria* spp. are weeds, which are common in crops and altered locations, such as on roadsides and pastures (Flores and Miotto, 2005).

Crotalaria plants are mainly used in green manure and folk medicine. However, their medicinal use is restricted due to the high content of alkaloids in leaves and seeds, which plays a vital role in the chemical defense of these plants, as they are toxic to vertebrates and unpalatable to some herbivorous insects (Hartmann, 1999; Flores et al., 2009; Pacheco and Silva-López, 2010). However, despite the recognized toxic effect attributed to this class of alkaloids, in some countries (Colombia, for example), *Crotalaria* species are used in folk medicine (Peñaloza and Peláez, 2008; Pacheco and Silva-López, 2010).

In Brazil, *Crotalaria* is widely used in agriculture, especially in the Southeast, Northeast and Midwest, although there is a lack of technical information about this application. These plants are mainly used for coverage purposes for biomass production in a no-tillage system or mulching formation (Flores and Miotto, 2005; Garcia et al., 2013; Lima Filho et al., 2014). Furthermore, the use of *Crotalaria* spp. in consortium with crops of economic interest can be observed, whether for nematode control, pollinator attraction or increased nitrogen supply (Garcia et al., 2013; Lima Filho et al., 2014). In addition to the green manure and cover crop, *Crotalaria* species are widely used in agriculture to manage phytonematodes in revegetation areas contaminated with toxic substances (such as arsenic, for example) and to produce textile fibers (Garcia et al., 2013). In general, they stand out for their high strength and hardiness, high dry mass production, high nitrogen fixation rate and nutrient cycling (Lima Filho et al., 2014). However, the degree to which these characteristics are expressed varies with species, the planting season, management practices, edaphoclimatic conditions, growing season, and stand (Lima and Castro, 2017).

3. Chemical Composition of *Crotalaria*: Monocrotaline and Its Role in Defense Against Herbivory

The distribution and adaptability of legumes (Fabaceae) around the world suggests that some biological characteristics promote their establishment and evolutive success. In the most of these plants, distinct classes of chemical compounds can act against herbivores and help to the species establishment throughout the time (Lima et al., 2017).

Plant species of the *Crotalaria* genus, for example, are known to have high levels of PAs, which, besides being an important line of defense, are potentially hepatotoxic, neurotoxic, and carcinogenic to animals and humans. Generated after ingestion, the effects are characterized by liver and lung damage and clinical neurological signs (Culvenor, 1978; Kempf et al., 2010; Pacheco and Silva-López, 2010). The exact mechanism of the toxicity caused by monocrotaline is not yet determined, but it is known that its biotransformation by the liver into the reactive metabolite of hydromonocrotaline is necessary, which interferes with DNA synthesis and proteins (Butler et al., 1970; Honório Junior et al., 2010).

Phytochemical studies conducted with different *Crotalaria* species have allowed the identification, isolation and characterization of a large number of secondary metabolites, including several types of alkaloids, such as quinolizidines and pyrrolizidines, in addition to amines, flavonoids, polysaccharides, chalcones, condensed and catechin tannins, lectins, among other substances, such as protease inhibitors, defense peptides (Wink and Mohamed, 2003). Thus, pyrrolizidine alkaloids, chalcones, defense peptides, and protease inhibitors stand out concerning herbivory defense (Pacheco and Silva-López, 2010).

More than 50 PAs have so far been isolated in *Crotalaria* seeds, which made the occurrence of this class of compounds one of the taxonomic characteristics considered for the division of sections of species of the genus (Flores et al., 2009), that is, used as a chemotaxonomic marker (Table 1).

In general, *Crotalaria* species stand out as they produce large amounts of monocrotaline (Hartmann, 1999); however, some species can produce greater amounts of senecionine (Table 1) (Flores et al., 2009). Other PAs, such as junceine, tricodesmine, dehydropyrrolizine, and dicrotaline, may be present in smaller amounts, as in monocrotaline-predominant species, and senecionine and its derivatives may also be identified and vice versa (Mattocks, 1986; Pacheco and Silva-López, 2010). Thus, monocrotaline can represent up to 5% of the dry weight of seeds in some *Crotalaria* species (Martinez et al., 2014), and the structure of this compound is represented by a retronecine base linked to monocrotalic acid (Martinez et al., 2014).

The production of monocrotaline by *Crotalaria* can be influenced by some factors, such as water availability, plant nutrition and pest and/or pathogen attack (Schramm et al., 2019), as well as occurs in other species of Fabaceae that produce chemical defense compounds (Almeida-Cortez et al., 2004; Lima et al., 2017). The main site of production of this compound in these plants are the roots (Irmer et al., 2015) and it is known that the dry mass of decaying *Crotalaria* can release monocrotaline and other compounds present in these plants to the soil, interfering with the diversity of the edaphic fauna (Leal et al., 2018) and soil pests (Oliveira et al., 2007; Gill et al., 2010), in addition to providing the toxic alkaloid to neighboring plants that do not produce these compounds (Nowak et al., 2016).

The quantification of monocrotaline levels present in *Crotalaria* species has been under-documented. However, a recent study successfully quantified the amount of monocrotaline present in leaves and seeds of *C. spectabilis* using the HPLC/MS/MS method (Scupinari et al., 2020). The amounts of monocrotaline quantified by the authors in *C. spectabilis* leaves were 0.141 $mg.g^{-1}$ (5.88%), and in the seeds, it was 17.393 $mg.g^{-1}$ (3.037%)

Thus, it is known that *C. spectabilis* plants have a symbiosis with nitrifying bacteria (characterized by the presence of rhizobial nodules) and produce monocrotaline which is not considered functionally involved in symbiosis (Irmer et al., 2015; Schramm et al., 2019). In this study, the absolute amounts of PA per plant were higher in leaves, followed by nodules, roots and stems, while the concentration was higher in nodules (1.97 $mg.g^{-1}$ of dry weight), exhibiting a 10-fold concentration higher than in leaves (0.21 $mg.g^{-1}$ of dry weight). Despite this, it is known that the plant is the producer of PA and not the microbiont; however, transcripts of the gene responsible for its synthesis (HSS) were detectable only in nodules, indicating that they are the only site of alkaloid biosynthesis and the source from which monocrotaline and other PAs are transported above ground (Schramm et al., 2019). Thus, it is believed that the production of monocrotaline by *Crotalaria* species can be influenced by the establishment of symbiosis with nitrogen-fixing bacteria (Irmer et al., 2015), in which the amount of the compound is dependent on the plant organ.

In the specific case of monocrotaline in *Crotalaria* plants, the relationship of this compound with the *U. ornatrix* moth mentioned above can be cited. In this regard, monocrotaline is assimilated by the insect and used to repel natural enemies, and these compounds are transferred to offspring during copulation (Trigo, 2000; Cogni and Trigo, 2016).

3.1. Pyrrolizidine alkaloids and their effect on associated organisms: what is known so far?

Pyrrolizidine alkaloids are an important class of secondary metabolites (allelochemicals) biosynthesized by a large number of plant species (Martinez et al., 2014). These compounds are particularly found in specimens of Senecioneae and Eupatorieae, within Asteraceae, several genera of Boraginaceae, Apocynaceae, Fabaceae (mainly the genus *Crotalaria*) and some genera of Orchidaceae (Hartmann and Ober, 2000; Ober and Kaltenegger, 2009; Schramm et al., 2019). Furthermore, PAs are also found in species of Ranunculaceae, Convolvulaceae, Celastraceae, Proteaceae and Poaceae, where they occur in only a few or even a single species (Hartmann and Witte, 1995; Hartmann and Ober, 2000).

Generally, plants produce several types of PAs, usually within the same structural group, and the amount can vary greatly between species and between plants of the same species (plant-plant variation), depending on their physiological state, the environmental conditions, development stage, type of plant tissue, environmental conditions and procedures used for extraction and quantification (Hartmann and Witte, 1995; Flores et al., 2009; Schramm et al., 2019). Thus, reported concentrations of PAs vary greatly from trace amounts to 19% of dry weight (Schramm et al., 2019).

PAs have toxic action and are therefore feared in human and animal diets due to their structural characteristics. These alkaloids are the substrate for cytochrome P450-dependent enzymes, located in the liver

Table 1. Species belonging to each section of the genus *Crotalaria* in Brazil, the most abundant type of pyrrolizidine alkaloid (PA) and the degree of specialization of the flowers.

Section	Species	Predominant PA	Flower specialization
Calycinae	*C. balanse*	Monocrotaline	Standard appendages restricted to the blade and the keel nipple twisted in a spiral
	C. breviflora		
	C. flavicoma		
	C. grandiflora		
	C. hilariana		
	C. juncea		
	C. martiana		
	C. otoptera		
	C. paulina		
	C. pilosa		
	C. stipularia		
	C. tweediana		
	C. velutiana		
	C. vespertilio		
Crotalaria	*C. mucronata*		
	C. ochroleuca		
	C. retusa		
	C. spectabilis		
	C. virgulata		
Chrysocalycinae	*C. harley*	Senecionine	No specializations
	C. holosericea		
	C. incana		
	C. maypurensis		
	C. micans		
	C. miottoae		
	C. reyipila		
	C. vitellina		
Hedriocarpae	*C. lanceolata*		
	C. pallida		
	C. trichotomata		

Adapted from Flores et al. (2009).

in vertebrates, but also in insects (Ober and Kaltenegger, 2009; Schramm et al., 2019). Furthermore, the resulting pyrrolic intermediates exhibit cell toxicity and react with biological nucleophiles such as proteins and nucleic acids (Fu et al., 2004). Not all PAs are toxic, but those affecting human health particularly have esters and unsaturated 1,2-hydroxymethyl-dihydropyrrolizidine (HDP) in their structure (Mattocks, 1986). Potentially toxic PAs exhibit essential characteristics in their structure, such as: (i) an unsaturated pyrrole ring; (ii) one or two hydroxyl groups attached to the ring; (iii) one or two ester groups; and (iv) the branched acid molecule (Pacheco and Silva-López, 2010). Despite their rich diversity, they all share a common structure: they are alkaloids composed of necine esterified with one or more nectic acids (Pacheco and Silva-López, 2010; Schramm et al., 2019).

To date, more than 500 structurally different PAs are known and can be grouped according to the number of ester bonds or according to their base portion (Boppré and Thoden, 2010; Pacheco and Silva-López, 2010). Thus, almost all PAs can be classified into five different major structural groups (Kempf et al., 2010). Thus, toxic PAs comprise a large group of related compounds (about 160 already described) that occur in plants, mainly in species of the genus *Crotalaria*, *Senecio, Heliotropium, Trichodesma, Symphytum, Echium* and in other genera related to the Asteraceae and Boraginaceae families, which mainly biosynthesize monocrotaline, senecionine, triangularine, licopsamine and falaeonopsine (Pacheco and Silva-López, 2010).

Regarding the action of PAs on insects, it was shown that they are strong feeding deterrents for many herbivores, in addition to their genotoxic action (Hartmann, 1999) and have a broad defense function against herbivory in plants (Ober and Kaltenegger, 2009; Macel, 2011; Martinez et al., 2014; Schramm et al., 2019). Strong support for the defensive role of PAs includes the adaptations of specialized insects to PA-containing plants, allowing them not only to deal with these plant toxins but also to accumulate them in their body for their own defense (Hartmann and Ober, 2000; Ober and Kaltenegger, 2009). However, the importance of these effects is difficult to measure, and often the most specific observation is that generalist herbivores do not feed on a plant that contains some type of PA (Hartmann, 1999).

The general metabolism of PAs in insects is similar to the metabolism that occurs in humans, including adverse physiological effects (Schramm et al., 2019). Several studies on the effects of individual PAs and general PA content of a given plant in the feeding of specialist and generalist herbivores were conducted, and it was observed that the influence of such a compound on insects depends on the type and concentration of the most abundant PA in the plant tissue (Wei et al., 2015). Furthermore, it is known that generalist herbivores consume PA-containing plants only in times of scarcity of nutritionally adequate food, whereas adapted insects are believed to be attracted (Hartmann, 1999; Cheng et al., 2013).

In the same way, insects can convert and store this PA load, maintaining them in the form of non-toxic alkaloids, and this modification is carried out in the intestine, and the compounds from this process are adsorbed as tertiary alkaloids (Hartmann, 1999; 2004). These, in turn, are synthesized exclusively in the form of N-oxide, which is the specific molecular form for long-distance translocation and storage in the vacuole. In this form, N-oxide, are incorporated by insects (Hartmann, 1999; Brückmann et al., 2000).

Thus, herbivorous insects have evolved through numerous adaptations to overcome the defensive barrier of PA-protected plants and to overcome the defensive barrier of PA-protected plants and sequestrate them and use them for their own defense against predators (Hartmann and Witte, 1995). These alkaloids are accumulated and used by various insects in various processes, such as chemical defense in which they become deterrent food or acquire aposematic coloring against predatory invertebrates such as spiders, ants, coccinellids, and wasps (Nishida, 2002). Moreover, some males can convert their alkaloid charge through a signaling process, providing females with a way to discriminate between males that have the alkaloid and males without them (Trigo, 2011).

Concerning PA N-oxide storage, insects developed a strategy to use these allelochemicals for their own defense against predators. An example of this adaptation is the neotropical Ithomiinae butterflies which, unlike moths, do not feed on PA plants or sequester them from the larval stage but absorb PAs exclusively through nectar or dry twigs (Macel et al., 2005; Schramm et al., 2019). This habitat protects them from the *Nephila clavipes* (Linnaeus, 1757) (Aranae: Nephilidae) spider, eliminating butterflies that have already ingested PAs from their own net (Silva and Trigo, 2002; Schramm et al., 2019). Sequestered PAs play an important role in the mating process of some tribes of the Lepidoptera family because they serve as a precursor to male pheromones (Boppré, 1990). This is also the case of the African grasshopper, *Zonocerus variegatus* (Linnaeus, 1758) (Orthoptera: Pyrgomorphidae), which, due to N-oxidation and accumulation of PAs in its hemolymph, circumvents the chemical defense of plants and uses the compost to protect itself against predators (Kubitza et al., 2018).

Through the described mechanisms, specialized insects can take advantage of plants containing PAs (Joshi and Vrieling, 2005) through: (i) specific sensory recognition for a given PA in the host; (ii) biochemical mechanisms to ingest, maintain, metabolize, accumulate and transfer alkaloids under controlled physiological conditions and; (iii) alkaloid-guided mating behavior (Hartmann, 2004).

In addition to their contribution to defense against insects, PAs also influence plant interactions with fungi, with an inhibitory effect on *Fusarium* and *Trichoderma* (Hol and Van Veen, 2002). Plants that have beneficial interactions with microorganisms, especially mycorrhizal fungi, and nitrogen-fixing bacteria, influence the PA synthesis process, affecting the production and concentration of these compounds, which generally start to be produced in larger quantities released into the soil later (Schramm et al., 2019). From that moment, plants that do not produce PAs, when cultivated in the same place, can absorb and accumulate these compounds through the roots, either if they grow concomitantly with PA-producing plants or if they grow under their decomposing dry mass (Nowak et al., 2016).

4. *Crotalaria* in Agroecosystems: Effects on Associated Organisms

Regarding the influence of *Crotalaria* on the fauna associated with agricultural crops, four characteristics that are possibly related to its activity can be listed: (i) floral characteristics (Meagher Junior et al., 2019); (ii) the presence of monocrotaline above ground (Hartmann, 1999; Scupinari et al., 2020); (iii) the peculiarities of its root system (Wang et al., 2002; Irmer et al., 2015; Leal et al., 2018) and; (iv) and dry mass decomposition products (straw) (Oliveira et al., 2007; Gill et al., 2010; Nowak et al., 2016) (Figure 1). A summary of the main effects of the *Crotalaria* species on the associated fauna is shown in Table 2.

The floral characteristics of *Crotalaria* are an important factor for the attractiveness of pollinators and natural enemies as they have many flowers at different developmental stages, with vibrant and attractive yellow flowers, in addition to the high availability of pollen and nectar (Meagher Junior et al., 2019). Several pollinator species have been observed to occur in *Crotalaria* species, including *Apis mellifera* (Linnaeus, 1758) (Hymenoptera: Apidae) (Marchini et al., 2001; Villalobos and Ramírez, 2010; Henrique and Figueiredo, 2018), *Xylocopa virginica*

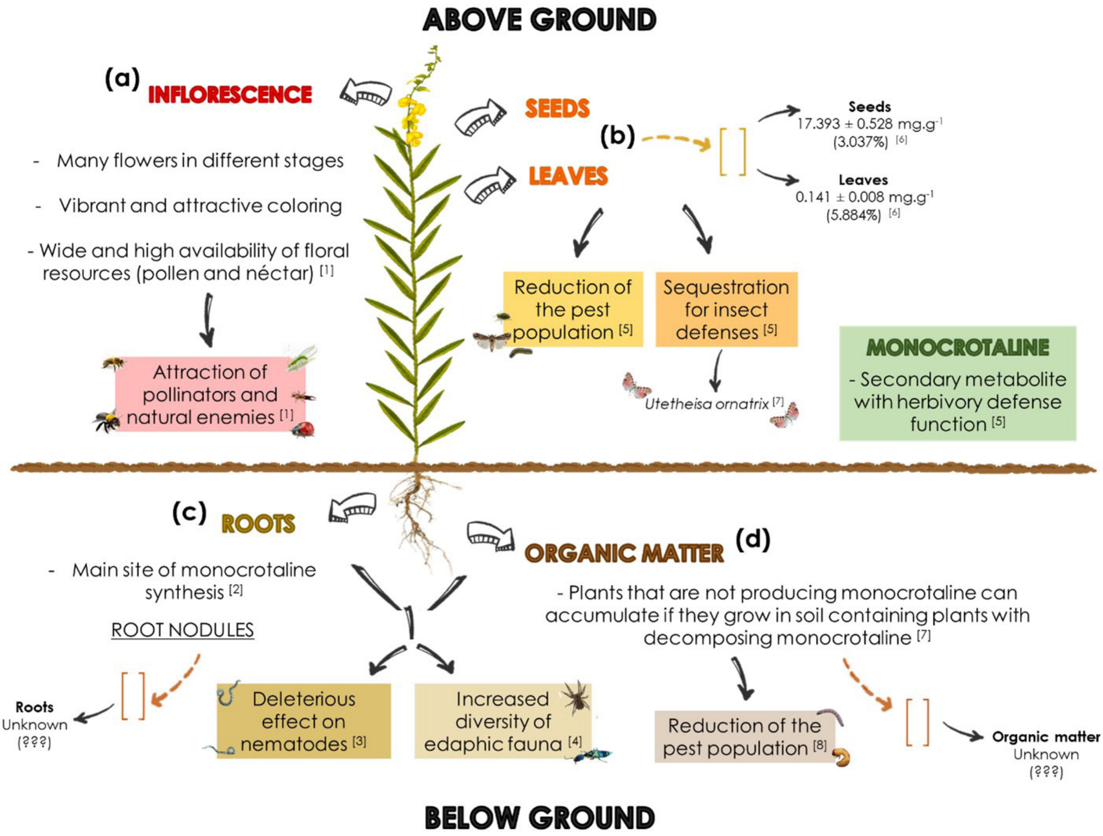

Figure 1. Influence of *Crotalaria* (Fabaceae) species on the associated fauna in agroecosystems: (a) inflorescence; (b) leaves and seeds; (c) roots; and (d) organic matter. According to [1]Meagher Junior et al. (2019); [2]Irmer et al. (2015); [3]Wang et al. (2002); [4]Leal et al. (2018); [5]Hartmann (1999); [6]Scupinari et al. (2020); [7]Nowak et al. (2016); [8]Oliveira et al. (2007) and Gill et al. (2010).

(Linnaeus, 1771) (Hymenoptera: Apidae) and *X. micans* (Lepeletier, 1841) (Henrique and Figueiredo, 2018; Meagher Junior et al., 2019), *X. brasilianorum* (Linnaeus, 1767) (Brito et al., 2010), *X. frontalis* (Olivier, 1789) and *X. grisesncens* (Lepeletier, 1841) (Jacobi et al., 2005; Henrique and Figueiredo, 2018), *Megachile mendica* (Cresson. 1878) (Hymenoptera: Megachilidae), *M. lanata* (Fabricius, 1775) and *M. sculpturalis* (Smith, 1853) (Hall and Avila, 2016; Meagher Junior et al., 2019), *M. bicolor* (Fabricius, 1781) and *M. georgica* (Cresson, 1878) (Meagher et al., 2019), *Augochlora* sp. (Smith, 1853) (Hymenoptera: Halictidae) (Brito et al., 2010), *Bombus morio* (Swederus, 1787) (Hymenoptera: Apidae) (Krueger et al., 2008) and *Trigona* sp. (Jurine, 1807) (Hymenoptera: Apidae) (Brito et al., 2010; Henrique and Figueiredo, 2018). This increase in the population of pollinators has conditioned a significant increase in the production of agricultural crops intercropped with Crotalaria species. In this context, the increase in the number of soybean pods (*Glycine max* L., Fabaceae) (Sholahuddin et al., 2019) and coffee pods (*Coffea* spp. L., Rubiaceae) (Setyawati et al., 2018; Supriyad et al., 2019) when cultivated in consortium with *Crotalaria juncea* stands out, which is a factor attributed to greater abundance of pollinators.

The attractiveness of *Crotalaria* plants also extends to natural enemies, which was already observed for several species of the genus. For example, *C. juncea* caused attraction of natural enemies in some crops, such as cowpea (*Vigna unguiculata* L., Fabaceae), corn (*Zea mays* L., Poaceae) and a variety of fruit trees (Trisnawati and Azis, 2017). This increase in the population of natural enemies was also observed in other studies, which found a significant increase in the number of chrysopids (*Chrysoperla externa* Hagen, 1861 (Neuroptera: Chrysopidae)), earwigs (*Doru luteipes* Scudder, 1876 (Dermaptera: Forficulidae)), spiders (*Nephila clavipes* (Araneae: Nephilidae)) and stink bugs (*Orius insidiosus* Say, 1832 (Hemiptera: Anthocoridae)) in *Crotalaria* in the single cultivation and in the consortia (Venzon et al., 2006; Tavares et al., 2011). Moreover, in this context, another study pointed out that *Trichograma papilionis* (Nagarkatti, 1974) (Hymenoptera: Trichogrammatidae) preferred to parasitize *Helicoverpa zea* (Boddie, 1850) (Lepidoptera: Noctuidae) eggs deposited on *C. juncea* than on corn (*Z. mays*), suggesting that there may be interference from semiochemical volatiles released from the plants when the eggs are placed on the leaves (Ali and Wright, 2020).

In turn, the presence of monocrotaline in plants influences the establishment and development of pests associated with crops (Hartmann, 1999), whether in crop

Table 2. Effects of *Crotalaria* species on the arthropods associated with agroecosystems.

Observed effect	*Crotalaria* species	Affected organisms			Reference
		Order	Family	Specie	
Pollinator attraction	*C. micans*	Hymenoptera	Apidae	*Apis melífera*	Villalobos and Ramirez (2010)
				Xylocopa spp.	
	C. juncea			*Bombus morio*	Krueger et al. (2008)
			Megachilidae	*Megachile sculpturalis*	Hall and Ávila (2016)
				Megachile lanata	Henson et al. (2019)
				Megachile bicolor	Meagher Junior et al. (2019)
				Megachile georgica	
				Megachile mendica	
			Apidae	*Xylocopa virginica*	
				Xylocopa micans	
	C. retusa			*Xylocopa frontalis*	Jacobi et al. (2005)
				Xylocopa grsescens	
	C. spectabilis			*Apis melífera*	Marchini et al (2001)
				Xylocopa sp.	Henrique and Figueiredo (2018)
	C. vitellina		Halictidae	*Augoclora* sp.	Brito et al. (2010)
			Megachilidae	*Megachile* spp.	
			Apidae	*Trigona* sp.	
				Xylocopa brasilianorum	
Increase of natural enemies populations	*C. juncea*	Hymenoptera	Trichogrammatidae	*Trichogramma* spp.	Manandhar and Wright (2016)
					Wright (2019)
				Trichogramma papilionis	Ali and Wright (2020)
		Dermaptera	Forficulidae	*Doru luteipes*	Tavares et al (2011)
		Coleoptera	Coccinellidae	*Eriopis connexa*	
		Aranae	Nephilidae	*Nephila clavipes*	
		Neuroptera	Chrysopidae	*Chrysoperla externa*	Venzon et al. (2006)

Table 2. Continued...

Observed effect	*Crotalaria* species	Affected organisms			Reference
		Order	Family	Specie	
		Aranae	-	-	Lima and Castro (2017)
		Coleoptera	Coccinellidae	-	
		Diptera	Asilidae	-	
			Dolichopodidae	-	
		Hymenoptera	Encyrtidae	-	
			Vespidae	-	
	C. lanceollata	Coleoptera	Coccinellidae	*Hippodamia convergens*	McSorley et al. (2009)
		Diptera	Asilidae	*Neiotamus* spp.	
	C. ochroleuca	Hymenoptera	Trichogrammatidae	*Trichogramma* spp.	Wright (2019)
	C. spectabilis			*Trichogramma* spp.	Trisnawati and Azis (2017)
					Wright (2019)
		Hemiptera	Pentatomidae	*Orus insidiosus*	McSorley et al. (2009)
Pest attack/damage reduction	*C. juncea*	Lepidoptera	Noctuidae	*Spodoptera frugiperda*	Dias et al. (2016)
					Guera et al. (2020)
			Pyralidae	*Elasmopalpus lignosellus*	Gill et. al. (2010)
		Hemiptera	Aleyrodidae	*Bemisia argentifolli*	Manandhar et al. (2009)
		Coleoptera	Scarabeidae	*Phyllophaga cuyabana*	Oliveira and Garcia (2003)
					Oliveira et al. (2007)
			Vesperidae	*Migdolus fryanus*	Bento et al. (2020)
		Thysanoptera	Thripidae	*Thrips tabaci*	Quintanilla-Tornel et al. (2016)
		Hemiptera	Aphididae	-	Hinds and Hooks (2013)
					Manandhar and Hooks (2011)
	C. pallida	Lepidoptera	Noctuidae	*Heliothis virescens*	Cogni and Trigo (2016)
	C. spectabilis			*Helicoverpa armigera*	Reigada et al. (2016)
			-	-	Burkart (1952)

Table 2. Continued...

Observed effect	*Crotalaria* species	Affected organisms			Reference
		Order	Family	Specie	
Sequestration of monocrolatine for defense	*C. juncea*	Lepidoptera	Arctiidae	*Utetheisa ornatrix*	Hartmann (1999)
	C. spectabilis				Schramm et al. (2019)
					Trigo (2000)
Reduction of nematode population	*C. juncea*	-	-	*Heterodera glycines*	Acharya et al. (2020)
		-	-	*Pratylenchus brachyurus*	Mathew and Opperman (2020)
		-	-	*Meloidogyne incognita*	Ferreira et al. (2020)
		-	-	*Meloidogyne javanica*	
	C. spectabilis	-	-	*Rotylenchulus reniformis*	El-Deriny et al. (2020)

rotation/succession or in polycultures or intercrops, in which the roots were the main production site of this compound in plants (Irmer et al., 2015). The decomposing dry mass of *Crotalaria* can release monocrotaline and other compounds present in these plants into the soil, interfering with the diversity of the edaphic fauna (Leal et al., 2018) and soil pests (Oliveira et al., 2007; Gill et al., 2010), in addition to providing the toxic alkaloid to neighboring plants that do not produce these compounds (Nowak et al., 2016).

Regarding pest control, studies show that *Crotalaria* plants affect herbivores in different situations, affecting their biology and development, as well as the behavior of associated herbivores (Dias et al., 2016). When it consisted of the only food available, different *Crotalaria* species significantly reduced the survival of *Spodoptera frugiperda* (Dias et al., 2016), *Helicoverpa armigera* (Reigada et al., 2016), and *Heliothis virescens* (Fabricius, 1777) (Lepidoptera: Noctuidae) (Cogni and Trigo, 2016) in laboratory studies.

When cultivated in an intercropped way with crops of economic interest, *Crotalaria* reduced the occurrence of pests. In pumpkin (*Cucurbita pepo* L., Cucurbitaceae) crops, the consortia with *C. juncea* reduced the occurrence of aphids and beetle-pests and provided an increase in the population of predators such as spiders (Hinds and Hooks, 2013). Similarly, studies have shown that *Crotalaria* in consortia with pumpkin reduced the incidence of whitefly (*Bemisia argentifolli* Bellows & Perring, 1994 (Hemiptera: Aleyrodidae)), while also reducing the incidence of the pumpkin mosaic virus, which is transmitted by the aforementioned insect (Manandhar et al., 2009; Manandhar and Hooks, 2011).

In this context, Crotalaria plants can be used in push-pull systems, as in the study conducted by Guera et al. (2020), who associated *C. juncea* with corn. As a result, the authors observed that when in intercrop, *C. juncea* made corn less attractive to fall *S. frugiperda* (Smith & Abbot, 1797), under laboratory and greenhouse conditions.

Among other cultivation modalities, whether in intercropping or crop rotation/succession, *Crotalaria* plants can be used to form mulching, that is mulch for the soil. In this system, it was observed that *C. juncea* mulching reduced the damage caused by thrips (*Thrips tabaci* Lindeman, 1889 (Thysanoptera: Thripidae) and leafminer (*Liriomyza* spp. Mik, 1894 (Diptera: Agromyzidae)) in the onion (*Allium cepa* L., Amaryllidaceae) crop (Quintanilla-Tornel et al., 2016). Moreover, in that same study, there was an increase in the diversity and richness of associated species, especially in the population of predators and parasitoids, particularly spiders.

Another potential use for *Crotalaria* plants is as a trap crop, to attract or repel pests, natural enemies and other organisms in cropping systems (Santos et al., 2008). The attraction of some groups of insects is due to the characteristics of the plant, such as the architecture of the stems and high ground cover. These factors have favored populations of organisms by increasing humidity and reducing the temperature, providing areas of refuge and shelter for parasitoids and predators, in addition to mites and beneficial nematodes (McSorley et al., 2009).

The negative effect on insect pests can be direct (repellent effect) or indirect, by attracting natural enemies, which affect the herbivore population (Smith and McSorley, 2000). These effects were observed in citrus crops, where *C. spectabilis* plants were attractive to caterpillars (Burkart, 1952), and in tobacco (*Nicotiana tabacum* L., Solanaceae) crops, where *C. juncea* plants acted as a trap to attract natural enemies throughout the whole culture cycle (Trisnawati and Azis, 2017).

Regarding the specific effect of monocrotaline on insects, *U. ornatrix* stands out. Males of *U. ornatrix* have a court pheromone, which is derived from pyrrolizidine alkaloids ingested in the larval stage of the host plant, *C. spectabilis* (Trigo, 2000). *U. ornatrix* larvae sequester pyrrolizidine alkaloids (monocrotaline) and retain them during the developmental stages. When an adult, the alkaloid derivative is transferred to the female during copulation, via spermatophore, and in oviposition, it transfers the alkaloids to the eggs, serving as protection (Trigo, 2000; Cogni and Trigo, 2016).

Regarding the fauna that inhabits the soil, there was an increase in the number of insects captured in pitfall traps in *C. spectabilis* and *C. ochroleuca* cultivation sites (Leal et al., 2018). An increase in the mesofauna and a greater diversification of the macrofauna were also observed, in which the order Hymenoptera was the one with the most captured representatives (Leal et al., 2018). Thus, regarding soil pest control, studies show that *Crotalaria* plants act on rhizophagous pests, such as *Phyllophaga cuyabana* (Moser, 1918) (Coleoptera: Melolonthidae) white grubs, which prefer to feed on *C. juncea* instead of on other cultures, highlighting the importance of intercropping with cultures where this insect-pest occurs (Oliveira and Garcia, 2003; Oliveira et al., 2007). Furthermore, *Crotalaria* plants increased the mortality of *Elasmopalpus lignosellus* (Zeller, 1848) (Lepidoptera: Pyralidae) pupae in soil covered with *C. juncea* mulching (Gill et al., 2010).

In addition to these characteristics mentioned so far, it is worth noting that *Crotalaria* plants are the most used green manures for the management of phytonematode (Inomoto and Asmus, 2014), and the efficiency in controlling such pests can be explained by the high amounts of monocrotaline present in plants (Scupinari et al., 2020). Thus, several experiments demonstrate that *Crotalaria* of different species, in different arrangements (single cultivation or consortia) and conditions (field or laboratory), affected important species of phytonematodes, such as *Meloidogyne incognita* (Kofoid & White, 1919) and *Meloidogyne javanica* (Treub, 1885) (Rhabditida: Meloidoginidae) (Ferreira et al., 2020), *Heterodera glycines* (Rhabditida: Heteroderidae) (Ichinohe, 1952) (Acharya et al., 2020), *Pratylenchus brachyurus* (Godfrey, 1829) (Rhabditida: Pratylenchidae) (Mathew and Opperman, 2020) and *Rotylenchulus reniformis* (Rhabditida: Rotylencgulidae)(Linford & Oliveira, 1940) (El-Deriny et al., 2020).

Crotalaria interactions with phytonematodes can occur in the following ways: (i) as a non-host or poor host; (ii) producing toxic allelochemicals or inhibitors; (iii) providing a niche for antagonistic fauna and flora; or (iv) trapping the nematode (Wang et al., 2002). However, the resistance mechanism in *Crotalaria* varies between plant species and target nematodes (Wang et al., 2002; Boppré and Thoden, 2010; Ntalli and Caboni, 2012; Scupinari et al., 2020). In addition, the contribution of biomass provided by *Crotalaria* to the soil generates a large increase in organic matter, which is considered an ally in nematode management, as decomposing organic material favors an increase in the population of nematophagous fungi and bacteria, in addition to the incidence of predatory nematodes (Wang et al., 2002).

The information cited so far gives support to highlight the potential use of *Crotalaria* species in maintaining biological control in agricultural areas, called conservative biological control. Since, the increase in plant diversity in an agroecosystem, by itself, is able to assist in maintaining the population of natural enemies and, consequently, reduce the number of potential pests individuals (Freitas and Mantovani, 2018).

5. Conclusion

Plants of the *Crotalaria* genus have been used for various purposes in agriculture and industry. Considering the facts presented throughout this review, we highlight the potential use of *Crotalaria* species in agroecosystems, whether in crop rotation/succession, in intercropping systems or as a trap crop, in crops in large areas (conventional), or even in cropping systems of organic or agroecological production, especially as a cultural management strategy in integrated pest management programs. However, further studies are needed concerning the concentration of monocrotaline in *Crotalaria* species and the elucidation of the effects of this compound on the main pest insects affecting the crops to be protected.

Still, despite the high concentration of PAs, few studies have been conducted on the pharmacological and biotechnological applicability of those isolated substances of this genus. Therefore, the study of new molecules and those previously isolated from *Crotalaria* constitutes a new and wide field of investigation on the functions of these components and their potential application in therapeutic treatments and as an insecticide.

Acknowledgements

The authors would like to thank the anonymous reviewers for their helpful comments. This research was supported by the National Institute of Science and Technology – Semiochemicals in Agriculture (FAPESP and CNPq – grants #2014/50871-0 and #465511/2014-7, respectively).

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