24 Diesel Trees

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24.1 INTRODUCTION

The natural history of diesel trees has a long interaction with humans in the realm of economic botany. Trees in the genus *Copaifera* belong to the subfamily Caesalpinioideae in the family Fabaceae. In total, there are more than 70 species of *Copaifera* distributed throughout the world with at least 30 species found in South and Central America, primarily in Brazil, four species in Africa, and one in Malaysia and the Pacific Islands (Dwyer 1951, 1954; Hou 1994). The first species in the genus *Copaifera* was described by George Marcgraf and Willem Pies in 1628, but no formal species name was ascribed to the plant, although later it was deemed *Copaifera martii* on the basis of the description by Veiga Junior and Pinto (2002). Oleoresin from a *Copaifera* tree was listed as a drug in the London Pharmacopoeia in 1677 and to the United States Pharmacopoeia in 1820, and Linnaeus first described the genus *Copaifera* in 1762 (Plowden 2004). Later, more descriptions of *Copaifera* species were completed by Hayne in 1825 and Bentham in 1876 (Dwyer 1951). The current taxonomy of the genus has been largely defined by Dwyer and Léonard, who resolved the differences between the genera *Copaifera* and *Guibourtia* and further developed the New World and African species descriptions in the early 1950s (Léonard 1949, 1950; Dwyer 1951, 1954). Some species are still difficult to identify in the field, even to specialists, because of an incomplete taxonomy and esoteric species differences that rely on intricate flower morphology and other transient characteristics than can be difficult to ascertain or collect compared with leaf morphology. To complicate this situation further, *Copaifera* trees have been known to only flower once every 2 or

3 years in Amazônia (Alencar 1982; Pedroni et al. 2002). Furthermore, most up-to-date references on *Copaifera* taxonomy are in Portuguese, which hampers the interchange of information amongst the mainstream of scientists.

Copaifera species found in Africa are biochemically distinct from those discussed above because they produce resins that harden into a solid copal, which fossilizes into amber, whereas New World species produce a liquid oleoresin because of the higher concentrations of sesquiterpenes (Langenheim 1973). Oleoresin, which results from tapping *Copaifera* trees, was listed as a drug in the London Pharmacopoeia in 1677 and to the United States Pharmacopoeia in 1820. In Brazil, the oleoresin produced by *Copaifera* trees has been used by native people as a local medicine for healing wounds; an antiseptic; to relieve pain; and for a host of skin, respiratory, and urinary ailments (Plowden 2004). They have also been used for more esoteric purposes such as a snake bite remedy, aphrodisiac, removal of intestinal parasites, and as a contraceptive.

More recently, several scientific studies have verified the medicinal properties of various *Copaifera* oleoresin fractions for anti-inflammatory activity (Veiga Junior et al. 2007), stomach ulcers and intestinal damage mitigation (Paiva et al. 1998, 2004), anticancer activity (Ohsaki et al. 1994; Lima et al. 2003; Gomes et al. 2008), reduced pain sensitivity (Gomes et al. 2007), and increased rate of wound healing (Paiva et al. 2002). The oleoresin and oils of *Copaifera* species have also been used in varnishes and lacquers, as lumber, cosmetic products, and tracing paper (Plowden 2004; Lima and Pio 2007).

Additionally, in 1980 the Nobel-Prize-winning chemist Melvin Calvin noted the oleoresin from *Copaifera* trees was being used as diesel fuel directly from the tree with no-to-minimal processing (Calvin 1980). Calvin began his search for plants that could produce liquid fuels to be used directly in engines after the 1973 oil embargo. He later wrote two more papers in 1983 and 1986 on the potential for production of hydrocarbon fuels from living plants, the issue of global warming, and the pressing need to address U.S. foreign oil dependency which now, some 20 years later, seems almost prophetic. Plantations of *Copaifera* trees were established in Manaus, Brazil to test the viability of biofuel production in the 1980s, but they were later shifted to focus on production of timber and the oleoresin for pharmaceutical and industrial purposes (Plowden 2005). The direct reasons for this shift were undoubtedly economic when diesel fuel returned to being relatively cheap.

AU: Plowden 2005 is not included in the reference list.

24.2 CHEMICALS PRESENT IN *COPAIFERA* OLEORESINS

Copaifera oleoresins, in general, are unique because they contain a greater fraction of sesquiterpenes compared with mono- and diterpenes. In *Copaifera multijuga*, approximately 80% of the oleoresin is composed of sesquiterpenes, whereas in *Copaifera guianensis* only approximately 44% of the oleoresin was composed of sesquiterpenes (Cascon and Gilbert 2000). These authors also noted that the majority ratio of diterpene acids and sesquiterpenes oscillated back and forth throughout the growing season in *Copaifera duckei*.

A wealth of original articles and review papers has focused on describing terpene biosynthesis. As such, only a brief description of the major terpene constituent characteristics and their biosynthesis in relation to conifer and *Copaifera* structures will be attempted here.

In short, isoprene units, the building blocks of terpenoids, are derived from either the mevalonic acid (MVA) pathway present in the cytosol of cells, or the 2-C-methylerythritol-4-phosphate (MEP) pathway, also known as the non-mevalonate pathway, which occurs in plastids (Lichtenthaler 1999). Condensation of isopentenyl diphosphate and its isomer dimethylallyl diphosphate, the products of the MVA and MEP pathways, leads to the formation of geranyl pyrophosphate (GPP), farensyl pyrophosphate (FPP), or geranyl geranyl pyrophosphate (GGPP), which are the common precursors for mono-, sesqui-, and diterpenes, respectively. These three intermediates are catalyzed to form mono-, di-, and sesquiterpenes by the action of terpene synthases (TPSs). Individual TPSs can generate either one product or multiple products that, in turn, can be linear or cyclic. Mono- and diterpenes are thought to be derived primarily from isoprenes made in the plastid through the MEP pathway, whereas sesquiterpenes are derived from isoprenes made in the cytosol where the MVA pathway occurs. Movement of intermediates between these two pathways has been demonstrated in plants (Cheng et al. 2007).

The chemical compounds present in *Copaifera* oleoresin varies not only with tissue type (Gramosa and Silveira 2005; Chen et al. 2009), but also seasonally (Cascon and Gilbert 2000; Zoghbi et al. 2007), and amongst species (Veiga Junior et al. 2007). Therefore, any future genomics-based characterization of *Copaifera* trees must be coupled with close biochemical analysis to correctly match major compounds present in each tissue at the time of sampling. Identification of particular chemicals responsible for the pharmaceutical effects of *Copaifera* oleoresins will be necessary in the future because high chemical variability within samples, seasons, and species will inherently affect the effectiveness, dosage, and safety for patients.

24.3 BIOSYNTHESIS OF *COPAIFERA* OLEORESINS: WHAT CONIFERS CAN TEACH US

Not much is known about the biosynthesis of *Copaifera* oleoresins because most studies have been focused on traditional ecology and forestry of the genus. However, conifer resins have been thoroughly studied for over 40 years. These oleoresins are essentially made of the same basic constituents as *Copaifera* oleoresin: mono-, di-, and sesquiterpenes. Conifer oleoresins usually have an equal part of mono- and diterpene compounds with lower concentrations of sesquiterpenes (Martin et al. 2002).

Monoterpenes are volatile components found in oleoresins. Monoterpene synthases have been extracted from the woody stems of ten conifer species, and their activities have been measured (Lewinsohn et al. 1991). Species with resin ducts showed the highest levels of monoterpene cyclase activity from wood extracts, suggesting that monoterpene synthesis for oleoresins occurs in epithethial cells surrounding the resin ducts. Diterpenoids themselves are not typically found in conifer oleoresins in large quantities. Instead, modifications such as hydroxylation and oxidation occur, so the alcohol, aldehyde, and predominantly acid products are present (Keeling and Bohlmann 2006). These modified diterpene products harden the resin and form rosin after the volatile constituents evaporate. Sesquiterpenes, like monoterpenes, are volatile and are major constituents of *Copaifera* oleoresin. The three major chemical constituents, based on percentage, of oleoresin from different species are presented in Table 24.1. Although the percentages vary, β-caryophyllene is the major sesquiterpene product of oleoresins throughout *Copaifera* species that have been studied to date. Other than these major three sesquiterpenes in each species, there is a great diversity of terpenoids produced in the oleoresin. Nuclear magnetic resonance (NMR) studies have found previously undescribed diterpenes (Monti et al. 1996, 1999) that seem to be unique in biology.

AU: Please confirm this revision correctly clarifies your meaning.

Conifers produce a myriad of specialized tissues to store and secrete oleoresins that range from simple resin blisters to intricate networks of resin ducts (Martin et al. 2002). *Copaifera* trees form resin ducts throughout their xylem tissue that can easily be seen in cross-sections (Calvin 1980). *Copaifera*, *Hymenaea*, and *Daniella* resin ducts display many structural similarities (Langenheim 2003).

Conifer oleoresins accumulate in resin ducts throughout their lifetimes, but a local response can also be induced during mechanical damage, herbivory, or even fungal inoculation. This response activates epithelial cells in resin ducts, signals for formation of special traumatic resin ducts in stem xylem tissue, and induces diterpene biosynthesis gene transcripts (Keeling and Bohlmann 2006). Methyl jasmonate can also induce this response (Martin et al. 2002). Oleoresin production can also be induced in *Copaifera* species. Younger trees that do not produce oleoresin on the first attempt have been known to produce a small amount on a second tapping, putatively through induction by mechanical damage (Plowden 2003; Medeiros and Vieira 2008). Medeiros and Vieira (2008) were also able to draw a weak correlation between trees with termite infestations and production of oleoresin, suggesting that insect damage can induce production of oleoresins.

TABLE 24.1
Three Major Sesquiterpenes Present in the Oleoresins of *Copaifera* Species

Species	Reference	Compound	Percentage in Oleoresin
Copaifera langsdorfii	Gramosa and Silveira 2005	β-Caryophyllene	53.3
		Germacrene B	8.7
		β-Selinene	6.5
Copaifera martii	Zoghbi et al. 2007	β-Caryophyllene	42.6[a]
		δ-Cadinene	15.7[a]
		β-Elemene	5.0[a]
Copaifera multijuga	Veiga Junior et al. 2007	β-Caryophyllene	57.5
		δ-Humulene	8.3
		β-Bergamotene	2.6
Copaifera cearensis	Veiga Junior et al. 2007	β-Caryophyllene	19.7
		δ-Copaene	8.2
		β-Bisabolol	8.2
Copaifera reticulate	Veiga Junior et al. 2007	β-Caryophyllene	40.9
		δ-Humulene	6.0
		β-Bergamotene	4.1
Copaifera trapezifolia	Veiga Junior et al. 2006	β-caryophyllene	33.5
		Germacrene D	11.0
		Spathulenol	7.6

[a] Number represents an average of 11 sampling dates.

The cellular mechanisms involved in transport, storage, and secretion of oleoresin constituents against the concentration gradient present in resin ducts are not well understood (Langenheim 2003; Keeling and Bohlmann 2006). Synthesis of terpenoids present in conifer oleoresins typically involves TPSs and cytochrome P450 oxygenases (P450). A conifer diterpene synthase (PtTPS-LAS) and the first diterpene P450 (PtAO) have been localized to plastids and the endoplasmic reticulum (ER) using a green fluorescent protein in tobacco leaf cells (Ro and Bohlmann 2006). On the basis of the lack of accumulation of diterpenes in cells, these authors suggest that a transport mechanism must be in place to move the diterpenes into the ER or cytosol of cells.

Although *Copaifera* oleoresin exudes from resin ducts during tapping, no experiments have confirmed which tissues are responsible for production of chemical constituents in the oleoresin. Calvin (1980) hypothesized that the constituents in *Copaifera* oleoresin must be synthesized in the canopy of the tree and seep down through the resin ducts. In Norway spruce, diterpene synthases have been localized to epithelial cells surrounding resin ducts using protein-specific antibodies (Keeling and Bohlmann 2006). Recently, we have found that the sesquiterpenes are present in *C. officinalis* oleoresin in leaves and stem tissue of seedlings as well as leaves, stems, and roots of 2-year-old saplings (Chen et al. 2009). The presence of sesquiterpenes in different tissues at different ages could indicate transport or changes in regulation of TPS gene scripts signaled by development. In addition, the terpenes detected in oleoresins also appear in other tissues such as seeds (Gramosa and Silveira 2005). The seeds also have different sesquiterpenes that are not seen in oleoresins such as γ-muurolene, perhaps suggesting that different TPSs function in different tissues.

24.4 BIOLOGICAL FUNCTIONS OF OLEORESIN

The principal chemical constituents of *Copaifera* oleoresin are terpenoids. Therefore, understanding the biological/ecological roles of terpenoids will allow us to understand the roles of *Copaifera* oleoresin. Terpenoids are the largest class of secondary metabolites produced in the plant kingdom. Approximately 50,000 of these have been structurally identified (McCaskill and Croteau 1997). This diverse group of plant metabolites is important for many aspects of plant biology and ecology (Tholl 2006; Yuan et al. 2009). For instance, some terpenoids function in plant defenses against herbivores and microbial pathogens (Gershenzon and Croteau 1991). Other terpenoids produced by flowers as volatiles are involved in attracting insect pollinators for plant cross-pollination (Raguso and Pichersky 1999). Some volatile terpenoids are emitted from herbivore-damaged plants and function as cues to attract natural enemies of the feeding herbivores (Yuan et al. 2008). *Copaifera* oleoresin is generally believed to be involved in plant defenses that can be mainly attributed to terpenoids. Depending on the mechanisms of production, oleoresins may act in constitutive defense, induced defense, or both. *Copaifera* oleoresin could be toxic to herbivorous insects, bacteria, or fungi. Because of high volatility, the terpenoids in *Copaifera* oleoresin may be released from the tree as infochemicals, which can deter potential insect pests. Oleoresin may also flow out of the wound to physically push the invading insects out of the entry wound or entomb them so the insects cannot cause further damage. The wound caused by insect herbivory can be a natural site for invasion of microbial pathogens that would need to be defended against. *Copaifera* oleoresin and its constituents have been documented to have antimicrobial and antifungal activity (Howard et al. 1988; Braga et al. 1998). *Copaifera* oleoresin produced upon insects feeding may therefore prevent further damage caused by pathogens.

Studies on *C. langsdorfii* populations have showed that seedlings have a higher sesquiterpene concentration than their parent trees (Macedo and Langenheim 1989b). Additionally, there was a 48% mortality rate of first-generation oecophorid larvae and pupae when they were reared on seedling leaves, but no mortality was seen on oecophorids reared on parent leaves. The oecophorids that survived feeding on seedling leaves also exhibited a significantly lower weight gain than those feeding on parent leaves. Seedlings had twice as much caryophyllene, the major sesquiterpene present in most species' oleoresin, in leaves when compared with their parents. It is still unknown how tapping *Copaifera* trees for oleoresin affects tree health in the long term. Initial tapping, or even multiple tappings, could harm the tree by removing a source of chemical defense against pathogens and insects and must be considered in future studies.

24.5 OLEORESIN PRODUCTION ECOLOGY

Extractive collection of the oleoresin from wild populations of *Copaifera* trees has long been touted as a means to supplement income for native people in rural and forest areas instead of participating in the destructive practices such as slash-and-burn agriculture and timbering. However, the viability of this practice has been called into question because of the intermittent presence of oleoresin amongst individual trees, low yields of oleoresin per tree, as well as reduced and questionable secondary harvests of trees that produce oleoresins on the first tapping (Plowden 2003; Medeiros and Vieira 2008). Sustainable production of quality oleoresin for medicine and other uses has many problems that must be considered. First, a management system that will maximize production and minimize impact on the forest where harvest is occurring must be described (Rigamonte-Azevedo et al. 2004). This matter is complicated by the fact that the genus *Copaifera* is made up of many species that can produce useful oleoresin, and each of these species will naturally respond differently to each possible management strategy. In addition, anecdotal evidence suggested that each tree could produce between 20 and 30 L of oleoresin from one drill hole every 6 months (Calvin 1980); however, these stories seem to be more myth than fact.

In a study of 43 *C. multijuga* individuals in the Adolpho Ducke Forest Reserve in Manaus, Brazil, about half produced some volume of oleoresin during three tappings (Medeiros and Vieira 2008). Six of these individuals never produced oleoresin at all. On average, trees with a diameter at breast height (DBH) more than 41 cm produced 1.8 L of oleoresin per tree on the first tapping and 0.5 L during the second tapping 1 year later. Trees of 30 and 41 cm DBH produced an average of 0.13 L during the first tapping and 0.16 L during the second tapping. Plowden (2003) studied *Copaifera* oleoresin production from three different species in Pará, Brazil on the Alto Rio Guamá Indigenous Reserve. Trees 55 to 65 cm DBH yielded the most oleoresin, averaging 459 mL after two holes were drilled.

Some of the highest recorded average yields per tree were seen in the southwestern Brazilian Amazon in *C. reticulata* and *C. paupera* trees with 2.92 and 1.33 L, respectively (Rigamonte-Azevedo et al. 2006). However, these numbers were averages amongst oleoresin producing individuals only. Only 27% of *C. reticulata* trees and 80% of *C. paupera* trees produced oleoresin. It is not clear whether the lack of uniformity in oleoresin production stems from tapping methodology or whether the oleoresin itself is just not produced constitutively in all trees. Significant variation, both natural and in response to herbivory, in chemical composition of *C. langsdorfii* leaves has been noted (Macedo and Langenheim 1989a, c). This variation, compounded by variation in climate, nutrient availability, and other factors, could also cause sporadic oleoresin production and therefore explain the variation seen in oleoresin collection.

Multiple harvests have also been considered to increase oleoresin yields. Cascon and Gilbert (2000) tapped 300–550 mL of oleoresin from a single *C. duckei* tree ten consecutive times at 4-month intervals, but they never depleted the tree of oleoresin at any point. However, it is impossible to determine how much oleoresin collected at each interval was residual material that had been stored in the tree and how much had been synthesized and replaced between tappings. Most studies suggest that primary tapping accesses oleoresin from accumulations in heartwood that have built up over long periods of time (Plowden 2004) and therefore would not quickly regenerate for a secondary major harvest as Calvin had originally hoped. The density of trees also ranges from 0.1 to 2.0 per hectare depending on location and forest type (Rigamonte-Azevedo et al. 2004).

AU: Revised to Rigamonte-Azevedo et al. 2004 to match reference list.

It is unknown how phenology plays a roll in oleoresin production. As mentioned before, the chemical composition of the *Copaifera* oleoresins change throughout the year, but no specific cause has been identified as the factor driving this change. Phenology studies of *Copaifera* species are rare and focus more on the flowering, seed set, and leafing patterns (Pedroni et al. 2002). Most of these types of studies have been in *C. langsdorfii*, a species native to the southern parts of Brazil. However, oleoresin collection for commercial products occurs more commonly in the northern half of Brazil and South America. From our experience, the species *C. multijuga* and *C. reticulata* are most commonly available for purchase outside of Brazil, although they are often mislabeled as *C. officinalis*.

In a recent visit to Brazil during July, we were able to observe the oleoresin collection process (Figure 24.1). The trees had to be drilled by hand, and reaching the core of the tree to access the heartwood where the oleoresins are stored was not easy. We observed the tapping of 12 *C. langsdorfii* trees, none of which produced oleoresin. It was suggested that these trees may not produce oleoresin at all, or that they may not be in season because July is during the winter or dry season. This again reinforces the notion that tree species native to the northern parts of Brazil are more suitable for production of oleoresin, or at least traditionally there is a more widespread culture of oleoresin collection in the north.

24.6 COMPARING OLEORESIN TO DIESEL FUEL

Diesel fuel, like gasoline, consists of many different compounds isolated from only one fraction of the greater mixture known as crude oil. Diesel fuel distills from crude oil between the temperatures of 200 and 350°C. Not all diesel fuel comes directly from primary distillation; processes such as catalytic cracking, which breaks larger, denser molecules into smaller ones, have been developed to generate more liquid fuels from crude oil barrels (Bacha et al. 2007). In general, diesel fuel is made up of paraffins (alkanes), naphthenes (cycloalkanes), olefins (alkenes), and aromatics. As mentioned before, *Copaifera* oleoresins consist primarily of sesquiterpenes hydrocarbons.

FIGURE 24.1 (See color insert) Identification and tapping of *C. langsdorfii* trees near Nova Odessa, Sao Paulo State, Brazil. (a) A *C. langsdorfii* tree growing near a farm in Nova Odessa, Brazil. The trees grow as single individuals rather than in stands, making it difficult to locate and tap multiple trees. (b) Tapping a *C. langsdorfii* tree with a manual drill. The oils collect in the heartwood and so the hole must be drilled to the very center of the tree, making collection difficult. (c) Botanical characterization of *C. langsdorfii*. Pictures of leaves, seeds with fleshy aril, and seed pods were taken to correctly identify the genus and species of the trees.

The important properties of diesel fuel are the cetane rating, low-temperature operability, and volumetric heating value. Diesel engines produce combustion by compressing air, which, in turn, heats the air; at a designated moment of compression, fuel is injected into the chamber as tiny droplets, which vaporize and ignite. The cetane rating measures the ignition quality of fuels, or how readily the fuel burns. A fuel's quality of ignition can have implications in starting engines in cold conditions, as well as emissions, smoothness of operation, noise, and misfires (Bacha et al. 2007).

Low temperatures can cause some constituents in diesel fuels to solidify (such as the paraffins). This, in turn, can clog the fuel filter and stop the flow of fuel to the engine. This effect is measured with "cloud points," the temperature at which the waxes in the mixture begin to solidify, or the "pour point," the temperature when the fuel becomes so thick it will no longer pour. The volumetric heating value measures how much energy the fuel has per volume. Volumetric heating values influence torque, horsepower, and to some degree, fuel economy.

Monoterpenes and sesquiterpenes are volatile cyclic hydrocarbons. The major sesquiterpene present in most *Copaifera* species, β-caryophyllene, has a chemical structure most similar to a cyclic olefin, or a naphthene, which contains two double bonds. In general, naphthenes have a midrange cetane rating, good low-temperature properties, and an acceptable volumetric heating value. Biofuels from oilseed sources such as soybean and canola have a pour and cloud point of approximately 0°C, making them impractical in areas with cold climates. In addition, fuel additives to improve low temperature properties are not very effective because of the high level of saturated compounds present in the oils (U.S. Department of Energy 2004). Addition of terpenoid components (such as sesquiterpenes) to these types of biofuels could increase their low-temperature properties and complement their high cetane ratings.

Not much is known about the chemical and physical properties of *Copaifera* oleoresin as a diesel fuel. Calvin (1980) submitted a sample of *Copaifera* oleoresin to the Mobil Corporation and obtained a cracking pattern: 50% aromatics, 25% liquid petroleum gas (LPG), 3–4% low-molecular-weight fuel gas, and coke. Later, cracking of *C. officinalis* oleoresin with a zeolite catalyst, ZSM-5, led to production of over 200 compounds from 34 sesquiterpenes present in the original oleoresin (Stashenko et al.1995). The great variety of resulting products could indicate the utility of these oleoresins in not only fuels but also additional value-added products from a renewable resource. As mentioned before, the seeds of *Copaifera* species produce sesquiterpene hydrocarbons, but also produce various fatty acids when pressed and extracted (Lima Neto 2008; Stupp et al. 2008). In *C. langsdorfii*, oleic acid (C18:1) made up 33.1% of the fatty acid profile whereas palmitic acid (C16:0) made up 20.2% of the fatty acid profile. According to Stupp et al. (2008), the major fatty acid that was extracted was linoleic acid (C18:2), which made up 45.3% of the fatty acids and oleic acid making up 30.9%. It would be interesting to test oil pressed from these seeds against other biodiesels and to compare their overall chemical structure to see how the percentages of sesquiterpenes versus fatty acids are present in the seed oil.

24.7 FUTURE SCOPE OF RESEARCH AND DEVELOPMENT

For reasons described earlier, it does not seem economically feasible to create plantations of *Copaifera* trees to produce oleoresin for biodiesel markets. In brief, long generation times, low and sporadic yields per tree, and their tropical nature limit production of oleoresin. Instead, characterization of the unique terpenoid biosynthesis pathway and expressing it in other species already suited for production of biodiesel offers a more reasonable avenue.

Why these oleoresins produce higher amounts of certain terpenoids, sesquiterpenes mostly, is not well understood. Possible mechanisms include differential regulation of sesquiterpene synthesis, or even higher TPS efficiency. Although there has been a lot of work accomplished to characterize the chemical nature of oleoresins, there has been a surprising lack of molecular biology and biochemistry research as to how these oleoresins are created, stored, and transported. Identification, isolation, and characterization of the TPS responsible for the production of the oleoresin constituents will be crucial in first determining how these proteins function, but also in localizing them within tissue types to understand production of oleoresin constituents.

Detailed studies on the emission and performance standards of oleoresins as a diesel fuel will also be necessary. The host of traditional diesel classification tests for physical and chemical properties including density, cloud and pour points, viscosity, heat of combustion, cetane number, etc., should be performed to gauge the usefulness of these compounds in today's markets with current engine technology. This work will also need to include several different possible species because each one has a varying chemical makeup and properties associated with that makeup. These studies will be instrumental in determining whether oleoresin constituents are better suited as a stand-alone biodiesel fuel or as an additive for other petroleum or biofuels.

However, there are barriers to further research on *Copaifera* species. Many publications and historical records about *Copaifera* trees are in Portuguese; this presents a barrier to the larger scientific community and hampers research efforts to assess available information. In addition, obtaining germplasm outside of the native range of the *Copaifera* genus has been exceptionally difficult. Collaborations on an international level will be crucial to establishing successful research initiatives.

We are performing genomics and biochemistry research to characterize and exploit the *Copaifera* terpene biosynthetic pathways. There are at least two end-goals of research. First, we need a better understanding of the basic biochemistry of this interesting genus and its oleoresins. Second, genes and gene regulation responsible for hydrocarbon production could be valuable with regards to their use in production of bioproducts and fuels. For example, key genes might be transferred to temperate oilseed species to complement and increase their biofuel production. Taken together, we expect the diesel trees to contribute to new fuels and products beyond diesel.

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