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The ecological role of alkaloids during reproductive stages of angiosperms: an overview

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ABSTRACT

Alkaloids are secondary metabolites whose primary biological function has been related as a chemical defense mechanism. Although its ecological role is widely studied in herbivory, its importance during reproductive phases has been neglected. Since flowering and fruiting are fundamental phases in perpetuating angiosperms, alkaloids' ecological role during these stages is summarized here. Besides, the dual role as deterrent and as attractant in biotic interactions such as pollination and dispersal, is highlighted.

Keywords : attractant, chromoalkaloids, defense, dispersal, pollination

The alkaloids are secondary metabolites with heterocyclic nitrogen structures derived from amino acids metabolic pathways (Aniszewski 2015, Wink *et al*. 2018).These secondary metabolites have various biosynthetic origins and are chemically characterized and classified by their hydrophilic nitrogenate structures (Fig. 1) (Guerrero-Rubio *et al.* 2020). Alkaloids' ecological role has been mainly related to defense as many of these compounds show toxic properties (Matsuura and Fett-Neto 2015, Roy 2017, Wink 2019, Zaman *et al*. 2019). Most studies about the role of alkaloids have been made in terms of herbivory or plant protection, but the activity of alkaloids during reproductive stages has received less attention. Pollination and dispersal are key events in plant reproduction with significant repercussions on their life cycle (Medel and Zamora 2009, Barrett 2015). The pollination allows the arrival of pollen to stigmas to form seeds (Abrol 2011), and the dispersal allows the movement of seeds to a suitable place to accomplish germination (McConkey and O'Farrill 2016).

Both pollination and dispersal can occur by abiotic or biotic vectors, but biotic interactions play a significant role in maintaining natural communities through the establishment of networks (Abrol 2011, Dellinger 2020). Thus, compounds like alkaloids that confer protection to reproductive structures as flowers or fruits are crucial to survival (Li *et al.* 2017). Although the role of alkaloids as a protection mechanism is well known, their role as attractant has been little studied. It has even been reported that the concentrations of alkaloids in floral organs are like those on vegetative tissues (Strauss *et al*. 2004, Cook *et al*., 2013). However, these few studies on alkaloid accumulation in reproductive structures mainly focus on pharmacological applications leaving aside their ecological roles. Thus, this review aims to gather information about the participation of alkaloids during plant reproductive stages highlighting their dual role as defense and attractant through flowering and fruiting stages.

The ecological roles of alkaloids in floral tissues—Sexual plant reproduction is the primary input of genetic variation within a population (Barrett 2015). Furthermore, populations rely on pollination to form seeds (Lampert *et al.* 2019). For pollination to occur, flowers exhibit strategies to attract visitors as specialized structures that offer rewards (Tolke *et al.* 2020). However, not just pollinators can be attracted to flowers, but florivores, pollinivores, or nectar robbers (Tsuji and Ohgushi 2018). The consumption of floral parts or rewards could affect pollinators and plant fitness (Soper and Adler 2016, Wakabayashi *et al.* 2018). As well as pollination, the plant also displays strategies that deter other floral visitors or pathogens (Li *et al*. 2017, Hope *et al*. 2018, Nadot and Carrive 2020, Tolke *et al*. 2020).

The alkaloids are the primarily secondary metabolites that act as defense mechanisms against herbivory or florivory (Kruse *et al.* 2017, Wakabayashi *et al.* 2018, Wink 2019, Wink 2020). Of the different alkaloids, only certain types have been reported to occur in reproductive structures (Fig. 1). Despite their ecological importance, these reports on alkaloids in flowers mainly focus on pharmacognosy (Fahmy *et al.* 2020). However, the function of alkaloids in floral tissues can be inferred from the reported in non-ecological studies. An example is the species of the genus *Erythrina* (Fabaceae) that have a high content of alkaloids (>100 compounds) in flowers and seeds (Zhao *et al.* 2018). Pharmacological studies demonstrated the paralyzing and cytotoxic effects of floral and seed alkaloids in small animals such as frogs and rodents (Fahmy *et al.* 2020); thus, *Erythrina* flowers could deter florivores through their toxicity.

Although floral parts are the sites of high concentrations of some alkaloids during floral development or mature flowers (Table 1), the synthesis can occur in other plant tissues as young leaves or roots (Kruse *et al.* 2017). Thus, the alkaloids are translocated to reproductive structures through the vascular system (Stagemann *et al.* 2019). The alkaloids can concentrate in the whole flower or specific organs such as ovaries (Kruse *et al.* 2017) or stamens (Chen *et al.* 2016).The accumulation of

Table 1. Examples of presence of alkaloids during angiosperm flowering and fruiting stages and their respective ecological roles as deterrent (-) or as attractant (+). Number in parentheses refers to the chemical structures in Fig. S1

Reproductive stage	Source (Family)	Alkaloid compounds	Alkaloid type	Site of synthesis	Site of accu-mulation	Ecological effects	References
Flowering	*Coffea spp*	Caffeine (1)	Purine	Leaves	Nectar	(-) Deter florivores and pathogens (+) Favors floral constancy by causing addiction to pollinators	Ashihara *et al.* 2008; Thomson *et al.* 2015
Flowering	*Echium vulgare* (Boraginaceae)	• Echimidine (2) • Echimidine-N-oxide (3) • Echivulgarine (4) • Echivulgarine-N-oxide (5)	Pyrrolizidines	Flower	Nectar Pollen	(-) Toxic pollen to consumers Protection of male gametes.	Lucchetti *et al.* 2016; Kast *et al.* 2018
Flowering	*Nicotiana species* (Solanaceae)	Anabasine (6)	Pyridine	Roots	Flowers Nectar	(-) Deterring florivores and robbers enhancer by improving pollinator learning skills	Kaczorowski *et al.* 2014; Richardson *et al.* 2015
Flowering	*Nicotiana attenuata* (Solanaceae)	Nicotine (7)	Pyridine	Roots	Flowers Nectar	(-) Deter florivores and nectar robbers (+) Confer protection against parasites to main pollinators (+/-) Affect the bacterial community in nectar	Adler *et al.* 2012; Aizenberg-Gershtein *et al.* 2015; Richardson *et al.* 2015
Flowering	*Order Caryophyllales*	Betalains (8)	Indole	Flowers	Vacuoles of tepals or petals	(+) Attract pollinators Photoprotection (+) Improved pollen germination and growth of pollen tubes	Fernández-López *et al.* 2020; Nadotand Carrive 2020 Berardi *et al.* 2013
Flowering Fruiting	*Symphytum officinale* (Boraginaceae)	7-Acetylintermedine (9) Intermedine (10) Lycopsamine (11)	Pyrrolizidine	Roots and young leaves	Ovaries Fruits	(-) Protection of reproductive structures against herbivores	Kruse *et al.* 2017; Stegemann *et al.* 2019
Fruiting	*Crotalaria spectabilis* (Fabaceae)	Monocrotaline (12) Axillaridine (13) Axillarine (14) Desoxyaxillarine (15) O-Senecioylretronicine(16)	Pyrrolizidine	Nodules	Seeds	(-) Provide defense to seedlings after germination	Toppel *et al.*, 1988. Irmer *et al.* 2015
Fruiting	*Dysoxylum-binectariferum* (Meliaceae)	Rohitukine (17)	Piperidine	Stem bark	Cotyle-dons	(-) Defense of seedlings	Kumara *et al.* 2015
Fruiting	*Nothapodytesni-mmoniana* (Stemonuraceae)	Camptothecin (18)	Quinoline	Leaves	Unripe fruits Seeds	(-) Defense against fungal pathogens	Shaanker *et al.* 2008; Sarika 2015
Fruiting	*Papaver somniferum* (Papaveraceae)	Codeine (19) Morphine (20) Oripavine (21) Thebaine (22)	Isoquinolines	Capsule (fruit)	Latici-fers (fruit)	(-) Defense against frugivory during seed development	Lee *et al.* 2013; Hope *et al.* 2019

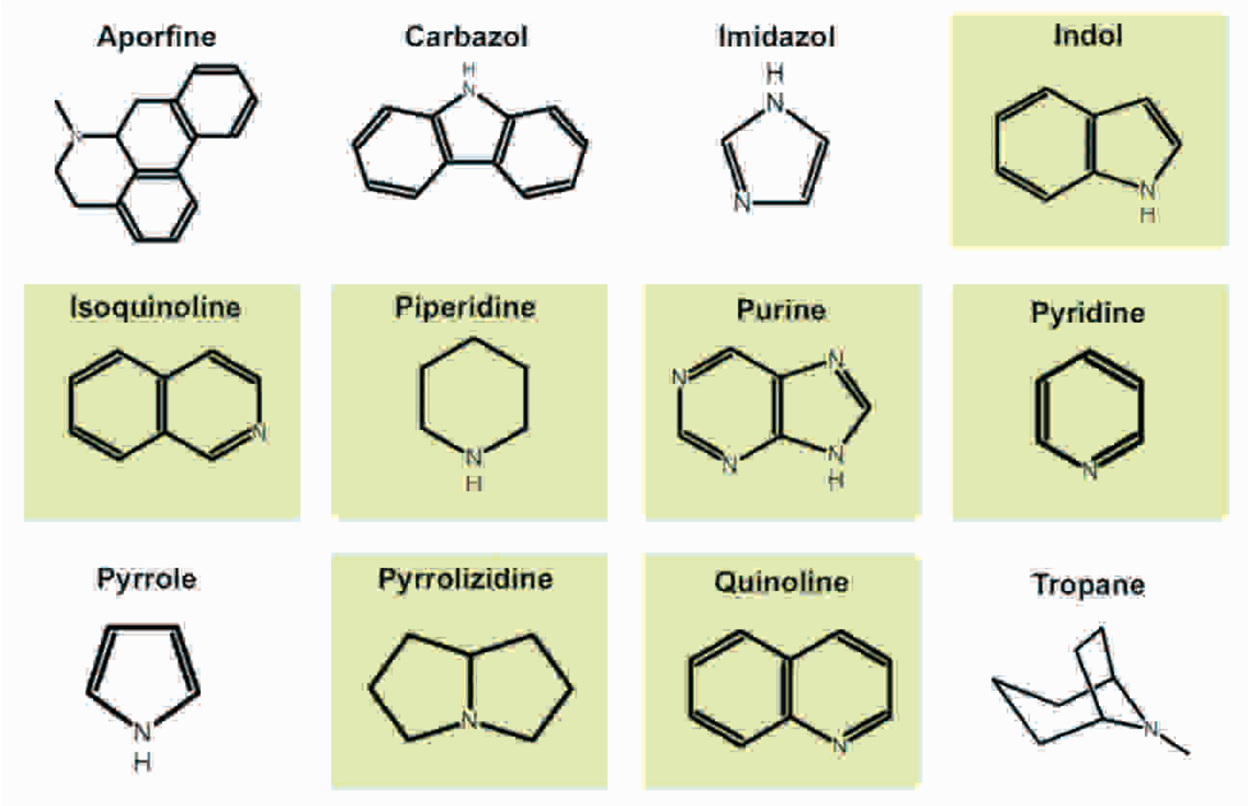

Fig. 1. Types of alkaloids according to their chemical structure. Alkaloids with relevance to reproductive structures of angiosperms (flowers and fruits) are marked in green.

alkaloids in specific organs such as ovaries is a way to protect the offspring and provide future seeds with a chemical defense against predators (discussed later), or as a nitrogen storage source for the embryo (Irmer *et al.* 2015, Wink 2020).

Toxic rewards during pollination : The floral rewards are crucial in the mutualistic pollination interaction (Roy *et al.* 2017). Nectar and pollen are the most common rewards that flowers offer to pollinators (Palmer-Young *et al*. 2018).These rewards provide nutrients to pollinators favoring their constancy to the visited flowers (Davis *et al.* 2019). In addition to nutrients, nectar and pollen may contain certain degree of toxicity caused by alkaloids that deter pollinivorous and nectar robbers (Baracchi *et al.* 2017, Barlow *et al.* 2017, Stevenson *et al.* 2017, Palmer-Young *et al.* 2018). In the particular case of pyrrolizidine alkaloids (PAs), it has been observed that they can be present in both nectar and pollen at relatively high concentrations (Lucchetti *et al*. 2016).

Other common alkaloids reported in nectar are anabasine, caffeine, and nicotine (Kerchner *et al.* 2015, Tölke *et al.* 2020) (Table 1). These alkaloids exhibit a dual role as deterrents and attractants, favoring a higher visitation rate with positive effects on plants and pollinators (Aizenberg-Gershtein *et al.* 2015). The anabasine in nectar favors the floral defense and provides defense against infectious diseases to pollinators such as bumblebees (Anthony *et al.* 2015, Richardson *et al*. 2015). The caffeine in nectar deters florivores or nectar robbers, but it causes addiction to pollinators in low concentrations. The addiction entails increasing the frequency of their visits, improving pollination success (Thomson *et al.* 2015). Nicotine is a volatile alkaloid highly reported in flowers, but its effect in deterring florivores can occur through olfactory signals as the floral scent (Adler *et al*. 2012, Thomson *et al.* 2015). Besides, the nicotine also positively affects pollinator behavior improving their learning skills and favoring more visits to flowers (Baracchi *et al.* 2017). In this sense, despite deterring chemicals in floral rewards, some specialized pollinators exhibit resistance to toxic compounds (de Oliveira 2015, Davis *et al.* 2019,Wink 2019). The resistance to alkaloids is a mutualistic strategy that has evolved through selective pressures that pollinators exert on flowers and vice versa (Kessler and Chautá 2020). Therefore, floral defense mechanisms by producing toxic rewards can be an adaptative trait in specialized pollination systems (Adler *et al*. 2012, Stevenson *et al.* 2017).

The chromoalkaloids as visual signals—The plants have developed strategies as advertisements to attract pollinators (Borghi *et al.* 2017). Those advertisements can be the rewards and olfactory or visual signals to lead the biotic vectors to flowers (Fishman and Hadany 2015). The visual attraction is mainly given by the flowers' coloration pattern (Borghi *et al.* 2017). These patterns are crucial in the communication between plants and animals as they can influence pollinator behavior (Renoult *et al.* 2015).

Compounds as flavonoids, terpenoids, and alkaloids give the coloring of flowers and fruits from secondary metabolism (Grotewold 2006, Nadot and Carrive 2020). The betalains are chromoalkaloids found in different structures such as roots, leaves, flowers, or fruits of some plants (Hussain *et al*. 2018). Betalains originate from L-tyrosine by the generation of betalamic acid and the formation of adducts with amino acids, generating two groups: betacyanins, when the adduct is formed with *cyclo*-DOPA, and betaxanthines when the adduct is formed with any other amino acid (Gandía-Herrero and García-Carmona 2013, Khan and Giridhar 2015, Arimura and Maffei 2016). Betaxanthines have a maximum absorption spectrum at 480 nm, and their molecules present colors ranging from yellow to orange. In comparison, betacyanins present a shift in electromagnetic absorption due to the *cyclo*-DOPA, showing a maximum absorption spectrum at 538 nm. The betacyanins have colorations ranging from red to violet (Grotewold 2006, Gandía-Herrero and García-Carmona 2013). The betalains are exclusively present in the order Caryophyllales in Aizoaceae, Amaranthaceae, Basellaceae, Cactaceae (Fig. 2), Chenopodiaceae, Didieraceae, Nyctaginaceae, Phytolaccaceae, and Portulacaceae (Arimura and Maffei 2016, Hussain *et al*. 2018, Wink *et al*. 2018). Furthermore, it is known that there is a mutual exclusion between the genes involved in the biosynthesis of anthocyanins and betalains; therefore, plants that synthesize betalains do not produce anthocyanins and vice versa (Brockington *et al*. 2011).

Although there are no studies explicitly focused on betalains' ecological role, it is well known that color is a crucial visual attractant in biotic interactions (Borghi *et al*. 2017, Fattorini and Glover 2020). The color of flowers provided by betalains allows the pollinators to discriminate viable and rewarding flowers and represent a long-range visual cue to floral visitors (Weiss and Lamont 1997). Also, it has been observed that betalains play a fundamental role in filtering the light transmitted by leaves and flowers, stimulating pollinators (Gandía-Herrero *et al*. 2005).Thus, the color in flowers and fruits is an adaptative trait resulting from the relationships between plants and their close pollinators and dispersers (Nadot and Carrive 2020).

A particular example of de role of betalains during reproduction occurs in *Mirabilis jalapa* (Nyctaginaceae), where the pollen grains from flowers with a high concentration of betacyanins (red and magenta flowers) perform better that pollen from other floral morphs (yellow and pink flowers) (Berardi *et al.* 2013). Additionally, the role of betalains in reproductive structures could also involve photoprotection and defense against oxidative stress like in vegetative tissues (Jain *et al*. 2015, Li *et al*. 2019, Fernández-López *et al.* 2020). Therefore, the attractant function of betalains is not the only role that these secondary metabolites can play during the reproductive stages of angiosperms.

The alkaloids through fruiting : from seed development to dispersal—The seeds resulting from reproduction will continue the life cycle of new individuals allowing species

Fig. 2. The betalains in flowers of Cactaceae. The variation in betalain colors from violet to red (betacyanins) and orange to yellow (betaxanthins).

perpetuation and habitat colonization and restoration (Emer *et al*. 2018, Simmons *et al.* 2018). To continue the cycle, seeds need to be dispersed to a new suitable place to accomplish germination (Suetsugu 2020). Therefore, seed dispersal is one of the most important phases in plants' life (Cayuela *et al.* 2018). As the seeds are mobile structures separated from the mother that contain the embryos, they have developed strategies against predators. Among the strategies are the toxic compounds as alkaloids that deter consumers (Hotti and Rischer 2017).

Seeds differentially accumulate the alkaloids at different developmental stages or tissues. For example, some fruits or seeds have specialized reward structures for dispersal called elaiosomes (Rios-Carrasco *et al*. 2019). In that sense, the accumulation of alkaloids occurs in seeds but not in elaiosomes (Stegemann *et al*. 2019). Thus, the reward is free of toxic compounds while the embryo remains protected from frugivores. Another scenario occursin fruits dispersed by frugivorous animals (endozoochory), where the alkaloids accumulate in developing seeds (Kumara *et al.* 2015). However, as the fruits reach maturity, the amount of alkaloids decreases favoring dispersal by endozoochory (Kumara *et al*. 2015). In addition to defense, the alkaloids in seeds are also an important nitrogen source for the developing embryo or the seedlings after germination (Irmer *et al.* 2015). This ecological function is common in Fabaceae seeds, where the source of nitrogen is limited during the earlier stages of the plants (Wink 2020).

As in flowers, the alkaloids in fruits or seeds are translocated from other plant tissues where they are primarily synthesized (Irmer *et al.* 2015). Nevertheless, the alkaloids also can synthesize *de novo* if precursors are available (Kumara *et al.* 2015, Hope *et al.* 2019). The alkaloid concentration in fruits varies depending on environmental conditions, influencing plant fitness (Hope *et al.* 2019).

Despite the great importance of alkaloids as a defense or attraction mechanism during the reproductive stages of angiosperms, much is still unknown. This review summarizes alkaloids' ecological functions during flowering and fruiting, including the importance of these secondary metabolites in the biotic interactions as pollination and dispersal. It is expected that future work will address research related to the dual role that alkaloids can have during plant reproduction. Since they are mostly focused on their role as deterrents, leaving aside their properties as attractants.

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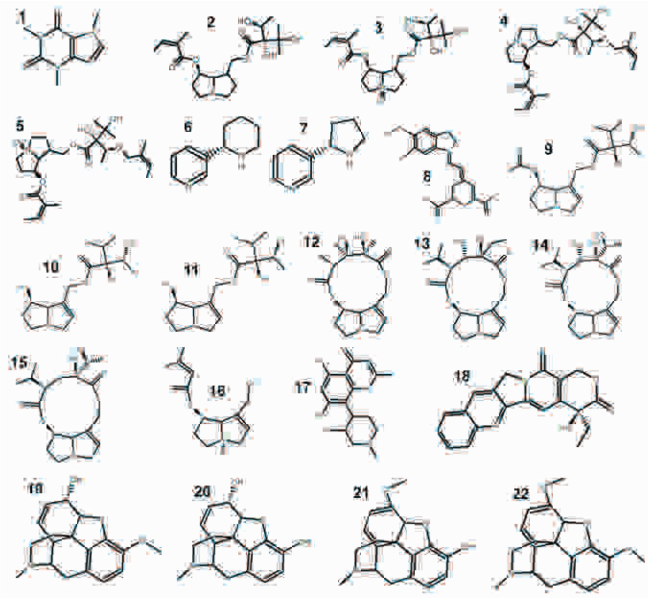

Fig. S1 Chemical structures of alkaloids present during angiosperm flowering and fruiting stages

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