Consiglio Nazionale delle Ricerche

PUBBLICAZIONE AI SENSI DELL'ART. 19 DEL D.LGS. N. 33 DEL 14 MARZO 2013, MODIFICATO DALL'ART. 18 DEL D.LGS N. 97 DEL 25 MAGGIO 2016 INTEGRATO DALL'ART.1 C. 145 DELLA LEGGE 27 DICEMBRE 2019 N. 160

BANDO N. 368.30 RIC – AREA STRATEGICA AGRICOLTURA AMBIENTE E FORESTE

CONCORSO PUBBLICO, PER TITOLI ED ESAMI, PER L'ASSUNZIONE CON CONTRATTO DI LAVORO A TEMPO PIENO E INDETERMINATO DI N. 3 UNITÀ DI PERSONALE PROFILO RICERCATORE - III LIVELLO PROFESSIONALE - PRESSO STRUTTURE DEL CONSIGLIO NAZIONALE DELLE RICERCHE

TRACCE DELLE PROVE D'ESAME ESTRATTE A SORTE

PER LA PRIMA PROVA VIENE ESTRATTA LA SERIE DI PUBBLICAZIONI CONTRADDISTINTE CON LA LETTERA "B"

PER LA SECONDA PROVA VIENE ESTRATTA LA TRACCIA CONTRADDISTINTA DAL N: 1

1. RHIZOSPHERE CHEMICAL DIALOGUES: PLANT-MICROBE INTERACTIONS

2. THE IMPACT OF ANATOMICAL CHARACTERISTICS ON THE STRUCTURAL INTEGRITY OF WOOD

3. COMPONENTS OF SUSTAINABLE ANIMAL PRODUCTION AND THE USE OF SILVO PASTORAL SYSTEMS

Rhizosphere chemical dialogues: plant–microbe interactions

Dayakar V Badri[1], Tiffany L Weir[1], Daniel van der Lelie[2] and
Jorge M Vivanco[1]

and parasitic interactions with other plants, pathogenic microbes and invertebrate herbivores. Plants release enormous amounts of chemicals through their roots, at a significant carbon cost, to combat pathogenic microorganisms and attract beneficial ones. Rhizosphere interactions are affected by many different regulatory signals, of which only a few have been identified, recalling a quote by Leonardo da Vinci that '*We know better the mechanics of celestial bodies than the functioning of the soil below our feet*' [2]. Rhizosphere interactions are not solely driven by roots but are highly integrated with and influenced by residing organisms and local edaphic factors. Soil-inhabiting mutualists and parasites, both prokaryotic and eukaryotic, are actively involved in signaling with a host (Figure 1). Therefore, rhizosphere interactions are very dynamic and can be altered by addition or loss of any of the players.

A large body of literature exists about rhizosphere interactions [3–5]. In this review, we summarize the current knowledge of rhizosphere chemical communication between plant roots and their associated microorganisms. Central to this discussion is the recent progress made in understanding rhizosphere chemical dialogues between plants and different components of the microbial community. We end with a discussion of how these chemical dialogues may improve plant fitness at the community level and discuss the new challenges faced by researchers.

Chemical signaling between plants and mutualists

Plant roots release a wide range of compounds that are involved in attracting beneficial organisms and forming mutualistic associations in the rhizosphere. These compounds include sugars, polysaccharides, amino acids, aromatic acids, aliphatic acids, fatty acids, sterols, phenolics, enzymes, proteins, plant growth regulators and secondary metabolites. The most important rhizosphere mutualisms described are between plants and mycorrhizae or rhizobacteria.

Mycorrhizal associations are present in almost all land plants and are essential biological constituents of the rhizosphere. Mycorrhizae are grouped into two categories: endomycorrhizae (arbuscular, AM) and ectomycorrhizae. The AM symbiosis represents the most widespread and ancient plant symbioses, originating about 450 million years ago [6]. About 6000 species in the Glomeromycotina, Ascomycotina and Basidiomycotina families have been recorded as mycorrhizal and with more sensitive molecular techniques this number is increasing [7]. Similarly, more

Introduction

Prokaryotes and eukaryotes have coexisted for millions of years on earth. It is estimated that humans have 10^{13} human cells and 10^{14} bacterial cells including the endogenous bacterial flora [1]. As a result of this long association, prokaryotes have developed both beneficial and detrimental relationships with eukaryotes. As autotrophic organisms, plants play a central role in sustaining all other life forms. Unlike mammals, plants are sessile, thus releasing an array of chemical signals to interact with other organisms. The root system, which was traditionally thought to provide anchorage and uptake of nutrients and water, is a chemical factory that mediates numerous underground interactions. These include mutualistic associations with beneficial microbes, such as rhizobia, mycorrhizae, endophytes and plant-growth promoting rhizobactertia (PGPR)

than 200 000 plant species host mycorrhizal fungi, but a relatively small number of mycorrhizal types are known [8]. The biotrophic interfaces that are formed between plant roots and the fungus result from recognition of exchanged cues. There is an extensive list of plant genes that are predicted to play a role in facilitating AM interactions [9*,10*], but comparatively few identified in the fungus [11*,12]. Thus little knowledge exists about signaling processes between symbionts, the pathways related to symbiosis-specific development of AM fungi in root tissues, or mechanisms of nutrient exchange between them [12,13,14,15*].

The establishment of AM symbioses begins with the colonization of a compatible root by hyphae produced by AM fungal soil propagules, asexual spores or mycorrhizal roots. This is followed by appressorium formation and entrance into the cortex to form specialized structures called arbuscles. Before colonization, it is assumed that a continuous dialogue of signals is exchanged between the symbionts to establish colonization. Since this symbiosis lacks host specificity it has been suggested that either the plant-derived signals are conserved throughout the plant kingdom or that a broad range of related compounds are involved. Plant-released compounds like sugars and amino acids are potential fungal stimuli but phenolic compounds, particularly flavonoids, are known as key signaling components in many plant–microbe interactions [16*]. There are vast quantities of data on the effect of flavonoids on AM hyphal growth, differentiation, and root colonization [16*], and specific effects depend on the chemical structure of the compound [17]. It was recently found that flavonoids exhibit a genus-specific and species-specific effect on AM fungi [18]. In addition, strigolactones, a group of sesquiterpene lactones exuded by *Lotus japonicus* roots, were shown to induce hyphal branching in AM fungi, a pre-requisite for successful root colonization fungi [19**]. Strigalactones present in the root exudates of a wide range of plants act display specificity as signals for AM fungi but did not affect other fungal species such as *Trichoderma*, *Piriformospora*, *Botrytis cinerea* and *Cladosporium* sp. [19**]. A further hypothesis is that strigolactones are not only involved in inducing AM hyphal branching factor but also act to attract AM fungi to roots [20]. However, more studies are needed to clarify both the specificity and roles of strigolactones in establishing mycorrhizal associations. The production and exudation of strigolactones are dependent on nutrient availability. Recently, Yoneyama *et al.* [21] reported that nitrogen and phosphorus deficiency enhanced the secretion of a strigolactone, 5-deoxystrigol in sorghum plants. Besides strigolactones, some studies demonstrate that calcium ions are an intracellular messenger during mycorrhizal signaling, at least in a pre-contact stage [22**].

Even less understood than the signaling between plants and mycorrhizae is the interaction of mycorrhizae with other soil microbes. It has been demonstrated that AM fungal exudates directly impact soil bacterial community composition [23], and some bacteria associated with AM can improve colonization, root branching and antifungal properties [7,24]. Future goals should include identifying all players of these signaling networks, particularly the signals and receptors that open the door to symbiosis formation. Other major challenges include unraveling the signaling events in tri-partite interactions (plant–AM–bacteria) to better understand how soil bacteria and AM fungi associate. Although, some structural properties that regulate interspecies interactions are known [25*] the bacterial–mycorrhizal network still remains to be elucidated.

As mentioned, flavonoids play a key role in the early signaling events of legume–rhizobia interactions [26]. The legume rhizosphere chemically attracts rhizobia by secreting flavonoids and related compounds [27]. Subsequently, the NodD protein of rhizobia perceives specific flavonoids through one or two-component regulatory systems, initiating transcription of nod genes that encode the biosynthetic machinery for a bacterial signal, the Nod factor. Nod factors are lipochitooligosaccharides consisting of β-1, 4-linked *N*-acetyl-glucosamine backbones with four or five residues with an acyl chain at C2 in the non-reducing end and decorated with acetyl, sulfonyl, carbamoyl, fucosyl or arabinosyl moieties at defined positions depending on the rhizobial strain [28]. Perception of the Nod factors by the plant induces multiple signaling pathways that initiate root hair infection and nodule formation. There are other nonflavonoid related compounds like xanthones, vanillin and isovanillin that induce NodD gene expression, but they are required at much higher concentrations than flavonoids [29*], and thus their importance in natural environments is questionable. Recently, Cai *et al.* [30**] reported that canavanine, a compound present in the seed coat and root exudates of various legume plants, is toxic to many soil bacteria but not to rhizobial strains that possess specific transporter to transport (detoxify) this compound. They also suggested that host legumes secrete canavanine into the rhizosphere to select beneficial rhizobia. Further studies are warranted to identify factors determining host–rhizobium specificity.

Molecular communication between host and pathogens

There are four main groups of plant pathogens [31] but only two of them are major players in the soil; fungi and nematodes. Comparatively fewer bacteria are considered to be soilborne plant pathogens; however, some well-studied exceptions include *Ralstonia solanacearum* (bacterial wilt of tomato) and *Agrobacterium tumefaciens*, the casual agent of crown gall disease [32,33]. Fungi and oomycetes, physiologically and morphologically similar but phylogenetically distinct groups of organisms, are the

Figure 1
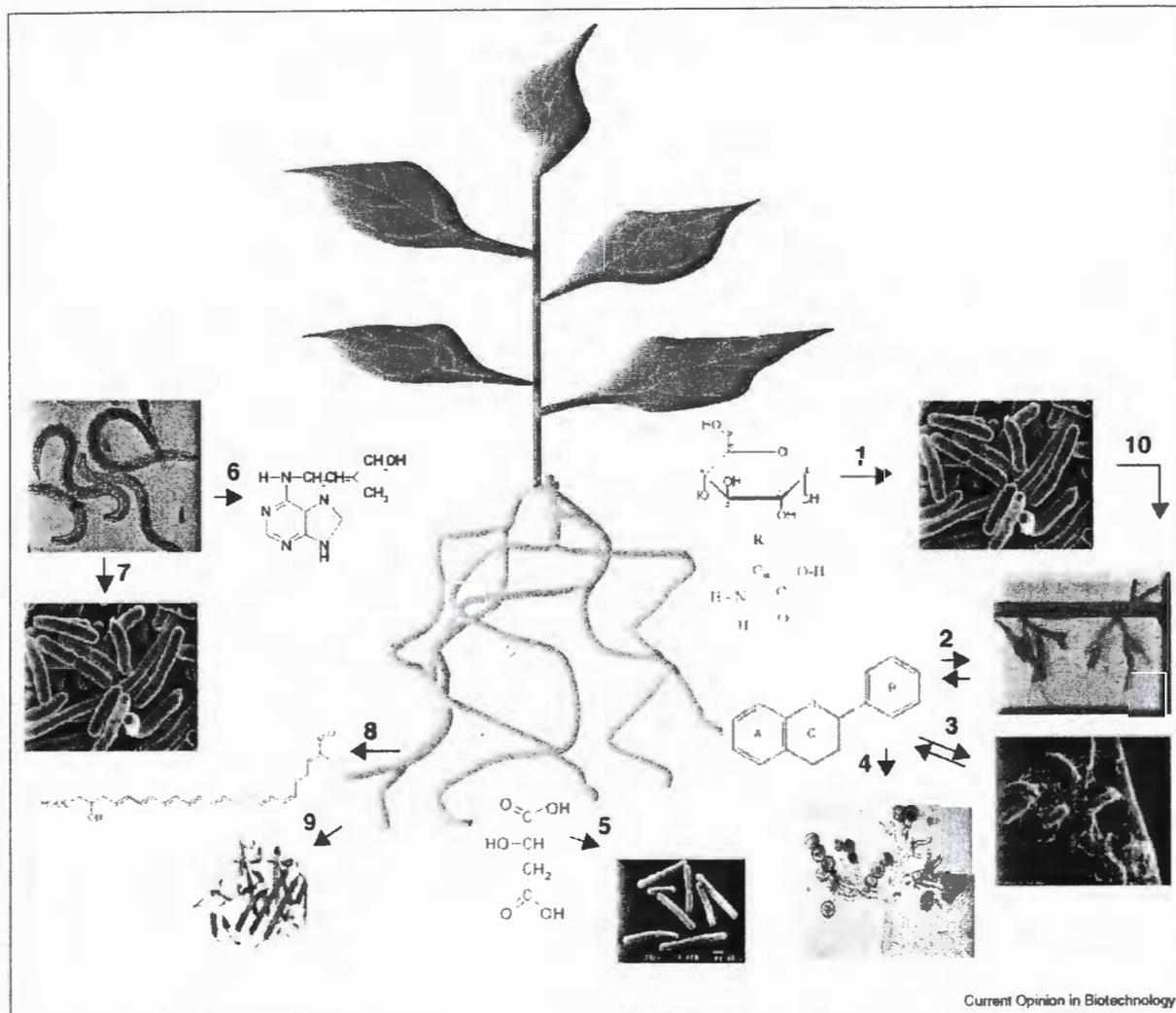
Pictorial illustration of the chemical communication that exists between plant roots and other organisms in the complex rhizosphere. Plant roots secrete a wide range of compounds, among those sugars and amino acids are engaged in attracting (chemotaxis) microbes (1), flavonoids act as signaling molecules to initiate interactions with mycorrhiza (AM fungi) (2), rhizobium (3) and pathogenic fungi (oomycetes) (4), aliphatic acids (e.g. malic acid) are involved in recruiting specific plant growth promoting rhizobacteria (*Bacillus subtilis*) (5), nematodes secrete growth regulators (cytokinins) that are involved in establishing feeding sites in plant roots (6) and nematodes secrete other compounds (organic acids, amino acids and sugars) involved in attracting bacteria and in bacterial quorum sensing (7). Knowledge of the roles of other types of compounds, such as fatty acids (8) and proteins (9), secreted by roots in the rhizosphere and other multi-partite interactions (10) remains unknown.

most predominant soilborne pathogens. Like plant–mutualist associations, pathogens also utilize chemical signals in early steps of host recognition and infection. Before the establishment of infection, *Phytophthora sojae* zoospores are chemically attracted by daidzein and genistein secreted by soybean [34]; however, the nature of the isoflavone receptor on the zoospores remains unknown. Most plants produce antimicrobial secondary metabolites, either as part of their normal program of growth and development or in response to pathogen attack and those antimicrobial compounds protect plants from a wide range of pathogens [35]. Preformed antifungal compounds, called phytoanticipins, occur constitutively in healthy plants and act as chemical barriers for fungal pathogens. By contrast, phytoalexins are antimicrobial compounds induced in response to pathogen attack but not normally present in healthy plants. These two groups of compounds have proven very effective for a wide range of fungal pathogens. However, most studies pertaining to these compounds were conducted in leaves,

(TraI/TraR) responsible for regulation of QS in *A. tumefaciens* occurs on the Ti (or tumor-inducing) plasmid, which is required for gall formation in host plants. An infection occurs when a segment of this plasmid is integrated into the nucleus of host plant cells, resulting in the production of opines that can then be utilized as a novel source of nitrogen and carbon [51]. The presence of opines, which are only found in the plant tumor, then upregulate expression of the bacterial TraR gene. Thus, the QS system, which allows for conjugation and replication of the Ti plasmid, is only effectively activated after infection, resulting in a questionable role for QS in *A. tumefaciens* pathogenicity [52]. To complicate matters, *A. tumefaciens* also produces a protein BlcC (formerly AttM) that has lactonase activity, which it was suggested may negatively regulate QS through signal degradation [53,54], a phenomenon called 'quorum quenching'. Another study showed that the presence of the plant defense metabolite salicylic acid resulted in increased expression of this lactonase and inhibition of virulence (vir) genes carried on the Ti plasmid [55]. However, the biological significance of this plant-induced lactonase to act as a quorum quencher was not substantiated by *in planta* data and appears to have only a transient effect [56•].

Quorum sensing has also been implicated as an important factor in the symbiotic association between legumes and rhizobia, although many details of its involvement are still emerging. Many rhizobia displaying mutations of their QS systems have reduced ability to infect root hairs and/or form nodules [57–59]. Additionally, several legumes have been shown to secrete compounds that can interfere with bacterial QS [60,61,62••], and *Medicago truncatula* responded differentially with regards to root exudation and protein expression to AHLs produced by its symbiont *Sinorhizobium meliloti* and an opportunistic pathogen *Pseudomonas aeruginosa* [63]. However, among the plant-produced QS agonists and antagonists that may play a role in legume/rhizobia interactions, the only one that has chemically identified is ʟ-Canavanine, an arginine analog [62••]; thus, it has been predicted that the observed QS inhibition may be an indirect effect potentially caused by protein misfolding of transcription regulators [59].

Endophytes as chemical factories
In addition to interacting with microorganisms in the rhizosphere, plants are internally colonized by endophytic bacteria and fungi. Endophytic bacteria can be defined as bacteria that reside within living plant tissue without causing substantive harm to their host. Diverse arrays of bacterial genera have been reported to be endophytic [64,65]. The community structure of endophytic bacteria was shown to be strongly affected by the plant species, up to the level of the cultivar [66], pointing to species-specific associations between endophytes and their plant host [67]. On the contrary, some endophytic bacteria

seem to be quite promiscuous when it comes to host plant colonization and plant beneficial effects, such as the *Burkholderia cepacia* Bu72, which was isolated from yellow lupine [68] but also significantly improved biomass production of poplar DN-34 [67,69]. Therefore, before applying plant growth promoting endophytic bacteria preliminary studies to confirm the plant growth promoting synergy of the selected endophytes and the plant species are required.

A close relationship exists between endophytic and rhizosphere bacteria and many facultative endophytic bacteria can also survive in the rhizosphere, where they can enter their host plant via the roots. Root colonization by rhizosphere bacteria involves several stages [70] and endophytic bacteria are hypothesized to follow a similar process. In the initial stage, bacteria move to the plant roots either passively via soil water fluxes or actively via specific induction of flagellar activity by plant-released compounds. In a second step, non-specific adsorption of bacteria to the roots takes place, followed by anchoring (third step), and resulting in the firm attachment of bacteria to the root surface. Specific or complex interactions between the bacterium and the host plant, including the secretion of root exudates, may arise that can result in the induction of bacterial gene expression (fourth step). Endophytic bacteria can subsequently (fifth step) enter their host plant at sites of tissue damage, which naturally arise as the result of plant growth (lateral root formation), or through root hairs and at epidermal conjunctions [71]. In addition, plant exudates leaking through these wounds provide a nutrient source for the colonizing bacteria.

Endophytic bacteria can improve plant growth and development in a direct or indirect way. Direct plant growth promoting mechanisms of endophytic bacteria may involve nitrogen fixation [65,72], the production of plant growth regulators such as auxins, cytokinins and gibberellins [73–75], suppression of the production of stress ethylene by 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity [76,77], and alteration of sugar sensing mechanisms in plants [78]. For instance, alteration of biosynthesis and/or metabolism of trehalose *in planta* have been shown to increase tolerance to drought, salt and cold [79]. It is therefore noteworthy that several endophytic bacteria from, for example, poplar were able to efficiently metabolize trehalose [67]. Endophytic bacteria can also indirectly benefit plant growth by preventing the growth or activity of plant pathogens through competition for space and nutrients [80], antibiosis [81], production of hydrolytic enzymes [82], inhibition of pathogen-produced enzymes or toxins [83] and through induction of plant defense mechanisms [84].

A systems biology approach to better understand the synergistic interactions between plants and their beneficial

the area where the soil and roots are in close proximity. Several lines of evidence implicate root-secreted exudates as signaling agents that play a key role in these interactions. Researchers have already identified some of the factors initiating the dialogues in the rhizosphere that drive these interactions. However, there are still numerous other factors/determinants yet to be identified to better understand these interactions at an ecological level. The rhizosphere is considered to be common ground for ecologists, molecular biologists and plant biologists to further explore these novel interactions occurring in this complex zone. Recent technology development in the areas of 'omics' such as proteomics, metabolomics, transcriptomics and secretomics allow us to further underpin these interactions efficiently for agricultural benefit. A combination of data analyses obtained from these 'omics' studies will further strengthen our capability to visualize a complete picture of these complex multi-species interactions.

Acknowledgements

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Invited Review

Components of sustainable animal production and the use of silvopastoral systems

Donald Maurice Broom¹*

Changing ideas about sustainability

The meaning of the term sustainable is now much wider than it was in the past. Systems were initially called unsustainable when a resource became depleted so much that it became unavailable to the system, or when a product of the system accumulated to a degree that prevented the functioning of the system. Now, the meaning of the term is much wider; for example, a system can be unsustainable because of negative impacts on human health, animal welfare, or the environment. A definition of sustainability is: a system or procedure is sustainable if it is acceptable now and if its expected future effects are acceptable, in particular in relation to resource availability, consequences of functioning, and morality of action (Broom, 2014 modified after Broom 2001, 2010). The development of new, sustainable systems is urgently needed because of industrial and livestock production practices. Consumers now include the ethics of food production in their evaluation of product quality (Broom, 2010). The opinion of the public is based on a range of components of sustainability, described briefly below.

What might make an animal usage system unsustainable? A consequence of the definition above is that any effect which the general public find unacceptable makes a system unsustainable. Members of the public in all parts of the world, particularly in developed countries, are now insisting on transparency in commercial and governmental activities and on changes in methods of producing various products (Broom, 2017). A production system might be unsustainable because of: inefficient usage of world food resources; adverse effects on human health; poor animal welfare; harmful environmental effects, such as low biodiversity or insufficient conservation; unacceptable genetic modification; not being "fair trade", in that producers in poor countries are not properly rewarded; or damage to rural communities. Any of these inadequacies could result in the quality of the product being judged

as poor. In the future, consumers are likely to demand that sustainable systems are used. If they are not, retail companies, production companies, and countries that do not produce good quality, sustainable products are likely to be boycotted and, hence, forced to change (Bennett et al., 2002, Broom, 2014).

The following subsections briefly describe components of unsustainable systems that are also factors leading to animal products being regarded as of poor quality. The section headed "Sustainable systems and welfare" summarises data on sustainability concerning some silvopastoral systems.

Efficient use of world resources

At present, some food for humans and for farmed animals is wasted. Much food that humans could eat is given to animals that will be eaten by people, a much less efficient process than for the humans to eat the food directly. What can be done in animal production to exploit existing resources better (Herrero et al., 2010)? The most important animals for food production are those that eat food that humans cannot eat. Hence, herbivores eating forage plants, not cereals, are much more important than pigs or poultry, which compete with humans for food (Broom et al., 2013). Similarly, herbivorous fish are more important than those fish that eat other fish.

Land used for agriculture is sometimes not exploited efficiently and too much energy from fossil fuels is used in cultivation and transport of feed and products. Maintaining resources, such as soil with good structure, and retaining water that might be lost from the soil are important objectives, as is minimising usage of carbon-based energy and imported fertilisers. Soil is often damaged by tillage and greenhouse gases emitted (Pagliai et al., 2004).

Adverse effects on human health

Some foods are regarded as being better for the health of the consumers because of the nutrients present in them. A major effect of attempts to provide a healthy diet on animal production in recent years has been the dramatic increase in the production of farmed fish, in part because they contain poly-unsaturated fats (Wall et al., 2010). As open-water fish management has failed in most parts of the world, fish-farming has increased and is likely to increase further. In the future, it is suggested for resource-usage reasons that herbivorous fish are likely to be the most important species and the welfare of fish and impact of farms on the environment will have to be fully considered for there to be public acceptance of the products.

In all aspects of farming, antibiotic use will have to decrease in most countries via legislation. This is because of the development of antibiotic resistance, largely because of misuse of antibiotics in human medicine, but partly because of widespread rather than just therapeutic use in livestock farming (Ungemach et al., 2006).

Negative impacts on animal welfare

Poor animal welfare is probably the third most important reason for unsustainable livestock production. Welfare is the state of the individual as regards its attempts to cope with its environment (Broom, 1986), so it can be measured scientifically. Measures of animal disease are often important, because health is a key part of welfare. Other measures, for example of behaviour, physiology, immune system function, and body damage, are described by Broom (2014) and Broom and Fraser (2015). Close confinement of animals, individual rearing of social animals such as pigs and cattle, and other systems for housing and managing animals that do not meet the needs of the animals are so much disliked by many consumers that they will not buy the animal products. Hence, some widely-used animal housing systems are unsustainable (Broom, 2017). The welfare of animals kept on pasture-only systems can be poor because of heat-stress, parasitic and other infectious disease, and low nutrient availability with associated competition (Petherick, 2005). The welfare of animals in feedlots is often worse than that of animals on pasture.

Harmful environmental effects

Agricultural methods that result in low biodiversity and the need for conservation are a consequence of widespread herbicide and pesticide use and perceived to be the norm by many farmers and some of the general public. However, biodiversity on farmland can be much increased in some systems. Livestock production can also result in pollution, locally and on a world-wide scale, e.g., via greenhouse gas production. Greenhouse gas production should be reduced and may have to be balanced against efficiency of use of world resources (Broom et al., 2013).

Unacceptable genetic modification

Many people in the world are unwilling to accept the use of genetically modified plants and few people accept the use of genetically modified or cloned animals. One reason for this is dislike of modifying what is natural. Another is that modified organisms may have allergenic proteins and

many of the public do not believe that proper checks on such possibilities are in place. Genetically modified animals may be more likely to have welfare problems so there should be checks using a wide range of welfare indicators before they are used for any purpose (Broom, 2008, 2014).

Not being "fair trade"

In recent years, consumers in many countries have been appalled to find that food producers in poor countries are often not properly rewarded for their work. Most profits from the sale of some basic products bought by many people are found to go to large companies. As a consequence of publicity about unfairness to poor producers, products like coffee, cocoa, and fruit are among those that are independently checked and have a Fair Trade label (Nicholls and Opal, 2005). Hence, the producers receive a larger part of the money paid by shoppers in relatively rich countries.

Damage to rural communities

When small-scale rural farmers are out-competed by large-scale production, local communities may disappear. The general public often find this unacceptable; so, schemes are introduced by governments to safeguard such communities. Consumers may also buy locally produced products, regarding this as a part of product quality. In the European Union, subsidies to preserve rural communities have prevented large cities from increasing in size (Gray, 2000; Broom, 2010).

Sustainable systems and welfare

Livestock in woodland

Agroforestry allows the use of spaces or clearings in woodland for livestock. The animal production is additional to woodland production and may have benefits when dung components are utilised by the plants (Mcadam et al., 2007). Animal welfare and other aspects of sustainability are better than the average in animal production, for example, when Andalucian or Portuguese pigs exploit woodland (Castro, 2009). The trees may be chestnut or oak (Table 2) and the pigs eat the fruits of the trees, either when these are produced or later. Agroforestry produces wood, as well as meat or other animal products (Tirapicos Nunes, 2007; Santos Silva and Tirapicos Nunes, 2013), and often provides an environment that results in good welfare for the animals.

The woodland may itself be made up of trees planted for production of a human resource. For example, in Malaysia cattle can utilise areas between trees in oil-palm plantations. Agropastoral combinations of soya or other crops and cattle can have various benefits. However, we have little knowledge of the effects on animal welfare.

Silvopastoral three level systems

Three-level plant production, including pasture, shrubs with edible leaves, and trees that may also have edible leaves, are an example of a silvopastoral system. A cattle production system whose characteristics and objectives include using three-level or other multi-level production of edible plants; managing the soil, taking account of worms and water retention; encouraging predators of harmful animals; minimising greenhouse gas emissions; improving job-satisfaction for stock-people; reducing injury and stress in animals and maximising good welfare; considering how to encourage biodiversity using native shrubs and trees; and utilising the potential for obtaining wood from trees is explained by Murgueitio et al. (2008, 2011), Giraldo et al. (2011), Naranjo et al. (2012), and Broom et al. (2013). Some of the species used as food for livestock in tropical and temperate silvopastoral systems are listed in Table 1 and Table 2. Some of the trees are used as "live fences" (Nahed-Toral et al., 2013; Villanueva-López et al., 2014).

Table 1 - Tropical and sub-tropical shrubs and trees that are eaten by sheep, goats, and cattle in South America

Species	Common name(s)
Gliricidia sepium	quickstick, mata ratón
Guazuma ulmifolia	bay cedar, guácimo
Morus alba	white mulberry, morera
Leucaena leucocephala	leucaena
Brosimun alicastrum	Maya nut, ramón
Tithonia diversifolia	tree marigold, botón de oro
Trichanthera gigantea	tricanthera, nacedero
Erythrina edulis E. poeppigiana	poroto, búcaro
Boehmeria nivea	ramie, ramio
Cratylia argentea	veranera
Malvaviscus penduliflorus	mazapan

Table 2 - Shrubs and trees that are used as forage for ruminants and pigs in temperate countries

Species	Common name(s)
Castanea sativa	sweet chestnut
Castanea mollissima	Chinese chestnut
Quercus pyrenaica	Pyrenean oak
Quercus ilex	evergreen oak
Quercus suber	cork oak
Olea europea	olive
Alnus nepalensis	Nepalese alder
Sesbania sesban	sesban
Chamaecytisus prolifer	tagasaste
Robinia pseudoacacia	black locust/frisia
Sambucus canadiensis	American elder
Helianthus tuberosum	Jerusalem artichoke (herb)

There are also possibilities for feeding tree and shrub leaves to pigs, poultry, or farmed fish. Where shrubs and trees are too high for animals to reach, branches can be cut and offered to the animals.

The production of leaves and other materials that can be eaten by the animals is much greater in silvopastoral systems than in pasture-only systems. Results from tropical and sub-tropical studies show that cattle production can be better. Three-level forage production produces more usable plant material than pasture only. Pasture plus *Leucaena* produced 29% more mass and 64% more protein than monoculture herbage-layer only systems. Nitrogen-fixing plants are used; so, less artificial fertiliser is needed. Animal production yields can be greater on silvopastoral systems than on semi-intensive silvopastoral systems than on monoculture systems (Table 3) (Murgueitio et al., 2011). Three-level silvopastoral systems generally have better soil structure, better water retention, and less soil loss (Murgueitio et al., 2008; Broom et al., 2013).

Three-level silvopastoral systems have much greater biodiversity than monoculture, single-level systems. The number of bird species increased by 200%, that of ants by 30%, and there were also increases in the numbers of butterflies. There was less pollution run-off because of water-holding properties of soil, 30% less methane production per kg meat, and better carbon sequestration. Workers on silvopastoral farms reported better job satisfaction (Broom et al., 2013).

The animal welfare in silvopastoral systems has been demonstrated to be better in various ways than that on pasture-only systems (Table 4) (Broom, 2015; 2016). The beneficial effects of shade are substantial in hot weather with cattle skin temperatures up to 4 °C lower than in pasture-only systems. High temperature increases water and energy loss and reduces foraging times in paddocks fully exposed to the sun (Améndola, 2013; Améndola et al., 2016). Less sun exposure results in less sun-burn, less cancer, and less photosensitisation (Rowe, 1989).

Anxiety and fear, including fear of humans, can be reduced when partial concealment is possible. This leads to better human-animal interactions and easier handling (Ocampo et al., 2011; Mancera and Galindo, 2011). More choice of food in silvopastoral systems results in more control by each individual animal of its environment and thus social behaviour is more normal (Améndola et al., 2016).

The increase in predators lowers the populations of ticks and injurious insects, such as horn flies, and hence reduces the incidence of diseases such as anaplasmosis, which has been shown to drop from 25 to <5% (Murgueitio and Giraldo, 2009). Reduction in diseases also leads to reduced antibiotic use. The presence of nitrogen-fixing shrubs, such as *Leucaena*, improves animal nutrition and this, together with the better water-retention by the soil, reduces the likelihood of thirst and starvation. Feeding behaviour is improved at high temperature and humidity if the animals are in a silvopastoral system (Ceballos et al., 2011). It may be that the improvement in dietary choice contributes to this beneficial effect (Manteca et al., 2008).

A study of welfare in three intensive silvopastoral systems was carried out in Colombia with *Leucaena*

Table 3 - Summary of comparisons between monoculture and semi-intensive silvopastoral systems

	"Improved" monoculture pasture	Semi-intensive silvopastoral system
Metabolisable energy (Mcal.ha^{-1})	56.9	70.2
Crude plant protein (tonne ha^{-1})	2.5	4.1
Milk per cow (kg day^{-1})	3.5	4.1
Meat (kg ha^{-1} year^{-1})	183	821
Methane (tonne of meat^{-1})	208	128
Bird species	24	75
Anaplasmosis (% of herd)	25	<5
Fights (% difference)	+37	
Social licking (% difference)		+65
Social interactions in shade (% difference)		+57

Re-analysed data from Murgueitio et al. (2008), Broom et al. (2013) and Améndola et al. (2013, 2016).

Table 4 - Summary of benefits of silvopastoral systems for animal welfare

Nutritional improvement because of shrub and tree intake	Murgueitio et al., 2011
Thermal comfort resulting from more shade	Mancera and Galindo, 2011
Less fear because of concealment	Ocampo et al., 2011
Better health because of more predators of ticks and flies	Murgueitio and Giraldo, 2009
Better body condition because of nutrients, shade, and less disease	Ocampo et al., 2011; Tarazona Morales et al., 2017
Improved food intake and social behaviour	Améndola, 2013; Améndola et al., 2013, 2016
Better human-animal interactions	Mancera and Galindo, 2011

leucocephala and *Guazuma ulmifolia* as shrubs for browsing at more than 8,000 shrubs/ha and several tree species (Tarazona Morales et al., 2017). The needs of the cattle were met, there being good food and water availability, effective body temperature control and physical comfort, good social behaviour, and low parasite levels. Some respiratory infection occurred on one farm, but this might be expected by chance.

When the social behaviour of cattle was compared in a silvopastoral system and a pasture-only monoculture system in the region of Merida, Yucatán, Mexico, there was more affiliative social behaviour in the silvopastoral system (Améndola, 2013; Améndola et al., 2013, 2016). Social licking was the main affiliative behaviour and was shown by 78% of the heifers in the silvopastoral system, but only 47% in the monoculture system. In the silvopastoral system, 57% of interactions occurred in the shade. Head-butting and chasing occurred in the silvopastoral system, but often did not develop into a fight. Social licking is known to occur after the animals have obtained food and shelter (Sato et al., 1991), but it does reduce the heart rate of the participants (Laister et al., 2011) and contributes to the stability of social relationships in cattle (Sato et al., 1993).

Conclusions

In relation to animal production throughout the world, there will be increasing demand from consumers for more efficient use of world resources and the avoidance of adverse effects on human welfare, animal welfare, and the environment.

Industry has to rapidly change policies relating to animal welfare and other aspects of sustainability. The animal production industry should be proactive.

Tropical and temperate livestock production should consider three-level silvopastoral systems, with shrubs and trees with edible leaves.

Animal welfare has been developing rapidly as a scientific discipline and the benefits of silvopastoral systems for animal welfare have been studied.

The animal welfare benefits of three-level silvopastoral systems include nutritional improvement because of shrub and tree intake; thermal comfort resulting from more shade; less fear because of concealment; better health because of more predators of ticks and flies; less risk of cancers and other diseases caused by too much direct sunlight; better body condition because of nutrients, shade, and less disease; improved food choice, food intake, and social behaviour; and better human-animal interactions.

Acknowledgments

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Article

The Impact of Anatomical Characteristics on the Structural Integrity of Wood

Lukas Emmerich *, Georg Wülfing and Christian Brischke

Wood Biology and Wood Products, University of Goettingen, Buesgenweg 4, D-37077 Goettingen, Germany;
georg.wuelfing@stud.uni-goettingen.de (G.W.); christian.brischke@uni-goettingen.de (C.B.)
* Correspondence: lukas.emmerich@uni-goettingen.de

1. Introduction

Most elasto-mechanical and rheological properties of wood are closely related to wood density and are therefore rather easily predictable. However, the anatomical features of wood, which can be wood species-specific, further affect especially dynamic strength properties such as the impact bending strength and shock resistance [1–3]. For instance, the large earlywood pores in ring-porous hardwoods such as English oak (*Quercus robur* L.), Sweet chestnut (*Castanea sativa* L.), Black locust (*Robinia pseudoacacia* L.) or Wych elm (*Ulmus glabra* Huds.) can serve as predetermined breaking points. Further deviations from an ideal homogeneous xylem structure such as large rays in European beech (*Fagus sylvatica* L.) or Alder (here: false rays, *Alnus* spp.), distinct parenchyma bands in Bongossi (*Lophira alata* Banks ex C. F. Gaertn.) or agglomerates of resin canals in Red Meranti (*Shorea* spp.), also have the potential to either strengthen or reduce the structural integrity of wood.

Similarly, wood cell wall modification affects different mechanical properties including the wood hardness and abrasion resistance, but also its brittleness and consequently its structural integrity. This has been shown previously with the help of High-Energy Multiple Impact (HEMI)-tests, where small wood specimens are subjected to thousands of dynamic impacts by steel balls in the bowl of a heavy vibratory mill. The fragments obtained are analyzed afterwards [4]. For instance, the weakening of cell walls by heat during thermal modification processes, especially in the middle lamella region,

leads to a steady decrease in the structural integrity of wood with increasing treatment intensity. The HEMI-test has further been used to detect fungal decay by soft rot, brown rot and white rot fungi (even in very early stages), the effect of gamma radiation, wood densification, wood preservative impregnation, wax and oil treatments, and different chemical wood modification processes [5].

It has previously been shown that the Resistance to Impact Milling (RIM), which serves as a measure of wood's structural integrity is very insensitive to varying densities, natural ageing, and the occurrence of larger cracks [5]. Furthermore, the RIM varies only little within one wood species, as shown for Scots pine sapwood (*Pinus sylvestris* L.) samples from trees in six Northern European countries [6]. However, the results from previous studies indicated that the structural integrity determined in HEMI-tests is not well correlated with wood density, since further variables such as wood species-specific anatomical characteristics of the xylem tissue interfere with the effect of density [7].

Objective

The aim of this study was to analyze the results from HEMI-tests of a wide range of softwood and hardwood species with an average oven-dry wood density in a range between 0.25 and 0.99 g/cm^3 and with multifaceted anatomical features.

2. Materials and Methods

One hundred replicate specimens of 10 (ax.) $\times$ 5 $\times$ 20 mm^3 were prepared from a total of 40 different wood species and separated between sapwood and heartwood, as listed in Tables 1 and 2.

To determine the oven-dry density (ODD), n = 10 replicate specimens of 10 (ax.) $\times$ 5 $\times$ 20 mm^3 per wood species were oven dried at 103 °C until a constant mass, weighed to the nearest 0.0001 g; the dimensions were then measured to the nearest 0.001 mm. The oven dry density was calculated according to the following equation:

$$\rho_0 = \frac{m_0}{V_0} \, [\text{g cm}^{-3}] \tag{1}$$

where:

ρ_0 is the oven-dry density, in g·cm^{-3};
m_0 is the oven-dry mass, in g;
V_0 is the oven-dry volume, in cm^3.

Table 1. The oven-dry density (ODD), Resistance to Impact Milling (RIM), degree of integrity (I), and fine percentage (F) of different softwood species. The standard deviation (SD) is in parentheses.

Name [1]	Botanical Name	ODD [g cm^{-3}]		RIM [%]		I [%]		F [%]	
Scots pine sw	*Pinus sylvestris*	0.41	(0.02)	88.2	(0.9)	67.4	(1.1)	13.5	(1.1)
Scots pine hw		0.58	(0.04)	84.5	(0.8)	41.9	(3.1)	1.3	(0.4)
Radiata pine sw	*Pinus radiata*	0.43	(0.02)	88.8	(0.5)	55.4	(2.1)	0.0	(0.0)
Carribean pine hw	*Pinus carribaea*	0.39	(0.04)	87.3	(0.4)	52.4	(1.8)	1.1	(0.3)
European Larch sw	*Larix decidua*	0.56	(0.02)	85.2	(0.4)	44.5	(2.2)	1.2	(0.3)
European Larch hw		0.51	(0.02)	80.8	(1.5)	35.5	(4.8)	4.1	(0.4)
Douglas fir sw	*Pseudotsuga menziesii*	0.63	(0.02)	86.3	(0.4)	45.6	(1.8)	0.2	(0.2)
Douglas fir hw		0.51	(0.02)	82.2	(0.5)	34.8	(1.3)	1.9	(0.3)
Norway spruce	*Picea abies*	0.43	(0.03)	82.9	(1.7)	35.9	(6.1)	1.5	(0.4)
Coastal fir	*Abies grandis*	0.40	(0.06)	80.6	(0.5)	26.5	(1.3)	1.4	(0.4)
Western hemlock	*Tsuga heterophylla*	0.42	(0.03)	83.8	(0.7)	40.0	(2.1)	1.6	(0.3)
Yew	*Taxus baccata*	0.60	(0.03)	84.5	(0.9)	43.9	(3.2)	1.9	(0.3)

[1] sw = sapwood, hw = heartwood; heartwood if not otherwise indicated.

Table 2. The oven-dry density (ODD), Resistance to Impact Milling (RIM), degree of integrity (I), and fine percentage (F) of different hardwood species. The standard deviation (SD) is in parentheses.

Name [1]	Botanical Name	ODD [g cm^{-3}]		RIM [%]		I [%]		F [%]	
English oak sw	Quercus robur	0.49	(0.02)	83.3	(0.5)	44.2	(1.7)	3.7	(0.4)
English oak hw		0.59	(0.01)	87.3	(1.2)	59.0	(4.3)	3.3	(0.4)
Black locust	Robinia pseudoacacia	0.68	(0.05)	83.5	(1.2)	41.0	(3.9)	2.3	(0.2)
Sweet chestnut	Castanea sativa	0.50	(0.03)	78.1	(2.3)	36.0	(4.2)	7.9	(1.8)
Ash	Fraxinus excelsior	0.62	(0.02)	83.1	(0.8)	40.4	(2.6)	2.7	(0.3)
Locust	Gleditsia sp.	0.66	(0.02)	86.7	(1.1)	52.6	(3.4)	1.9	(0.4)
Common walnut	Juglans regia	0.63	(0.02)	85.2	(0.5)	49.8	(2.1)	2.9	(0.3)
Wild cherry	Prunus avium	0.55	(0.01)	86.7	(0.7)	53.0	(2.2)	2.0	(0.3)
Black cherry	Prunus serotina	0.64	(0.04)	87.7	(0.6)	54.9	(2.1)	1.4	(0.2)
Beech	Fagus sylvatica	0.66	(0.02)	88.0	(0.4)	55.9	(2.2)	1.4	(0.3)
Maple	Acer sp.	0.61	(0.01)	89.1	(0.6)	58.0	(2.3)	0.5	(0.1)
Lime	Tilia sp.	0.44	(0.01)	90.1	(0.8)	61.1	(2.6)	0.2	(0.3)
Birch	Betula pendula	0.57	(0.02)	87.9	(0.4)	54.2	(1.6)	0.8	(0.1)
Hazel	Corylus avellana	0.68	(0.02)	86.9	(1.0)	52.8	(3.9)	1.8	(0.2)
Boxwood	Buxus sempervirens	0.96	(0.01)	90.3	(0.9)	64.1	(3.7)	0.9	(0.0)
Poplar	Populus nigra	0.39	(0.02)	86.3	(0.3)	50.5	(0.9)	1.8	(0.3)
Alder	Alnus glutinosa	0.48	(0.01)	86.9	(0.9)	54.6	(3.3)	2.3	(0.5)
Kiri	Paulownia tomentosa	0.25	(0.02)	80.9	(1.5)	40.0	(4.0)	5.5	(0.9)
Shining gum	Eucalyptus nitens	0.74	(0.11)	83.2	(1.5)	46.7	(4.5)	4.6	(0.9)
Teak	Tectona grandis	0.63	(0.09)	84.1	(0.7)	48.0	(2.1)	3.9	(0.8)
Ipe	Handroanthus sp.	0.93	(0.02)	86.0	(0.5)	51.8	(1.2)	2.6	(0.7)
Merbau	Intsia spp.	0.74	(0.03)	68.1	(2.4)	27.9	(1.8)	18.6	(2.7)
Bangkirai	Shorea laevis	0.79	(0.05)	87.7	(0.7)	54.9	(1.9)	1.4	(0.4)
Balau	Shorea spp.	0.92	(0.03)	84.3	(1.1)	51.7	(2.8)	4.8	(1.0)
Bongossi	Lophira alata	0.97	(0.03)	85.9	(1.0)	51.9	(2.7)	2.8	(0.7)
Amaranth	Peltogyne sp.	0.88	(0.01)	88.6	(0.7)	57.9	(2.7)	1.1	(0.0)
Basralocus	Dicorynia sp.	0.81	(0.02)	84.8	(0.6)	50.9	(1.9)	4.0	(0.4)
Garapa	Apuleia sp.	0.76	(0.04)	86.7	(1.1)	53.0	(3.3)	2.1	(0.5)
Limba	Terminalia superba	0.50	(0.03)	83.2	(1.2)	45.1	(2.7)	4.1	(0.9)
Kambala	Milicia sp.	0.62	(0.03)	79.7	(0.7)	45.2	(2.7)	8.8	(0.4)
Massaranduba	Manilkara bidentata	0.99	(0.04)	85.9	(0.6)	53.2	(2.5)	3.2	(0.2)
Greenheart	Chlorocardium rodiei	0.96	(0.02)	85.9	(1.5)	49.9	(5.3)	2.1	(0.8)

[1] sw = sapwood, hw = heartwood.

Afterwards, selected density specimens were cut with a traversing microtome and used for digital reflected-light microscopy with a Keyence Digital microscope VHX 5000 (Keyence Corporation, Osaka, Japan). Cross section photographs were taken at a magnification of 30×, and the diameter of the earlywood vessels, the vessel density, and the wood ray density were determined at a magnification of 200× for both the soft- and hardwoods. For the tropical species, the listed anatomical features were determined at a magnification of 100×. Therefore, $n = 10$ replicate measurements were conducted per wood species to determine the ray density and vessel density. The earlywood vessel diameter was determined on $n = 30$ vessels.

Five times 20 specimens of 10 (ax.) × 5 × 20 mm^3 were submitted to High-Energy Multiple Impact (HEMI)–tests. The development and optimization of the HEMI-test have been described by [4] and [8]. In the present study, the following procedure was applied: 20 oven-dried specimens were placed in the bowl (140 mm in diameter) of a heavy-impact ball mill (Herzog HSM 100-H; Herzog Maschinenfabrik, Osnabrück, Germany), together with one steel ball of 35 mm diameter for crushing the specimens. Three balls of 12 mm diameter and three of 6 mm diameter were added to avoid small fragments from hiding in the angles of the bowl, thus ensuring impact with smaller wood fragments. The bowl was shaken for 60 s at a rotary frequency of 23.3 s^{-1} and a stroke of 12 mm. The fragments of the 20 specimens were fractionated on a slit sieve according to [9], with a slit width of 1 mm, using an

orbital shaker at an amplitude of 25 mm and a rotary frequency of 200 min^{-1} for 2 min. The following values were calculated:

$$I = \frac{m_{20}}{m_{all}} \times 100 \; [\%] \tag{2}$$

where:

I is the degree of integrity, in %;
m_{20} is the oven-dry mass of the 20 biggest fragments, in g;
m_{all} is the oven-dry mass of all the fragments, in g.

$$F = \frac{m_{fragments<1mm}}{m_{all}} \times 100 \; [\%] \tag{3}$$

where:

F is the fine percentage, in %;
$m_{fragments<1mm}$ is the oven-dry mass of fragments smaller than 1 mm, in g;
m_{all} is the oven-dry mass of all the fragments, in g.

$$RIM = \frac{(I - 3 \times F) + 300}{400} \; [\%] \tag{4}$$

where:

RIM is the Resistance to Impact Milling, in %;
I is the degree of integrity, in %;
F is the fine percentage, in %.

3. Results and Discussion

3.1. Structural Integrity

The Resistance to Impact Milling (RIM) varied between 68.1% (Merbau) and 90.3% (Boxwood). In contrast, the degree of integrity (I) varied significantly more, i.e., between 26.5% (Coastal fir) and 67.4% (Scots pine sapwood), as did the fine percentage (F): i.e., between 0.0% (Radiata pine) and 18.6% (Merbau). The data for the RIM, I, and F are summarized in Table 1 for the tested softwood species and in Table 2 for the hardwood species. Besides differences between the wood species, the three indicators showed differences in the variation within one species, here expressed as the standard deviation (SD). The highest variation was obtained for F, followed by I and RIM. This supports previous findings pointing out the benefit of using the combined measure RIM, which is of higher sensitivity to differences in the structural integrity paired with less scattering of data compared to I and F [4,7]. In total, the SD of the RIM was between 0.3% (Poplar) and 2.4% (Merbau), corresponding to coefficients of variation (COV) between 0.4% and 3.5%, which is very low compared to mechanical properties such as the bending or impact bending strength (e.g., [7]).

3.2. Impact of Oven-Dry Density on Structural Integrity

A clear relationship between the ODD and structural integrity did not become evident, as shown for all the examined wood species and separately for the softwoods, ring- and semi-ring-porous hardwoods and diffuse-porous hardwoods in Figure 1. The RIM seemed to be at least superposed by further parameters such as structural features and anatomical characteristics. This coincides with the data for the Ash, Scots pine and Beech previously reported by [8], who showed that the density and RIM were not even correlated within one wood species. More recently, [7] reported that the density and RIM were also poorly correlated when considering ten different wood species representing a range of ODD between 0.37 and 0.77 g/cm^3. However, according to [7] the RIM was fairly well correlated with the impact bending strength (IBS, $R^2 = 0.67$) and modulus of rupture (MOR, $R^2 = 0.56$), as determined

on axially matched specimens, which indicates that these strength properties are also at least partly affected by similar anatomical characteristics as the RIM is.
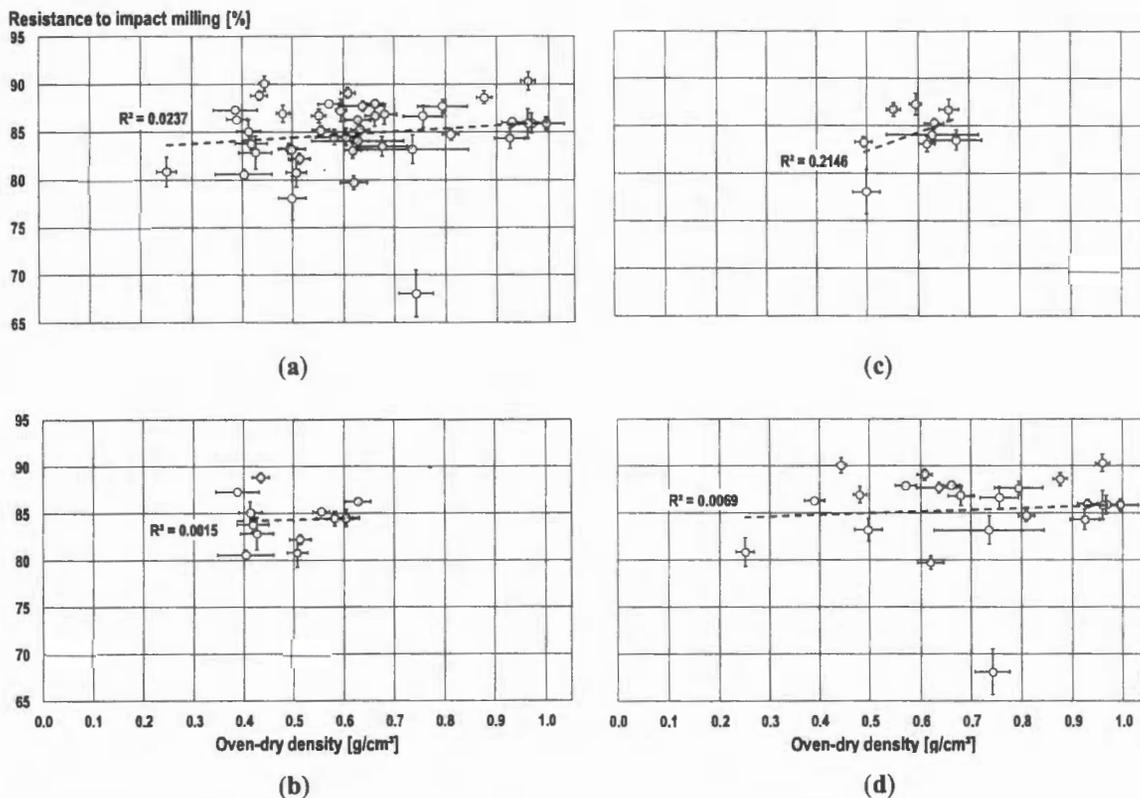

Figure 1. The relationship between the average oven-dry density and Resistance to Impact Milling (RIM): (a) all wood species (y = 3.1629x + 82.887); (b) softwoods (y = 1.1035x + 83.791); (c) ring- and semi-ring-porous hardwoods (y = 19.634x + 72.545); and (d) diffuse-porous hardwoods (y = 1.8475x + 84.086).

3.3. Impact of Anatomical Characteristics on Structural Integrity

The tested softwood species had a rather homogeneous and uniform anatomical appearance compared to the different hardwood species. However, even within this group the RIM varied between 80.6% and 88.8%. As summarized in Table 3, the softwood species differed also in the average tracheid diameter and in wood ray density. Nevertheless, the fracture patterns observed during the HEMI-tests were rather uniform, and fractures occurred predominantly along the growth ring borders in a tangential direction and along the wood rays and resin canals in a radial direction. The wood species showing an abrupt transition between the earlywood and latewood, such as the Larch and Scots pines, did not show a lower structural integrity compared to the species with a more gradual transition, such as the Norway spruce and Douglas fir, as one might expect due to a more sudden change of density within the tracheid tissue of one annual ring. Consequently, no fractures were observed along the transition line between the earlywood and latewood. In contrast to other softwood species, the Carribean and Radiata pines showed fractures in a tangential direction not only at the growth ring borders, but also where the resin canals ran in an axial direction.

As exemplarily shown for the heartwood of the Scots pine and Douglas fir in Figure 2, the major weak points, where fractures predominantly occurred, were the following: (a) the growth ring borders, where the less dense earlywood follows the dense latewood, and (b) the wood rays, which (1) consist of parenchyma cells, and (2) are running orthogonal to the main cell orientation in the tracheid tissue.

Table 3. The anatomical characteristics (tracheid diameter, ray density) and description of fractures during the HEMI-tests of different softwood species (standard deviation in parentheses).

Wood Species	Tracheid Ø [µm]		Wood Ray Density [mm^{-1}]		Fracture Behaviour		Remarks
					tang.	rad.	
Scots pine sw	29	(6)	4.6	(1.2)	GR	RC	wider rings compared to hw
Scots pine hw	25	(5)	3.7	(1.5)	GR	RC	-
Radiata pine sw	22	(4)	4.4	(1.3)	GR	R, RC	-
Carribean pine hw	28	(4)	5.2	(1.2)	GR	R, RC	-
European larch sw	35	(7)	5.9	(1.7)	GR	R	wider rings compared to hw
European larch hw	35	(6)	4.4	(1.1)	GR	R	-
Douglas fir sw	25	(6)	4.3	(1.2)	GR	R, RC	-
Douglas fir hw	23	(5)	3.9	(1.2)	GR	R	-
Norway spruce	25	(5)	4.5	(1.0)	GR	R	-
Coastal fir	28	(5)	5.7	(1.3)	GR	R	-
Western hemlock	25	(5)	5.0	(1.3)	GR	R	-
Yew	10	(3)	7.1	(1.4)	(GR)	(R)	Irregular fracture pattern

GR = along growth rings, R = along rays; RC = along resin canals; tang. = tangential growth direction; rad. = radial growth direction.
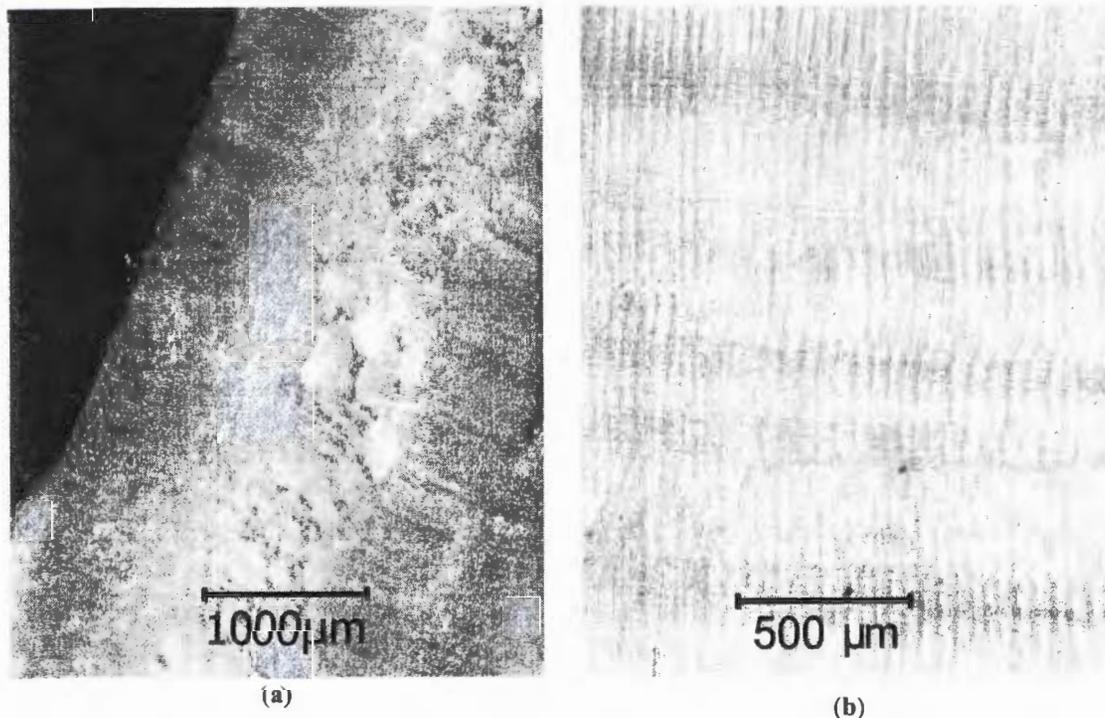

Figure 2. The fracture pattern in the softwoods: (a) Cross section of the Scots pine heartwood, fracture along a growth ring border; (b) The radial fracture section of the Douglas fir heartwood, fracture along the rays.

The fractures in the ring-porous hardwood species often followed the wide-luminous earlywood vessels, such as in the English oak, Sweet chestnut, Ash, Locust, and Black locust (Table 4). The specimens consequently broke apart in a tangential direction. In addition, the fractures occurred along the latewood vessel fields where high portions of paratracheal parenchyma were present (Figure 3). The ring-porous hardwoods with broad wood rays, such as the English oak, also showed fractures running parallel to the latter. Finally, the average diameters of the earlywood vessels were not correlated with the structural integrity, although, in the earlywood of all the ring-porous hardwoods, the fractures occurred preferentially in a tangential direction following the vessel rings.

Table 4. The anatomical characteristics (earlywood vessel diameter, vessel density, ray density) and description of fractures during the HEMI-tests of different hardwood species (standard deviation in parentheses).

Wood Species	Earlywood Vessel Ø [μm]		Vessel Density [mm^{-2}]		Wood Ray Density [mm^{-1}]		Fracture Behaviour [1]	
							tang.	rad.
English oak sw [2]	247	(51)	7.7	(1.6)	8.4	(2.0)	EW	P
English oak hw	202	(49)	10.1	(2.0)	10.7	(1.8)	EW	P
Black locust	190	(40)	11.5	(1.0)	6.9	(1.1)	EW	R *
Sweet chestnut	209	(30)	7.7	(1.5)	11.8	(1.5)	EW	V-V
Ash [3]	169	(21)	13.8	(1.7)	6.6	(0.8)	EW	n.a.
Locust	165	(25)	16.1	(2.0)	4.4	(1.2)	EW	R, P *
Common walnut	134	(32)	7.7	(2.0)	5.7	(1.3)	V-V	V-V
Wild cherry	33	(8)	171.6	(31.3)	6.1	(1.4)	GR	R
Black cherry [4]	33	(9)	67.9	(22.9)	5.6	(1.3)	n.a.	R
European beech	40	(8)	131.9	(15.4)	3.0	(1.3)	GR *	n.a.
Maple [5]	46	(7)	54.5	(3.4)	7.9	(1.7)	GR	R *
Lime [6]	39	(9)	104.7	(14.0)	4.8	(1.1)	n.a.	n.a.
Birch	54	(13)	45.2	(8.5)	8.3	(2.4)	n.a.	R
Hazel	28	(6)	98.9	(20.2)	11.6	(2.5)	GR	n.a.
Boxwood [6]	10	(4)	213.9	(14.0)	11.0	(2.5)	n.a.	R *
Poplar [7]	58	(13)	33.7	(6.4)	11.0	(1.5)	n.a.	R
Alder	41	(10)	108.0	(16.7)	11.7	(2.0)	GR *	R
Kiri	164	(55)	5.2	(2.0)	2.4	(0.8)	V-V	V-V, R *
Shining gum [2]	144	(25)	7.6	(3.2)	11.3	(1.1)	V-V	V-V
Teak [8]	184	(57)	6.3	(1.7)	4.1	(0.7)	V-V	R *
Ipé [2]	103	(9)	23.2	(2.7)	7.8	(0.9)	P *	V-V
Merbau	250	(40)	4.0	(1.4)	4.2	(0.9)	V-V, P *	V-V, P *
Bangkirai	207	(32)	7.3	(1.7)	3.7	(1.3)	P	V-V, R *
Balau	137	(13)	11.9	(2.9)	9.1	(1.2)	P	V-V, R *
Bongossi	232	(41)	2.9	(1.1)	9.9	(1.2)	P	V-V, P *
Amaranth	109	(16)	4.4	(1.7)	6.9	(1.7)	P, V *	R
Basralocus [2]	190	(33)	2.8	(1.0)	7.9	(1.0)	P *	V-V
Garapa	121	(19)	15.2	(3.0)	8.3	(1.3)	P	V-V
Limba	139	(28)	4.4	(1.7)	10.2	(1.0)	n.a.	R
Kambala	193	(41)	2.8	(0.8)	4.4	(1.0)	(P)	R
Massaranduba	113	(18)	13.1	(3.3)	10.5	(1.5)	(P)	R
Greenheart [2]	90	(16)	14.0	(2.0)	7.5	(0.9)	n.a.	V-V

[1] n.a. = not available (no clear pattern evident), GR = along growth rings, R = along rays, RC = along resin canals, EW = along earlywood vessels, P = in parenchyma tissue, V-V = vessel to vessel, V = at vessels, * = characteristic plays minor role; remarks related to fracture patterns: [2] radial, parallel to rays; [3] no clear radial pattern; [4] very often parallel to rays; [5] parallel to growth rings; [6] irregular fracture pattern; [7] samples often compressed; [8] often at growth ring border.

This stands to some extent in contrast to findings by [2], who studied the perpendicular-to-grain properties of eight North-American hardwood species and found that the earlywood vessel area fraction negatively influenced the radial maximum stress and strain, whereas the ray width and area fraction were positively related to the maximum radial properties. The rays also affected the transverse stiffness significantly.

Studies conducted by [10] showed that wood rays have a positive effect on the tensile strength of English oak and European ash wood. However, as shown for the fragments obtained in the HEMI-tests, the latewood vessel fields turned out to be weak spots when it comes to dynamic loads in different anatomical directions. Therefore, the potentially positive effect of the wood rays on the structural integrity might be superposed by other anatomical features.

Finally, the RIM of the heartwood of the English oak (87.3%) was significantly higher than that of its sapwood (83.3%), which is to some extent surprising since sapwood is often considered to be less brittle than heartwood [11]. While the fine percentage (F) of both English oak materials was almost equal, the degree of integrity (I) of the heartwood was remarkably higher than that of the

sapwood, which might be related to the potential 'gluing' effects of the tylosis which were present in the earlywood vessels in the heartwood (Figure 3b), but were absent in the sapwood. Whether and to what extent the formation of tylosis has a positive effect on structural integrity would need to be further investigated using different generally tylosis-forming wood species.
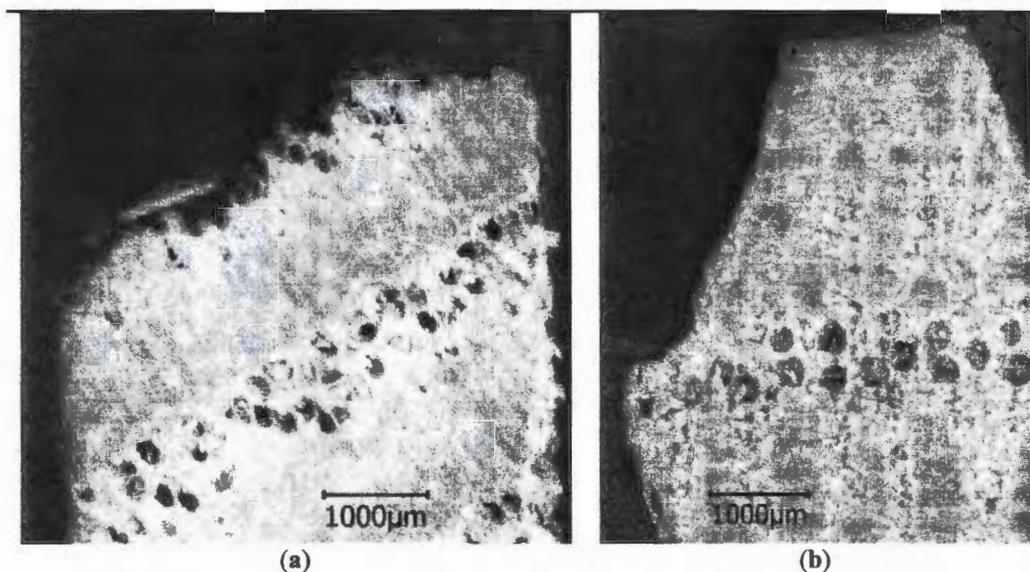

Figure 3. The fracture pattern in the ring-porous hardwoods: (**a**) Cross section of the Ash, fracture within a ring of the earlywood vessels; (**b**) Cross section of the English oak heartwood, fracture along the field of the latewood pores and the adjacent parenchyma cells.

By far, the Sweet chestnut showed the lowest RIM among the ring-porous hardwoods, which might be related to its high wood ray density (Table 4), but no clear correlation between the ray density and structural integrity became evident (Figure 4). Furthermore, the radial fractures in the Sweet chestnut were also running from one vessel to the next. More likely, the higher percentage of vessels and axial parenchyma leads to a higher number of weak points within the xylem of the Sweet chestnut compared to the other ring-porous species within this study.

The group of semi-ring-porous hardwood species, which was represented by the Teak, Wild cherry and Walnut in this study, takes an intermediate position between the ring- and the diffuse-porous species. This also became evident when analyzing the fracture patterns obtained through the HEMI-test. As shown in Figure 5a for the Wild cherry, the fractures occurred along the growth ring borders but did not run through the earlywood vessel rings.

In the diffuse-porous hardwoods, the RIM varied most, i.e., between 80.9% (Kiri) and 90.3% (Boxwood), respectively. Although these two species also represent the extremes in ODD, the latter was not correlated with the structural integrity, as shown in Figure 1. Nevertheless, in contrast to the ring-porous hardwood species, the average earlywood vessel diameter of the diffuse-porous hardwood species was correlated with the RIM ($R^2 = 0.4704$), as shown in Figure 6. [12] studied angiosperm wood species and concluded that the tissue density outside the vessel lumens must predominantly influence wood density. Furthermore, they suggest that both the density and the vessel lumen fraction affect the mechanical strength properties.

It became also obvious that in different wood species such as the Kiri, Walnut, Shining gum and further tropical species, the fractures occurred between the vessels, both in the radial and tangential directions (Table 4). Consequently, the vessels turned out to be general weak points in the fiber tissue of the hardwoods, where the weakness increases with an increasing vessel diameter. Figure 7a shows, as an example for the Bongossi, that the vessels served as a starting point for the fractures independently from its anatomical orientation. Tropical species with comparatively small vessels such as the Amaranth, Bangkirai, Garapa, and Ipé showed a rather high RIM. On the extreme end of

the scale, the Merbau showed the lowest RIM and also the largest vessel diameters of all the species. Furthermore, distinct parenchyma bands and wood rays appeared to be weak (and therefore starting points for fractures) in tropical species as well, as also shown in Figure 7. The fractures cutting the wood rays appeared only where the rays were deflected by the vessels from their straight radial orientation.
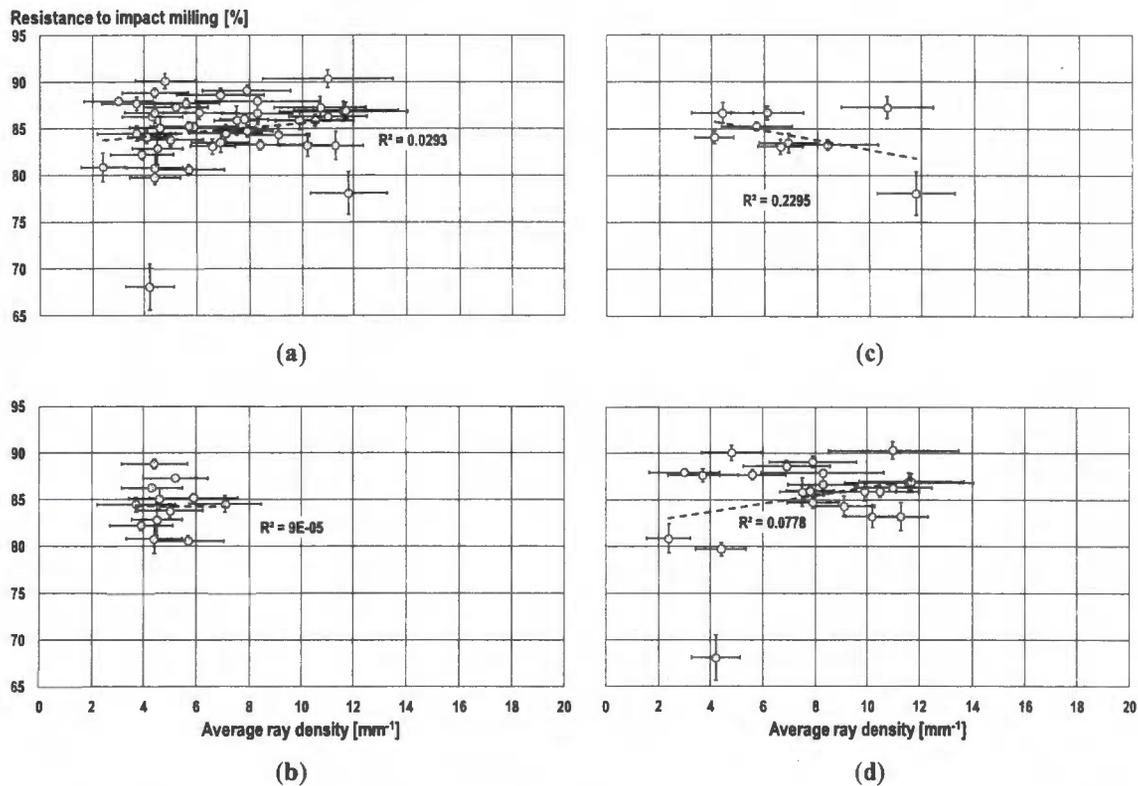

Figure 4. The relationship between the average ray density and the Resistance to Impact Milling (RIM): (**a**) all wood species (y = 0.2354x + 83.247); (**b**) softwoods (y = −0.0252x + 84.454); (**c**) ring- and semi-ring-porous hardwoods (y = −0.5083x + 87.875); (**d**) and diffuse-porous hardwoods (y = 0.4365x + 81.997).
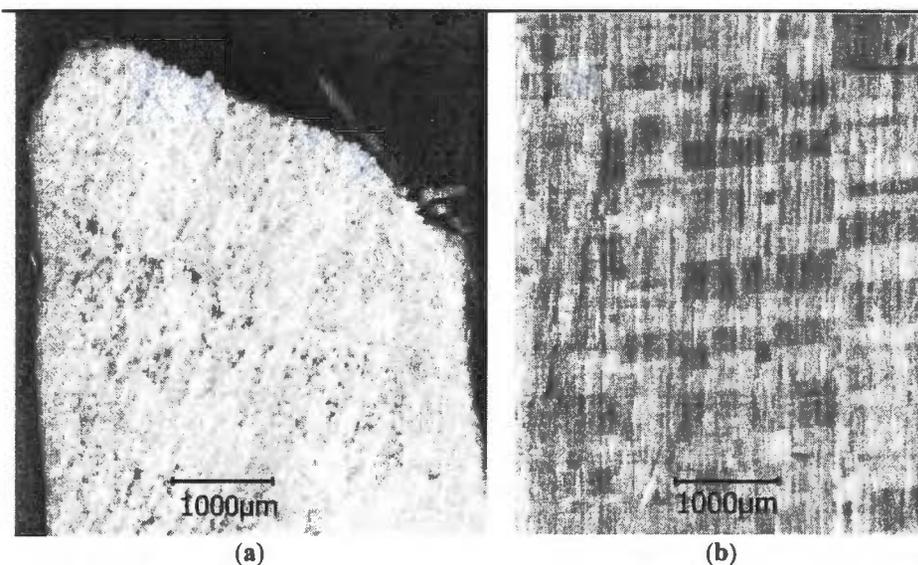
Figure 5. The fracture pattern in semi-ring-porous and diffuse-porous hardwoods: (**a**) the cross section of the Wild cherry, the fracture along a growth ring border; (**b**) the radial fracture section of the Alder, the fracture along the rays.
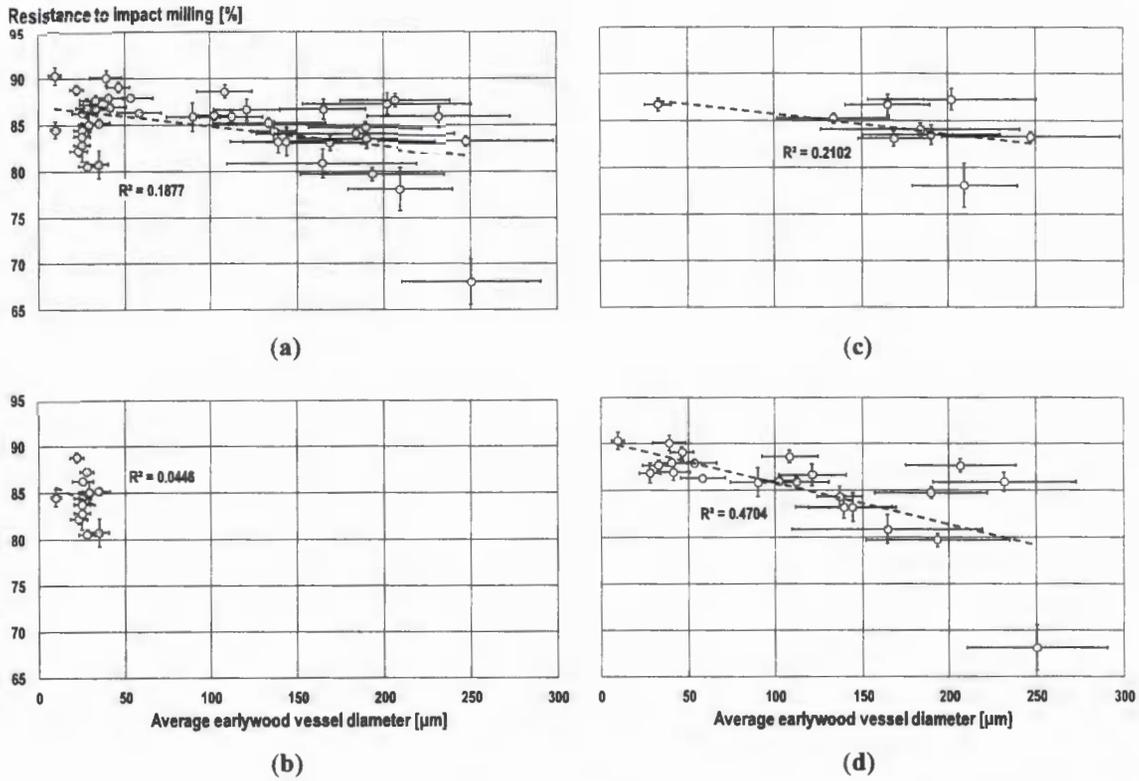
Figure 6. The relationship between the average earlywood vessel diameters and the resistance to impact milling (RIM): (**a**) all wood species (y = −0.0213x + 86.982); (**b**) softwoods (y = −0.0814x + 86.425); (**c**) ring- and semi-ring-porous hardwoods (y = −0.0213x + 87.857); and (**d**) diffuse-porous hardwoods (y = −0.0445x + 90.309).
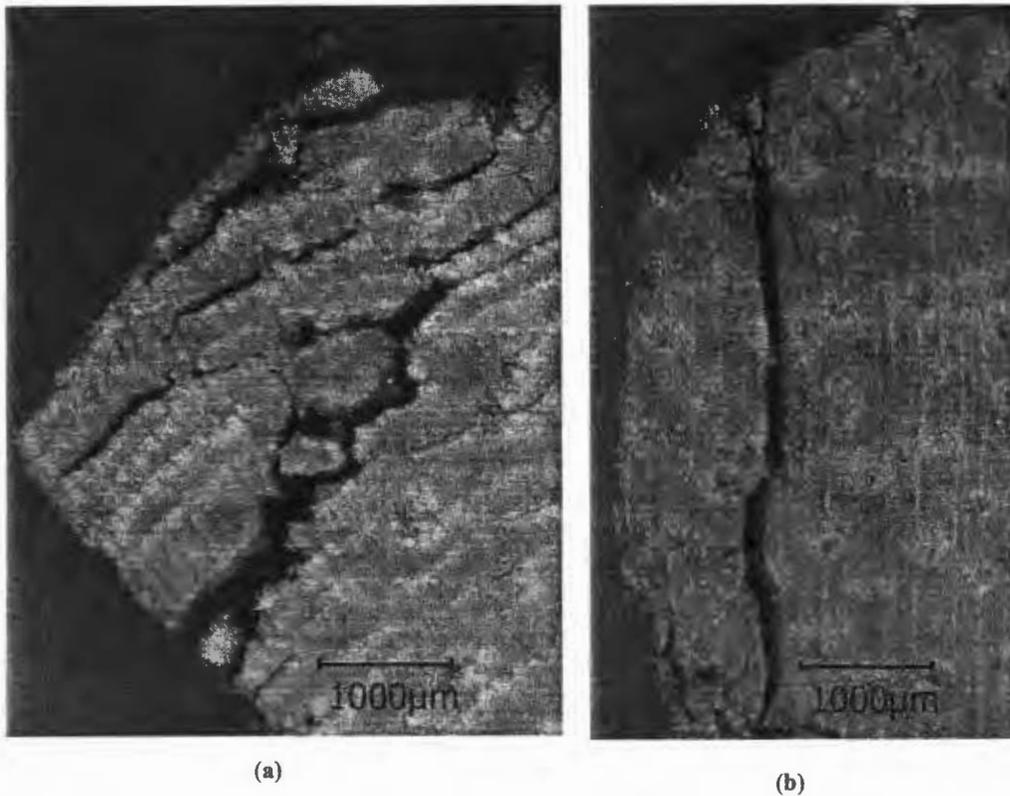

Figure 7. The fracture pattern in the diffuse-porous hardwoods: (**a**) the cross section of the Bongossi, the tangential fractures; (**b**) the cross section of the Amaranth—the radial fractures along the rays.

4. Conclusions

In this study, we showed that the differences in the structural integrity of wood and thus in the brittleness are predominantly affected by anatomical characteristics. The size, density and distribution of the vessels as well as the ray density of the wood were found to have a significant impact on the structural integrity of the hardwoods. The structural integrity of the softwoods was, on the other hand, affected by the number of growth ring borders and the occurrence of resin canals. The density affected the Resistance to Impact Milling (RIM) of neither the softwoods nor the hardwoods.

Consequently, for applications where the brittleness of wood is more relevant than its elasto-mechanical properties, which are generally strongly correlated with wood density, other anatomical characteristics need to be considered for assessing wood quality. In particular, where dynamic loads impact on wooden components, the brittleness of wood becomes a critical issue. Dynamic loads paired with long-term wear and abrasion can be expected, for instance, on outdoor flooring. Furthermore, during wood processing, machining and handling during industrial processes, numerous dynamic impacts occur and affect the structural integrity of wood.

Wood quality is consequently strongly purpose-specific and cannot be simply derived from wood density data. Anatomical features showed a high potential to serve as better indicators for the structural integrity of wood. Additional influences such as the occurrence of reaction wood, alternating rotational growth and other types of fiber deviations likely affect the structural integrity of wood to a similarly extent. In summary, the findings from this study confirmed the need for test methods other than standard strength tests. As long as the common knowledge about wood anatomy and its effects on mechanical wood properties is incomplete, methods are needed that are sensitive, reliable, and accurate enough to characterize the structures of wood in a comprehensive manner. As shown with the HEMI-method applied in this study, indicators can be delivered for instance of the structural integrity of wood. However, further tests are needed, paired with more detailed analyses of the anatomical and chemical constitution of the wood samples being tested, to achieve a fully satisfactory insight on the relationship between wood anatomy and its structural integrity.

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1) IL CANDIDATO IN BASE ALLE PROPRIE COMPETENZE E CONOSCENZE IMPOSTI UN PROGETTO DI RICERCA SULLA TEMATICA DEGLI "EFFETTI DEI CAMBIAMENTI CLIMATICI SUGLI AGRO-ECOSISTEMI", METTENDO IN EVIDENZA BREVEMENTE:

- STATO DELL'ARTE
- OBIETTIVI
- APPROCCIO METODOLOGICO
- RISULTATI ATTESI

(2) IL CANDIDATO IN BASE ALLE PROPRIE COMPETENZE E CONOSCENZE IMPOSTI UN PROGETTO DI RICERCA SULLA TEMATICA DEI "SERVIZI ECOSISTEMICI", METTENDO IN EVIDENZA BREVEMENTE:

- STATO DELL'ARTE
- OBIETTIVI
- APPROCCIO METODOLOGICO
- RISULTATI ATTESI

③ IL CANDIDATO IN BASE ALLE PROPRIE COMPETENZE E CONOSCENZE IMPOSTI UN PROGETTO DI RICERCA SULLA TEMATICA DELLA "SOSTENIBILITA' DELLE PRODUZIONI AGRICOLE E FORESTALI" METTENDO IN EVIDENZA BREVEMENTE:

— STATO DELL'ARTE

— OBIETTIVI

— APPROCCIO METODOLOGICO

— RISULTATI ATTESI