Current knowledge and future research perspectives on cassava (*Manihot esculenta* Crantz) chemical defenses: An agroecological view

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A B S T R A C T

Cassava (*Manihot esculenta* Crantz) is one of the most important staple crops worldwide. It constitutes the major source of carbohydrates for millions of low-income people living in rural areas, as well as a cash crop for smallholders in tropical and sub-tropical regions. The Food and Agriculture Organization of the United Nations predicts that cassava plantations will increase and production systems will intensify in the future, highlighting the need for developing strategies that improve the sustainability of production. Plant chemical defenses hold the potential for developing pest management strategies, as these plant traits can influence the behavior and performance of both pests and beneficial arthropods. Cassava plants are well-defended and produce a number of compounds involved in direct defense, such as cyanogenic glycosides, flavonoid glycosides, and hydroxycoumarins. In addition, volatile organic compounds induced upon herbivory and the secretion of extrafloral nectar act as indirect defense against herbivores by recruiting natural enemies. Here, cassava chemical defenses against pest arthropods are reviewed, with the aim of identifying gaps in our knowledge and areas of research that deserve further investigation for developing sound pest control strategies to improve sustainable production of this crop, and how these defenses can be used to benefit other crops. Cyanogenic content in cassava is also highly toxic to humans, and can cause irreversible health problems even at sub-lethal doses when consumed over prolonged periods. Therefore, the promotion of chemical defense in this crop should not aggravate these problems, and must be accompanied with the education on processing methods that reduce human exposure to cyanide.

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1. Introduction

Cassava (*Manihot esculenta* Crantz) is a perennial shrub belonging to the Euphorbiaceae family. From a socio-economic point of view, it is one of the most important crops in tropical and sub-tropical regions worldwide. In its native range of South America, where it was domesticated many thousands of years ago (FAO, 2013; McKey et al., 2010), and in Africa, where it was introduced during the 16th century, cassava is one of the most important staple food sources for low-income families in rural areas and a primary cash crop for smallholders. In contrast in Asia, cassava was introduced much later, about two hundred years ago (Howeler, 2000), and is mainly grown for exporting industrialized products (dried chips, pellets and starch for animal feed and industry) as well as for fuel production (FAO, 2013; Zhou and Thomson, 2009). In terms of food security, the importance of this crop relies on several plant traits. Cassava accumulates starch in the root parenchyma, making this starchy organ an important source of carbohydrates. Roots also contain significant levels of vitamin C, riboflavin, thiamin and niacin (FAO, 1997). Currently this crop feeds more than 800 million people worldwide (FAO, 2013). Moreover, plants are adapted to grow fairly well in marginal soils and withstand severe conditions of heat and drought, which makes cassava a key crop in arid regions, as well as under the predicted changes in climatic conditions (Burns et al., 2010; Rosenthal et al., 2012). Smallholders seldom spray pesticides in cassava plantations, as chemical control is not economically viable. However, since plantations of cassava are expected to expand in the future (FAO, 2013), this situation may

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change, due to the pressures to intensify production under modern agricultural paradigms.

For increasing sustainability of production systems, pest control strategies that are environmentally friendly and economically accessible to growers need to be developed and implemented. Plants are active components of multitrophic interactions and in order to develop sound pest management strategies, it is essential to understand the ecology of diversified agroecosystems (Lewis et al., 1997) and exploit the plant's own defenses. Chemical defenses against herbivores in plants include an array of volatile and non-volatile compounds that can negatively affect behavior and performance of arthropod herbivores (direct defenses) or enhance the activity of natural enemies that exert biological control (indirect defenses) (Mithöfer and Boland, 2012). These defenses can be expressed constitutively, without requiring any abiotic or biotic stress for synthesis. In addition to constitutive expression, chemical defenses can be induced upon herbivore damage or other types of environmental stress (Chen, 2008; Karban and Baldwin, 1997; Turlings and Wäckers, 2004).

Cassava plants are well-defended and possess an array of chemical strategies involved in both bottom-up (resource-based) and top-down (enemy-based) herbivore regulation. It has been argued that chemical defenses of cassava underlie the agronomical advantages of this crop (McKey et al., 2010 and references therein). Perhaps, because of the occurrence of these defense mechanisms, there is a number of herbivores associated with this plant (Table 1) but only a few species have the potential to cause yield losses (Bellotti et al., 1999; FAO, 2013). Cyanogenic glycosides comprise the most studied group of chemical defenses, probably because of the well-known toxicity of these compounds to arthropods and other animals. Cyanide produced from the breakdown of the glycoside is an effective poison even for plants. Due to the cyanogenic potential of this species, consumption of insufficiently processed cassava poses risks to human health. Acute intoxication with cyanide can cause vomiting, tachypnea, tachycardia dizziness, headache, abdominal pain, diarrhea, mental confusion and convulsions (Cliff et al., 1997). In addition, prolonged exposure to cyanide at sub-lethal doses, such as continued consumption of insufficiently or incorrectly processed cassava, can cause irreversible neurological diseases, such as Konzo and tropical ataxic neuropathy (TAN), among other serious disorders (Nhassico et al., 2008). In addition to cyanogenic glycosides, however, cassava leaves and/or roots contain other compounds such as different phenolics and terpenoids (Blagbrough et al., 2010; Montagnac et al., 2009). Moreover, cassava plants emit volatile organic compounds (VOCs) and secrete extrafloral nectar (EFN), all of which play an important role in biological control. Burns et al. (2010) suggest that boosting natural chemical defenses of cassava plants can improve sustainability of production of this crop. Here, the current state of knowledge on the chemistry of cassava plants is reviewed from the perspective of the ecology of plant-insect interactions focusing on plant defenses against arthropod herbivores. Therefore, the aim is to identify knowledge gaps and areas of research that deserve further attention in the exploitation of chemical defenses of cassava in the light of sustainable agricultural production.

2. Direct defenses

2.1. Cyanogenic glycosides

Cyanogenic glycosides are relatively widespread in the plant kingdom and constitute an important group of secondary metabolites involved in plant defense against arthropod herbivores (Gleadow and Møller, 2014). Chemically, they are composed of an α-hydroxynitrile type aglycone and a sugar moiety (mostly D-glucose) (Vetter, 2000). In cassava, L-valine-derived linamarin (**1**) and L-isoleucine-derived lotaustralin (methyl linamarin) (**2**) (Fig. 1) are the most abundant cyanogenic glycosides and account for, respectively, over 90% and under 10% of total cyanogenic compounds in cassava (McMahon et al., 1995). There is, however, considerable genetic variation in the levels of cyanogenic glycosides (Burns et al., 2012). In addition, environmental factors such as nitrogen supply (Jørgensen et al., 2005) and water deficit (Cardoso et al., 1999) contribute significantly to the cyanogenic content of plants. The biosynthesis of these compounds occurs commonly in shoots from where they translocate to roots (Jørgensen et al., 2005), though low quantities of cyanogenic glycosides are also biosynthesized in the roots (McMahon et al., 1995). Therefore, cyanogenic glycosides are present in all plant tissues but mainly in the leaves (Jørgensen et al., 2005; White et al., 1998). The enzyme linamarase is synthesized in the laticifers (Pancoro and Hughes, 1992) and is concentrated in the latex where its activity has been reported to be more than 300-fold higher than in leaves (Nambisan, 1999). Cyanogenesis in cassava starts when the vacuole releases linamarin (**1**) and lotaustralin (**2**) upon cell rupture, which commonly occurs during mechanical damage or feeding by chewing herbivores, and these compounds encounter the hydrolyzing enzymes. Degradation of cyanogenic glycosides by linamarase results in the formation of an unstable cyanohydrin (**10**). This compound can be broken down enzymatically by α-hydroxynitrile lyase (HNL), or be spontaneously decomposed at pH greater than 5.0 or temperatures over 35 °C (Fig. 2) (White et al., 1998) to form acetone and hydrogen cyanide (HCN), both being highly toxic to herbivores (Mithöfer and Boland, 2012).

The role of linamarin (**1**), lotaustralin (**2**) and the linamarin-deglycosylated form acetone cyanohydrin in plant defense has been studied in several combinations of herbivores and plants (for a review, see Gleadow and Woodrow, 2002; Nahrstedt, 1985). As a general rule, strongly cyanogenic plants are protected from generalist rather than specialist herbivores (Ballhorn et al., 2010; for a review, see Gleadow and Woodrow, 2002). Cyanogen content in cassava leaves is known to deter the generalist grasshopper *Zonocerus variegatus* (Orthoptera: Pyrgomorphidae) from feeding (Bellotti and Riis, 1994; Bernays et al., 1977). This species prefers to feed on senescent or wilting leaves (Bernays et al., 1977) that release HCN more slowly compared to younger growing leaves. Only starved adult grasshoppers can feed on growing cassava leaves, but they progressively lose weight, whereas nymphs do not accept growing leaves at all (Bernays et al., 1977). Negative effects of cyanogenic glycosides are also reported for the generalist burrowing bug *Cyrtomenus bergi* (Hemiptera: Cydnidae), a root feeder in cassava plantations. Intracellular penetration of the stylet in the root parenchyma during feeding results in the accumulation of linamarin (**1**) in the hemolymph causing greater nymphal mortality, particularly during early instars. Adults fed on clones with high cyanogenic levels showed lower longevity and fecundity but not significantly higher death rates (Bellotti and Riis, 1994; Riis et al., 2003). The longer stylet of adults allows them to feed beyond the outer layers or cortex of the roots, where the larger linamarin (**1**) contents occur, which results in better performance of adults compared to nymphs (Bellotti and Arias, 1993). A study on the preference of whiteflies *Bemisia tabaci* (Hemiptera: Aleyrodidae) for cassava varieties with different levels of "bitterness" found fewer insects settled on the most "bitter" varieties (Dengel, 1981) suggesting that preference is negatively affected by higher cyanogenic glycoside contents. Beyond these few examples, we are not aware of other studies assessing the effect of cyanogenic glycosides on other herbivores associated with cassava. However, cyanogenic glycosides may also be involved in the resistance against other generalist herbivores attacking this crop. From studies in *Lotus*

Table 1
Major arthropods (mites and insects) of cassava plants.[a]

Herbivore	Damage[a,b]
Mites	
Acari (Tetranychidae)	
Mononychellus tanajoa	Leaves with yellow spots and deformed. Necrosis of stems and leaves progressively from top to bottom.
Oligonychus gossypii *Oligonychus peruvianus* *Oligonychus pratensis*	Small white spots along the central and lateral leaf veins and margin. Damage more pronounced on lower leaves.
Tetranychus cinnabarinus *Tetranychus desertorum* *Tetranychus marianae* *Tetranychus truncates* *Tetranychus urticae*	Yellow dots along the main leaf vein, beginning with the basal leaves. Severely infested leaves dry and drop, and plants may die.
Insects	
Orthoptera	
Zonocerus elegans (Pyrgomorphidae) *Zonocerus variegatus* (Pyrgomorphidae) *Conocephalus cinereus* (Tettigoniidae) *Eutropidacris cristata* (Acrididae) *Orphulella punctata* (Acrididae) *Schistocerca cancellata* (Acrididae) *Schistocerca gregaria* (Acrididae) *Schistocerca nitens* (Acrididae)	Complete defoliation of plants can occur, and heavy outbreaks result in stripping of the bark. They may also act as vectors of cassava bacterial blight.
Isoptera (Rhinotermitidae)	
Coptotermes curvignathus *Heterotermes convexinotatus*	They feed on propagative material, roots or growing plants. Principal damage appears to be loss of cuttings.
Thysanoptera (Thripidae)	
Corynothrips stenopterus *Dendrothripoides innoxius* *Frankliniella melanommatus* *Heliothrips haemorrhoidalis*	Yield reductions range from 5.6 to 28.4%, depending upon varietal susceptibility.
Hemiptera	
Amblypelta cocophaga (Coreidae) *Amblyoelta lutescens* (Coreidae) *Leptoglossus gonagra* (Coreidae) *Pseudotheraptus devastans* (Coreidae) *Corythaica carinata* (Tingidae) *Corythuca gossypii* (Tingidae) *Vatiga illudens* (Tingidae) *Vatiga manihotae* (Tingidae) *Cyrtomenus bergi* (Cydnidae) *Edessa meditabunda* (Pentatomidae)	Foliar damage with yellow spots that eventually turn reddish brown, resembling mite damage.
Whiteflies	
Aleurodicus dispersus (Aleyrodidae) *Aleurotrachelus socialis* (Aleyrodidae) *Bemisia tabaci* (Aleyrodidae) *Bemisia tuberculata* (Aleyrodidae)	Particular importance as vectors of cassava mosaic disease. May cause yellowing and necrosis of the lower leaves.
Scale Insects	
Aonidomytilus albus (Diaspididae) *Pinnaspis strachani* (Diaspididae) *Ceroplastes cirripediformis* (Coccidae) *Coccus viridis* (Coccidae) *Parasaissetia nigra* (Coccidae) *Saissetia coffeae* (Coccidae) *Dysmicoccus brevipes* (Pseudococcidae) *Ferrisia virgata* (Pseudococcidae) *Nipaecoccus nipae* (Pseudococcidae) *Paracoccus marginatus* (Pseudococcidae)	Leaves on attacked stems turn yellow and drop; in severe attack, the plants are stunted and stems can desiccate, causing plant mortality. Heavy infestations may cover the stem and lateral buds.
Mealybugs	
Phenacoccus madeirensis (Pseudococcidae) *Phenacoccus manihoti* (Pseudococcidae) *Phenacoccus herreni* (Pseudococcidae) *Stictococcus vayssierei* (Stictococcidae)	High populations cause defoliation of cassava plants and drying of stem tissue, resulting in loss of planting material.

Table 1 (*continued*)

| Herbivore	Damage[a,b]
Erythroneura cassavae (Cicadellidae) *Jacobiasca formosana* (Cicadellidae)	Young plant shoots, which retards bud growth and causes yellow-green bud curling.
Coleoptera	
Anomala antiqua (Rutelidae) *Anomala viridis* (Rutelidae)	Adults are leaf feeders, and larvae live in the ground, feeding on roots.
Coelosternus alternans (Curculionidae) *Coelosternus sulcatulus* (Curculionidae) *Diaprepes abbreviatus* (Curculionidae) *Prepodes quadrivittatus* (Curculionidae) *Sternocoelus tardipes* (Curculionidae)	Larvae penetrate the stems and branches and may dry and break. They reduce root production and quality of planting material.
Dihammus rusticator (Cerambycidae) *Ecyrus hirtipes* (Cerambycidae) *Lagocheirus obsoletus* (Cerambycidae) *Lagocheirus araneiformis* (Cerambycidae) *Phyllophaga hogardi* (Scarabeidae) *Leucopholis rorida* (Scarabeidae)	Destruction of the bark and buds of recently planted cuttings and the presence of tunnels in the woody part. These cuttings may rot and die.
Diptera	
Anastrepha manihoti (Tephritidae)	Larvae bore into the fruit, destroying the seed. Larval tunneling in the stem results in brown galleries.
Jatrophobia braziliensis (Cecidomyiidae)	A severe attack causes yellowing of leaves, retarding plant growth; roots become thin and fibrous.
Silba chalybea (Lonchaeidae) *Silba perezi* (Lonchaeidae) *Neosilba nigrocaerula* (Lonchaeidae) *Neosilba pendula* (Lonchaeidae)	Retards plant growth, breaks apical dominance, and causes germination of side buds.
Lepidoptera	
Erinnyis ello (Sphingidae)	One of the most serious pests of cassava, and can rapidly defoliate plants.
Chilozela trapeziana (Crambidae)	Larvae feed on the leaves.
Haritalodes derogata (Crambidae)	Larvae eat the leaf margins, causing the leaves to curl and drop.
Eumeta variegata (Psychidae)	Commonly known as defoliator of forest species.
Parasa lepida (Limacodidae) *Phobetron hipparchia* (Limacodidae)	Severe defoliation.
Agrotis ipsilon (Noctuidae) *Feltia subterranea* (Noctuidae)	It damages seedlings by cutting off the stem at the soil surface, and older plants by feeding on foliage.
Spodoptera albula (Noctuidae) *Spodoptera litura* (Noctuidae) *Spodoptera ornithogalli* (Noctuidae)	Damage arises from extensive feeding by larvae, leading to complete stripping of the plants.
Hymenoptera (Formicidae)	
Atta spp. *Acromyrmex* spp.	Defoliated plants. Outbreaks frequently occur during the early months of the crop.

[a] CABI (2015).
[b] Plantwise (2015).

corniculatus and *Phaseoulus lunatus* (Fabaceae), there is evidence that linamarin (**1**) content affects the feeding preference of various generalist grasshoppers (Compton and Jones, 1985) and the progeny sex ratio of the two-spotted spider mite *Tetranychus urticae* (Acari: Tetranychidae) (Rojas and Morales-Ramos, 2010), a herbivore of cassava plants.

Herbivores regarded as major cassava pests include specialist Neotropical arthropods such as the cassava hornworm *Erinnyis ello* (Lepidoptera: Sphingidae), the cassava green mite *Mononychellus tanajoa* (Acari: Tetranychidae), the cassava mealybug *Phenacoccus manihoti* (Hemiptera: Pseudococcidae), as well as whiteflies and thrips species specialized on this crop (Table 1). However, some of these species have been accidentally introduced into other regions. The cassava green mite caused serious economic damage in Africa before the introduction of Neotropical species of predatory mites, and more recently, *P. manihoti* has recently been recorded in Asia and threatens cassava plantations in that part of the world (Parsa et al., 2012). In contrast to generalists, specialist arthropods have

co-evolved with their hosts and they are able to tolerate or detoxify defensive chemicals. Some species can even ingest these compounds as a source of nitrogen for the biosynthesis of proteins (for a review, see Gleadow and Woodrow, 2002). Furthermore, cyanogenic glycosides can be exploited as oviposition or feeding stimulants (Calatayud, 2000; Honda et al., 1997), or sequestered by specialist herbivorous arthropods for their own defense (Nahrstedt, 1989; Zagrobelny et al., 2004). There is little evidence on how chemical defenses drive plant-specialist herbivore interactions in cassava. Based on interviews with South American Indians, however, Wilson (2003) concluded that there is no difference between cultivars with low and high levels of cyanogenic glycosides in the incidence of the attack of *E. ello*. The developmental time of *M. tanajoa* was not adversely affected by the genotype when comparing cultivated cassava, wild *Manihot peruviana* and *M. flabellifolia*, which may contain higher contents of cyanogenic glycosides than cultivated cassava (Boaventura et al., 2009; Wang et al., 2014). However, larger numbers of cassava green mites
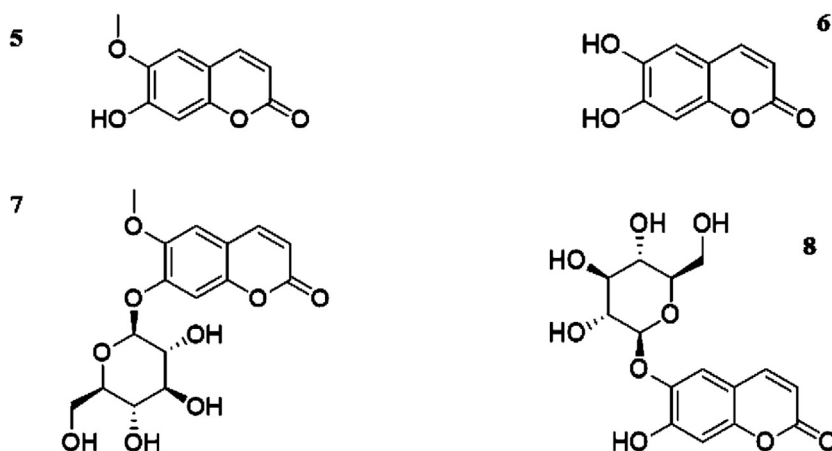
CYANOGENIC GLYCOSIDES
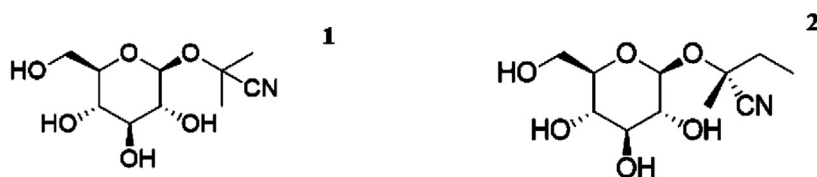
FLAVONOIDS
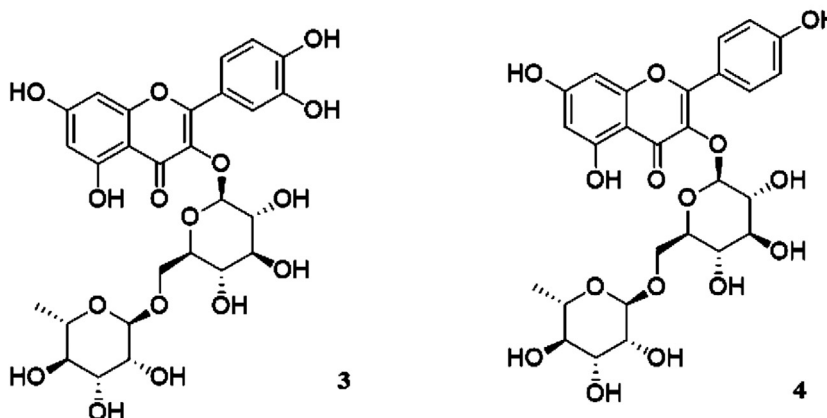
HYDROXYCOUMARINS

Fig. 1. Molecular structures of main chemical compounds involved in direct defenses found in cassava plants: linamarin (**1**), lotaustralin (**2**), rutin (**3**) and its isomer kaempferol-3-*O*-rutinoside (**4**), scopoletin (**5**), esculetin (**6**) and their glucosides, scopolin (**7**) and esculin (**8**).

were previously reported in "bitter" varieties compared with "sweet" varieties thereby suggesting a preference for highly cyanogenic plants (Shukla, 1976). The cassava mealybug *P. manihoti* performs equally well when fed on artificial diets containing a range of concentrations of linamarin (**1**) (Calatayud, 2000). More interesting is the fact that this species exploits cyanogenic compounds present in the phloem sap of cassava plants as phagoestimulants (Calatayud, 2000 and references therein). According to Calatayud et al. (1997), there might be bacteria present in the midgut of the insect that produce a variant of linamarase. Insects with sap-sucking mouthparts may have a competitive advantage over chewing insects when feeding on cyanogenic plants, since their mode of feeding causes little mechanical damage to plant tissue, and therefore little or no degradation of cyanogenic glycosides into more toxic compounds (for a review, see Gleadow and Woodrow, 2002). Nevertheless, specialized chewing insects have

evolved feeding behaviors to circumvent poisonous plant defenses (Després et al., 2007), such as severing (i.e. 'trenching') the laticifers upstream of the feeding site (Fig. 3).

2.2. Phenolic compounds

Phenolic compounds found in cassava leaves and roots include two groups, flavonoid glycosides and hydroxycoumarins. Both are bioactive groups that play important roles in plant defense against not only arthropod herbivores, but also pathogens. In cassava leaves, the flavonoid glycoside rutin (**3**) (quercetin-3-*O*-rutinoside) and a kaempferol-containing isomer (**4**) (kaempferol-3-*O*-rutinoside) (Fig. 1) have been identified. Rutin (**3**) occurs in several plant species, and depending on the concentration, this compound can act as a phagostimulant or a feeding deterrent for many generalist herbivores (for a review, see Simmonds, 2003). Detrimental effects
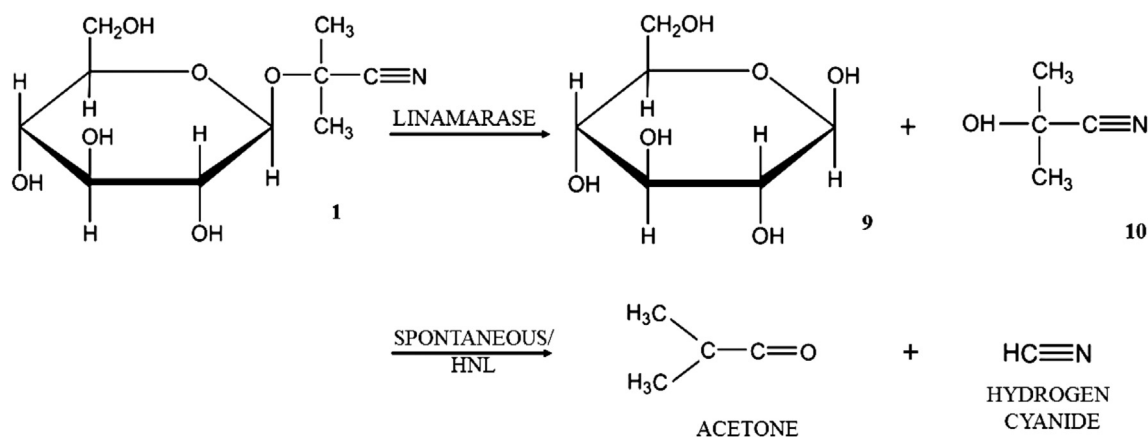
Fig. 2. Cyanogenesis of linamarin (**1**).

of rutin (**3**) occur upon hydrolyzation of the glycosidic bond that results in the release of the aglycone quercetin, which can inhibit mitochondrial ATPase and cytochrome P450-dependent mixed function oxidases (for a review, see Simmonds, 2001). In cassava, this flavonoid glycoside is induced upon herbivory by *P. manihoti* and can negatively affect weight and increase the developmental time of this specialist mealybug (Calatayud et al., 1994a). Negative effects are more evident as the ratio of rutin (**3**)/linamarin (**1**) in a diet increases.

Hydroxycoumarins function as phytoalexins in plants and are commonly induced upon pathogen attack and wounding (Yamane et al., 2010). They have recently been involved in the response of plants to abiotic oxidative stress, which activates biochemical pathways also known to be involved in plant responses to biotic defenses (Bourgaud et al., 2006; Petrov et al., 2015). In cassava leaves and roots, four hydroxylated coumarins are found: scopoletin (**5**), esculetin (**6**), scopolin (the 7-β-D-glycoside of **5**), (**7**) and esculin (**8**) (glycoside of **6**) (Fig. 1) (Blagbrough et al., 2010; Gómez-Vásquez et al., 2004). These compounds have been associated with the physiological process of root deterioration upon harvesting cassava (Blagbrough et al., 2010). There is, however, evidence from other plant-herbivore systems indicating that hydroxycoumarins are also involved in induced plant resistance to herbivores. For example, scopoletin (**5**) is induced after feeding by the beetle *Zygogramma exclamationis* (Coleoptera: Chrysomelidae) in sunflower, which is a non-cyanogenic plant. This induction can confer resistance against the beetle by decreasing feeding preference (Olson and Roseland, 1991). Scopoletin (**5**) has also proved to be a feeding deterrent and has growth inhibition potential against *Spilarctia obliqua* (Lepidoptera: Noctuidae) (Tripathi et al., 2011). Recently esculin (**7**) has been involved in the inhibition of trehalases in insects, which may impair insect development (Silva et al., 2006).

3. Indirect defense

3.1. Herbivore-induced plant volatile organic compounds

The recruitment of natural enemies (e.g. predators and parasitoids) upon plant herbivore damage is a widespread phenomenon in nature and has been documented in a number of studies involving different tritrophic systems (see recent reviews by Gols, 2014; Heil, 2014; McCormick et al., 2012). The list of predators and parasitoids responding to these signals grows annually. In cassava, several studies have shown the attraction of natural enemies to herbivore-damaged plants. These beneficial organisms

include natural enemies of the cassava mealybug, such as the encyrtid parasitoids *Anagyrus lopezi, Apoanagyrus diversicornis, Aenasius vexans* (Hymenoptera: Encyrtidae) and the predator *Exochomus flaviventris* (Coleoptera: Coccinellidae) (Bertschy et al., 1997; Le Rü and Makosso, 2001; Nadel and van Alphen, 1987; Souissi, 1999). In addition, the predatory mites *Typhlodromalus manihoti* and *Typhlodromalus aripo* (Acari: Phytoseiidae) (Gnanvossou et al., 2003), which are predators of the cassava green mite, have been shown to be attracted to *M. tanajoa*-damaged plants. These two predator species were successfully introduced in Africa and are nowadays an important component in the management of this pest in several African countries.

The recruitment of natural enemies is mediated by changes in plant volatile organic compounds (VOCs). Broadly speaking, these

Fig. 3. Feeding behavior of the cassava hornworm *Erinnyis ello*. Leaf-trenching by a larva (**A**) upstream of the intended feeding site on cassava plants (**B**) (photos by DM Pinto-Zevallos).

include an array of terpenoids (mono-, sesqui- and homo-terpenoids), C6 alcohols, aldehydes, ketones and esters derived from fatty acids (commonly referred to as green leaf volatiles — GLVs), phenylpropanoids and, to a lesser extent, amino-acid (others than phenylalanine) derived compounds (Dudareva et al., 2006) emitted in a blend to which natural enemies respond. In cassava, compounds found to be induced upon *M. tanajoa* feeding include linalool (**11**), methyl salicylate (**13**) (MeSA) and (3*E*,7*E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (**15**) [(*E,E*)-TMTT], but particularly (*E*)-β-ocimene (**12**) and (3*E*)-4,8-dimethyl-1,3,7-nonatriene (**14**) [(*E*)-DMNT] (Fig. 4) (Hountondji et al., 2005). Though it is demonstrated that herbivore-induced VOCs are involved in the orientation of predatory mites during prey location, it is not known which compounds or combination of compounds are exploited by predators. Most of these compounds, however, are recognized as infochemical cues in another mite-based model system (De Boer et al., 2004), and it is quite likely that predatory mites in cassava agroecosystems exploit a combination of volatile compounds that allow specific recognition of herbivore-damaged plants (Bruce et al., 2005). Other compounds identified in the headspace of cassava plants include the GLVs (*Z*)-3-hexen-1-ol (**16**) and (*E*)-2-hexen-1-ol (**17**), but their emission upon mite infestation does not differ when compared to emission from uninfested plants (Hountondji et al., 2005). Feeding by chewing insects, however, usually causes larger mechanical damage compared to feeding by mites or sap-sucking insects, which results in larger emissions of GLVs that may act as important cues for higher trophic levels (see Scala et al., 2013 for a review on GLVs). In addition to arthropod natural enemies, *M. tanajoa*-induced plant VOCs can promote sporulation of *Neozygites tanajoae*, an entomopathogenic fungus of the cassava green mite (Hountondji et al., 2005).

3.2. Extrafloral nectar (leaf exudate)

Many plants secrete an aqueous sugar-rich solution from vegetative parts commonly referred to as extrafloral nectar (EFN). Usually, EFN is secreted by specialized structures (extrafloral nectaries) and contains high levels of mono- and disaccharides (mainly glucose (**9**), fructose and sucrose) in addition to small quantities of amino acids (Wäckers et al., 2005). In cassava, stems, midribs, petioles and the veins on the abaxial sides of the leaves secrete EFN, especially when the leaves are young (Fig. 5). Though Pereira and Splittstoesser (1987) did not observe specific structures for secreting EFN in these plant parts, more recent studies in cassava leaves, revealed circular extrafloral nectaries with a discernible cavity outlined by a rim, and pointed to the palisade parenchyma cells on the tissue as the ones responsible for secreting EFN (Ogburia, 2003). Cassava EFN or cassava leaf exudate, as it is commonly known, contains a major fraction of fructofuranosides (63.3% of dry weight on average among six cultivars) and a minor fraction of reducing oligosaccharides (23.8% of dry weight on average among six cultivars) (Pereira and Splittstoesser, 1987). Amino acids are present in much lower amounts, usually constituting less than 1% of the dry weight of the exudate. In addition, cassava leaf exudate contains substantial levels of bound cyanide, as well as traces of rutin (**3**) and two kaempferol derivates (Calatayud et al., 1994b), which may confer particular organoleptic properties to the nectar, or make it deterrent or toxic to consumers. The secretion of EFN in cassava varies among plant parts (Ogburia, 2003), plant genotype (Pereira and Splittstoesser, 1987) and season (Toko et al., 1994) even though plants produce EFN throughout the growing season.

Recently Heil (2015) reviewed several aspects of EFN in the context of plant-insect interactions. Like herbivore-induced VOCs, EFN acts as indirect plant defense 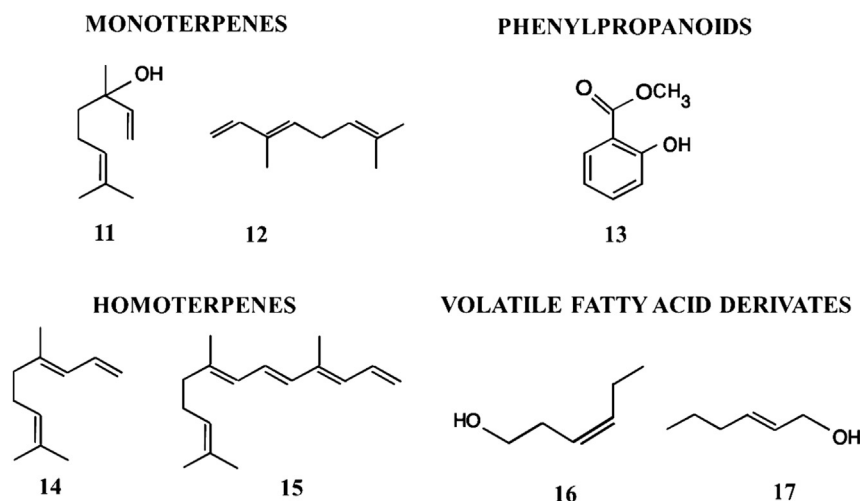by recruiting and supplementing herbivore natural enemies with alternative food resources. In addition to ants (Wäckers et al., 2005 for a review), predatory arthropods and parasitoids belonging to a number of taxonomic groups exploit and benefit from EFN (see Heil, 2015 for a review). In cassava, few studies have addressed the importance of alternative food sources for predatory mites associated to this crop. Cassava leaf exudate arrests foraging adults (Bakker and Klein, 1992), supports nymphal development and enhances adult longevity (Bakker and Klein, 1992; Toko et al., 1994) of the predatory mite *Typhlodromalus limonicus* (Acari: Phytoseiidae). However, adults of this species feeding exclusively on leaf exudate do not lay eggs (Toko et al., 1994). In a similar study, Gnanvossou et al. (2005) found that cassava leaf exudate enhances the development of *T. aripo* and *T. manihoti* in the absence of prey but reproduction does not occur in *T. manihoti*. Together these results highlight the importance of EFN as a source of carbohydrates to maintain predatory mites during periods of low prey density, as well as the importance of protein-rich food sources (e.g. pollen and prey) to build up the population of predatory mites. In the field, the secretion of cassava exudate occurs in younger tissues where normally colonies of the green mites occur even at very low densities, and close to the upper bud where predatory mites usually hide during the day (Onzo et al., 2009). In addition, the largest secretion of EFN occurs during the rainy season (Toko et al., 1994) when, interestingly, the lowest prey densities occur. These spatio-temporal variations in the secretion of this food source supports the role of EFN as a plant indirect defense mechanism (Heil, 2015).

4. Defenses in cassava in an agricultural context. Further directions

Here, the review of the role of different defense strategies of cassava plants against herbivorous arthropods was motivated for several reasons. First, cassava is one of the most important crops for food security and there is no doubt that issues related to sound pest management strategies are of great interest. Second, small farmers rarely use the external inputs characteristic of intensive agriculture (pesticides and fertilizers) in this crop, and rely on natural processes and polycultures for providing ecosystem services such as soil fertility and pest control. Therefore, ecologically-oriented pest management practices are feasible and necessary in cassava. Third, as the planted area of cassava expands (FAO, 2013) and production intensifies, new pest and disease problems are likely to appear. Growers will be more prone to use pesticides, since these are still marketed as sure-fire methods for pest control, despite the enormous problems of pest resurgence and resistance that are threatening production systems worldwide. Here, four areas are highlighted as potentially important for future research on cassava: the importance of direct defenses, tritrophic interactions, cassava as an integral part of polycultures in small-scale farming and ecological and nutritional trade-offs involved in the implementation of ecological pest control methods.

4.1. Direct defenses in cassava

To our knowledge, few studies (most of them referenced in this review) have focused on direct chemical defenses in this crop and behavioral or biological responses have not been coupled to phytochemical analyses. This is surprising since our understanding of the evolution, chemistry and ecology of plant defense mechanisms, particularly induced defenses, has increased tremendously during the last three decades. Understanding how plants react to herbivore-associated stimuli, and which plant-mediated intra- and interspecific interactions are involved could broaden the knowledge base necessary for developing new pest management

MONOTERPENES

PHENYLPROPANOIDS

11   12

13

HOMOTERPENES

VOLATILE FATTY ACID DERIVATES

14   15

16   17

Fig. 4. Main volatile organic compounds (VOCs) induced upon feeding by the cassava green mite *Mononychellus tanajoa* on cassava plants. Linalool (**11**), (*E*)-β-ocimene (**12**), methyl salicylate (**13**) (MeSA), 4,8-dimethyl1,3*E*,8-dimethylnonatriene (**14**) (DMNT) and 4,8,12-trimethyl-1,3*E*,7*E*,11-tridecatetraene (**15**) (TMTT). The fatty acid derivates (*Z*)-3-hexen-1-ol (**16**) and (*E*)-2-hexen-1-ol (**17**) have also been found in the headspace of herbivore-damaged plants (from Hountondji et al., 2005).

strategies that are both ecologically sustainable and socially just. Studying the interactions between other secondary metabolites such as flavonoid glycosides (Calatayud et al., 1994b) is a promising area of research when considering plant defenses against specialist herbivores, or considering phloem-sucking insects that hardly cause cell rupture and therefore, low HCN formation. Additionally, it would be interesting to understand how multiple metabolites may interact against herbivores and pathogens (Osbourn, 1996). Particular traits involved in direct defense of plants can be incorporated into breeding programs. Alternatively, induction of formation of particular compounds can be boosted by the use of external elicitors (Thaler, 1999) or simply by suitable neighboring plants through chemically-mediated associational resistance (Barbosa et al., 2009).

Environmental conditions can largely influence not only physical characteristics of plant tissues but also the quality and quantity of phytochemical traits. Lighting conditions have a direct impact on photosynthesis whereas water and nutrient supply are major factors limiting carbon fixation and nutrient uptake. In addition,

abiotic stress such as extreme heat or excessive light, water deficit and salinity, as well as air or soil pollution, can trigger plant defense mechanisms similar to those observed in response to herbivore and pathogen attack, leading to temporal accumulation of particular compounds involved in plant defense (Atkinson and Urwin, 2012; Suzuki et al., 2014). Burns et al. (2010) have already reviewed and discussed some knowledge gaps relating to the impact of abiotic factors on the sustainability of this crop. As mentioned in Section 2.1, nitrogen supply favors the accumulation of cyanogens in cassava (Jørgensen et al., 2005). Drought can result in increased cyanogenic potential of this crop, a condition which is reversed when water supply is restored (Cardoso et al., 1999). As phytochemical traits can influence herbivore oviposition and feeding preferences as well as herbivore performance (Berenbaum and Feeny, 2008), understanding how environmental factors affect plant-insect interactions is of great interest. This should be particularly important for cassava, which can grow in poor soils and withstand water deficit and heat, and upon which people are expected to rely more in the predicted conditions of climate change (Burns et al., 2010). Abiotic stress can increase or decrease plant defenses against biotic stress such as herbivores (for a review, see Suzuki et al., 2014).

In the context of pest management in agricultural crops, secondary metabolites from cassava hold the potential to control pests in other agroecosystems. One possibility is in exploiting its toxic compounds for pest control in associated crops. *Manipueira* is a liquid formed during processing of cassava roots for flour. Linamarin (**1**) from *manipueira* rapidly degrades, but the acetone cyanohydrin (**10**) formed is stable. In Brazil, *manipueira* has been used to control nematodes for decades (Chitwood, 2002 and references therein). Recently, this root extract was shown to be lethal to the brown citrus aphid *Toxoptera citricida* (Hemiptera: Aphididae) (Gonzaga et al., 2008) and the cabbage aphid *Brevicoryne brassicae* (Hemiptera: Aphididae) (de Jesus and de Mendonça, 2012). Therefore, cassava root extract offers potential as an environmentally friendly strategy to control pests and this area of research warrants more attention.

4.2. Tritrophic interactions for enhancing biological control

From studies with cassava green mites, there is chemical

Fig. 5. Extrafloral nectar (EFN) droplets in cassava in early morning plants (photo by DM Pinto-Zevallos).

evidence that herbivore attack induces changes in VOC emissions in cassava plants. However, basic knowledge on how the plant modulates VOC emissions upon attack by other herbivores is missing. Coupling chemical analyses with behavioral and electrophysiological studies (electroantennography − EAG) involving key natural enemies, could give new insights as to which compound or mixture of compounds are exploited by predators and parasitoids to find their host or prey. Equally important is to understand how variations in plant signals affect natural enemy behavior. VOC blends are known to vary depending on several plant and herbivore traits such as density of the attacking herbivore (De Boer et al., 2008; Guerrieri et al., 1999) or plant age (Rostás and Eggert, 2008) and physiological state (Gouinguené and Turlings, 2002). Predatory mites and parasitoids from cassava agroecosystems modulate their response depending on prey identity, density and plant genotype (Gnanvossou et al., 2001, 2002; Onzo et al., 2012; Souissi, 1999), but the chemical basis for such modulations remains unknown. For major pests, there is a good number of predators and parasitoids attacking different life stages already identified and studied (see Bellotti et al., 1999 for a review). Therefore, chemical ecological studies of above ground tritrophic interactions are a promising area of research in cassava. In the case of below ground pests, such as the burrowing bug *C. bergi*, the entomopathogenic nematode *Steinernema carpocapsae*, has shown potential as a biological control agent under laboratory conditions (for a review, see Bellotti et al., 1999). To what extent this species exploits plant volatiles as previously shown for other species (Rasmann et al., 2005) is still unknown, and deserves investigation. Genetic variation in inducibility of plant VOCs (Degen et al., 2004) could be exploited for breeding plants with a stronger tritrophic signal or to identify candidate plants to develop push-pull programs (Khan et al., 2014).

Several other ways to enhance biological control via manipulating indirect plant defenses are possible (Rodriguez-Saona et al., 2012). Boosting herbivore-induced VOCs and EFN are likely to be possible via priming or by endogenous applications of elicitors such as jasmonic acid, as well as other jasmonates (see Heil, 2015 for a review; Thaler, 1999), though still not commercially applied. In cassava agroecosystems, this would be particularly important for recruiting parasitoids of major pests. Additionally, understanding whether natural enemies can exploit and benefit from EFN or whether secondary metabolites present in the sugary solution can adversely affect higher trophic levels found in cassava plantations is an important step towards keeping biological control agents in the field. Simultaneous exploitation of these strategies is feasible and may be a good strategy to enhance biological control (Stenberg et al., 2015).

4.3. Cassava as a part of polycultures

The polyculture systems planted by small-scale farmers in the tropics provide broad nutritional content, and cassava often constitutes the major source of carbohydrates. These intercropping systems usually include maize, upland rice and legumes such as beans, cowpeas, mungbeans and groundnuts (FAO, 2013). Polyculture systems can provide important agricultural ecosystem services, such as biological control and pollination as crop diversification may boost the abundance and activity of beneficial arthropods. Furthermore, they are essential in improving matrix quality for conservation of biodiversity, since they constitute habitats that can be exploited by native species (Perfecto et al., 2009). However, different polycultures can show different qualities, and understanding agroecosystem function within different polycultures can provide us with important information that can be appropriated by the farmer in order to create resilient production systems.

Herein, described is how cassava EFN could be important for improving biological control on this crop. However, in polycultures, traits in one species can affect the whole system, and an important goal in agroecosystem management is to understand how these traits can be manipulated to enhance ecosystem services. An important question is whether EFN in cassava attracts visitors that spill over onto the other crops in the system. Thus, cassava could provide a reservoir for natural enemies that forage more broadly than the cassava plant itself. To our knowledge, no studies have addressed how carnivorous arthropods, other than mites, exploit cassava EFN, and whether the presence of toxic compounds may or may not affect behavior or performance of consumers. Cassava is commonly grown in association with other crops, for example maize and beans (Altieri and Nicholls, 2004). Schulthess et al. (2004) showed how intercropping cassava with maize can drastically reduce pest incidence and increase parasitism of maize pests, and productivity of the maize-cassava intercrop as whole per unit area was greater than the monocrops. The converse question is just as important: How does EFN from associated plants affect the natural enemy community on cassava? The association of different EFN-bearing plants could provide redundancy and complementarity in biological control, enhancing or ensuring control despite absences of individual biological control agents.

Another important question is how associated plants in the polyculture can affect induced direct and indirect defense in cassava or vice-versa. EFN and VOC production are known to be inducible through plant-plant interactions (Kost and Heil, 2006). Different associated plants will have unique interactions, and this could be an important tool for planned biodiversity in agroecosystems. Some combinations of crops are known to confer associational resistance (Barbosa et al., 2009), and there could be chemical signaling behind these well-known combinations. Elucidating these mechanisms and reaching generalizations about traits involved in effective signaling could provide an important transferable tool for ecosystem management.

4.4. Plant defense and nutritional and ecological trade-offs

In order to adopt an ecological approach towards pest control in cassava and in any other crop, a series of ecological and nutritional challenges need to be overcome. Dependence on direct defenses, in particular cyanogenic compounds, brings with it a major concern relating to human health. Ingestion of cyanide can lead to acute intoxication and to irreversible neurological diseases such as Konzo and tropical ataxic neuropathy (TAN) and other health disorders if consumed at sub-lethal dosages over prolonged periods. While Konzo disease causes irreversible paralysis of the legs mainly in children and woman in eastern, southern and central Africa, TAN causes loss of vision, deafness and loss of sensation in hands (Nhassico et al., 2008). TAN occurs in West Africa, the West Indies and tropical Asia. Due to these effects of cyanides on food safety, sole reliance on direct defenses is inviable and possibly dangerous.

Genomic studies have recently shown that during the process of domestication of cassava the levels of cyanogenic glycosides in roots and leaves have been drastically reduced (Wang et al., 2014). Nevertheless, cyanide content in varieties in Amazonia and Africa ranges from 1 to 1550 ppm in root parenchyma (Cardoso et al., 2005), and varieties with depleted cyanogenic compounds are still sought (Jørgensen et al., 2005; Siritunga and Sayre, 2004). Reduction or removal of cyanogenic compounds in cassava roots and leaves is possible by genetically manipulating the biosynthesis of cyanogenic glycosides or by accelerating cyanogenesis and cyanide volatilization during root processing (Siritunga and Sayre, 2004). While the first strategy would deprive the plant of part of its natural defenses and possibly result in the larger pesticide

inputs, the second would not. Furthermore, genetic manipulation of plants can result in pleiotropic effects or mutations, affecting other plant traits, including those involved in defense (Strapasson et al., 2016). Contradictory as it may sound, South American Indians prefer to grow plants with larger amounts of cyanogenic glycosides (Wilson and Dufour, 2002). According to the interviewees, one of the reasons for such a choice is that "bitter" cassava varieties are less attacked by pests. Likewise, farmers in Africa prefer bitter cassava because they are less prone to predation (Cliff et al., 1997). According to McKey et al. (2010), the major problem of human cyanide toxicity is the result of introduction of cassava in Africa without proper knowledge of processing methods. Methods used by South American Indians and smallholders are more efficient, and probably reflect millennia of domestication in the Neotropics. However, new methods such as the simple wetting and rapid wetting can reduce cyanogen content in cassava flour (Bradbury and Denton, 2010; Cumbana et al., 2007) to below 10 ppm in cassava flour, the safe level recommended by the World Health Organization (WHO) (FAO/WHO, 1991). In Africa, Konzo disease is associated to periods of wars and drought, when cassava is harvested earlier and the processing time of cassava to reduce cyanide is shortened (Cliff et al., 1997), and to deficient intake of protein-rich food that provides sulphur donors needed in the process of cyanide destoxification (Nzwalo and Cliff, 2011).

Another major concern in the use of plant defense for pest control is how it might trade-off with crop production. It is known that chemical defense can be negatively correlated to biomass accumulation and other plant growth processes important for guaranteeing sustained productivity (Züst et al., 2015). Thus, investing in plant defense could lead to reduced yields. However, if a whole-system approach is adopted, including indirect defenses such as tritrophic interactions, this potential cost can be overcome, especially in systems such as cassava where direct chemical defense (cyanogenesis) brings with it a potential risk to human health. Cyanogenesis in lima bean is negatively correlated with plant competitiveness, though this negative effect disappears in the presence of herbivores (Ballhorn et al., 2014). Cyanogenesis in lima bean is also correlated with other types of defense, positively with trichomes, and negatively with EFN and VOC production (Ballhorn et al., 2008, 2013). This trade-off between direct and indirect defense should be viewed as an opportunity, and not a hindrance, for the development of ecologically based pest control in cassava, since it can result in acceptable levels of cyanogenic compounds in cassava while emphasizing biological control through tritrophic interactions. In order to achieve this in cassava, more research into the relationships between cyanogenic compounds and indirect defense is needed, focusing on how multiple defenses co-vary (Ballhorn et al., 2013). Incorporating an ecological, whole-system approach to pest control (Lewis et al., 1997) will reduce reliance on cassava direct defenses, since understanding the ecological interactions in the agroecosystem will allow the continued use of low-cyanide varieties without compromising biological control.

5. Final remarks

Cassava chemical traits and production systems offer a good ground for developing sound pest management strategies. Pest control based on the ecological understanding of agroecosystems can be developed in association with growers, thus including them in the generation of knowledge-based agroecosystem management. This is highly empowering to the growers, since they understand and are able to adapt their pest control techniques upon changing conditions, creating independence from external, high-cost inputs, as well as avoiding problems such as pest resistance to insecticides, pest resurgence, and human and environmental contamination. Knowledge empowerment is essential to develop resilient agroecosystems that guarantee local food production in the face of a world facing rapid social and environmental changes.

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