Are defensive structures good predictors of tree mortality under drought and insect pressure?

Master Thesis

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Master Thesis

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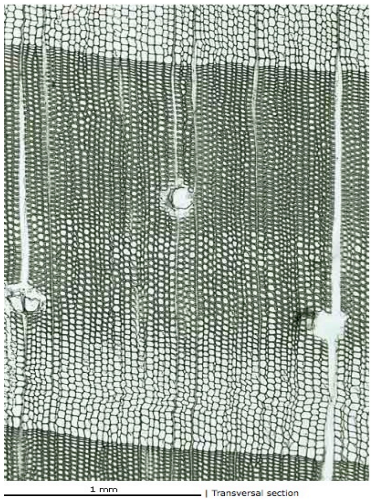

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Tucson/Bern, February 11th, 2011

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Left: Pair of live and dead *Pinus edulis* near White Rock, New Mexico, USA.
Picture by Alison Macalady, October 2010.

Right: Transversal section of *Pinus sylvestris* with vertical resin ducts in the stemwood.
Picture from http://www.woodanatomy.ch.

"Maybe – maybe not.
They's quite a pile that we dunno."
(Brand, 1954)

1 Abstract

Droughts are one of the major consequences of global change. In the southwestern USA, they are projected to increase in frequency and severity, causing large and rapid die-off of the overstory tree species *Pinus edulis* (Pinyon pine). To estimate the various negative impacts of "global-change-type droughts" on pinyon-juniper woodlands, better understanding of the mechanisms of tree mortality under drought is needed, in order to define explanatory variables for prediction of tree mortality and thus to improve models of future vegetation dynamics.

Despite the large negative implications of die-off of overstory trees and despite recent strong interest due to increased drought-induced tree mortality, the exact mechanisms leading to individual tree death are still relatively poorly understood. However, tree growth characteristics have proven to be useful for addressing mortality issues and long-term low growth rates seem to be a commonly accepted trait of dying trees. But under the influence of drought and drought-related insect pressure, growth variables may lose their predictive power and carbon investment in defensive structures like resin ducts are suggested to be more important determinants of tree mortality than investment in growth. Hence, the scope of this master thesis is to investigate the effects of a severe drought with subsequent bark beetle attacks in the southwestern USA on the resin-based defensive system of *Pinus edulis*. To present the addressed issues in a broader context and to build up the necessary knowledge for conducting this study, a preliminary literature review is carried out. The literature review encompasses leading theories of tree mortality under drought, the processes of bark beetle attacks under drought and the defensive system of pines. Afterwards, paired samples of live and dead Pinyon pines from three sites in New Mexico, USA, were used to measure characteristics of xylem based vertical resin ducts. These characteristics are presented and their relationships and temporal variations are investigated. To test if carbon allocation to defense and therefore to resin ducts is a relevant determinant of tree mortality under drought and insect pressure, resin duct characteristics between live and dead trees are statistically compared. To test whether and to what extent resin duct variables are good predictors of tree mortality under drought, logistic single variable regression models are established and externally validated.

The investigated relationships showed no strong indicators for large trade-offs between growth and defense, but some evidence for defense-internal trade-offs could be found. The size of resin ducts with its assumed positive correlation to resin flow is proposed as a crucial factor for resin-based defense of Pinyon. Relationships between resin ducts and tree size (DBH) suggest a possible tree size-dependent scaling effect by showing that bigger trees host bigger resin ducts. Over time, mean values of resin duct variables show moderate to high variation, indicating considerable climatic influence on resin duct formation and that resin duct variables could be used as dendroclimatological variables. However, genetic variation between and within Pinyon pine stands may also influence the values of the resin duct variables and further investigations of environmental and genetic influence on resin duct formation and configuration are suggested. The comparison of measures of vertical resin ducts between live and dead trees from two sites showed significant differences: Live trees have more and larger resin ducts than trees that died. These results suggest that carbon allocation to xylem-based resin defense is an important determinant of tree mortality and existing mechanisms of tree mortality under drought should be complemented and enhanced by the role of tree defense mechanisms and processes. However, the underlying ecological and tree physiological processes could not be revealed within this master thesis and need more attention. The established logistic regression models demonstrate that resin duct variables are good predictors for tree mortality under drought and insect pressure, both in the presence and in the absence of growth-mortality relationships, and could constitute valuable variables for modeling future vegetation dynamics. However, external validations show that the established single variable models can currently not be generalized to Pinyon pine populations across New Mexico, USA, and thus need further improvement.

2 Introduction

2.1 Forests and climate change

Past, ongoing and future emission and atmospheric accumulation of greenhouse gases have major impacts on the global climate; the consequences of global change are broad and severe. Droughts are one of the major effects and are projected to increase in frequency, intensity and/or severity under warming temperatures (IPCC, 2007) and will affect many landscapes all over the globe. Forests cover 30% of the world's land surface (FAO, 2006), and many societies rely on forests for their multiple services (MEA, 2005): *Provisioning services* (e.g. timber, food, fiber and fuel), *regulating services* (e.g. climate regulation, protection from natural hazards, water purification), *supporting services* (e.g. primary production, nutrient cycling) and *cultural services* such as recreation. Droughts and heat stress could fundamentally alter the composition, structure and biogeography of forests in many regions (Allen et al., 2010). Of particular concern is regional-scale mortality of overstory trees, which can rapidly change ecosystem type, associated ecosystem properties and land surface conditions for decades (Allen, 2009; Breshears et al., 2005). A global overview of drought and heat induced-tree mortality (Allen et al., 2010) has revealed many examples of forest mortality that were driven by climatic water/heat stress since 1970. Examples of recent die-offs are particularly well documented for southern parts of Europe and for temperate and boreal forests of western North America (Allen et al., 2010), where widespread death of many tree species in multiple forest types has affected well over 10 million ha since 1997 (Raffa et al., 2008).

Although temperature and water availability are not the only environmental factors driving climate-induced forest mortality (Fig. 2-1), drought and heat-driven mortality is often documented in relatively dry regions, and forests in such semi-arid regions may display substantial growth declines or increases in mortality under climate change (Allen, 2009).

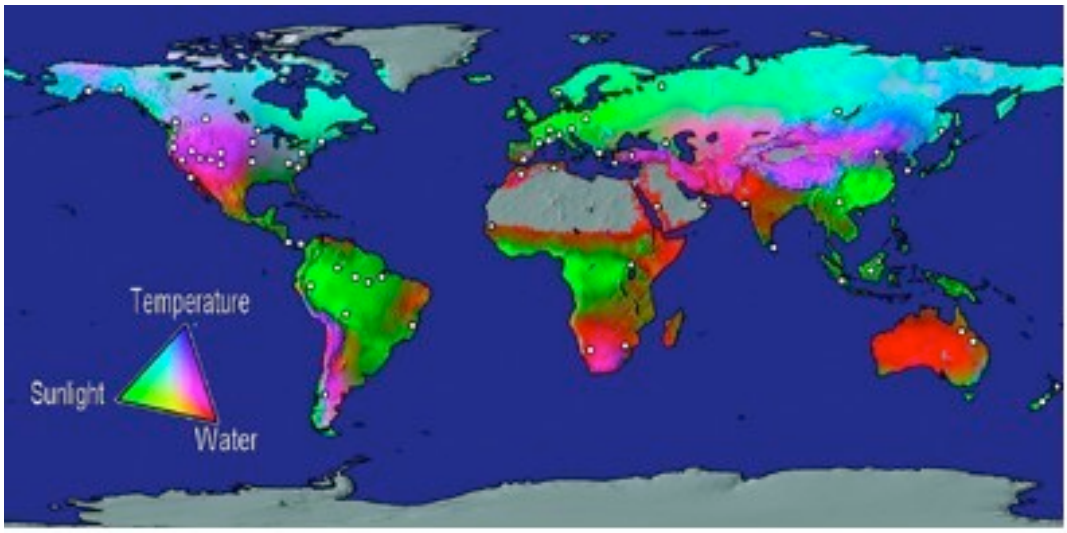

Fig. 2-1: Potential environmental limits to vegetation net primary production (background map) and documented localities (dots) with forest mortality related to climatic stress from drought and high temperatures (Boisvenue and Running, 2006).

For these regions, models predict a further decline of the annual mean runoff (Fig. 2-2), which could worsen the situation and lead to amplified forest mortality due to droughts.

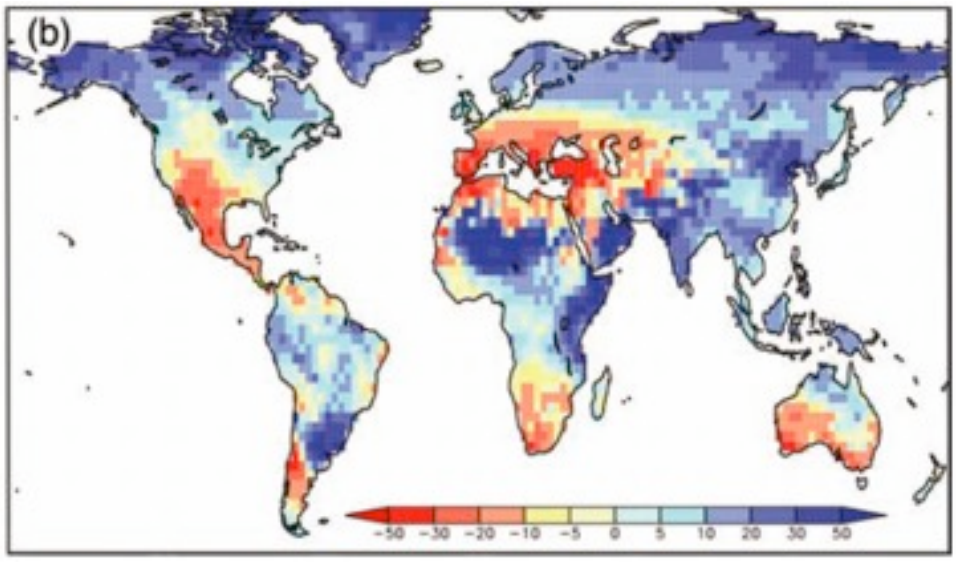

Fig. 2-2: Ensemble mean percentage change of annual mean runoff between present (1981 to 2000) and 2100 (Easterling et al., 2007).

2.1.1 The southwestern USA

Fig. 2-1 shows many well-documented localities with forest mortality due to drought and/or high temperatures, including localities in the southwestern USA. For this region, the predicted decrease of the annual mean runoff of about 20 to 50 percent until 2100 could lead to further and larger die-offs. Within the southwestern Four Corner States Arizona, New Mexico, Colorado and Utah, droughts are not uncommon events (Fig. 2-3).

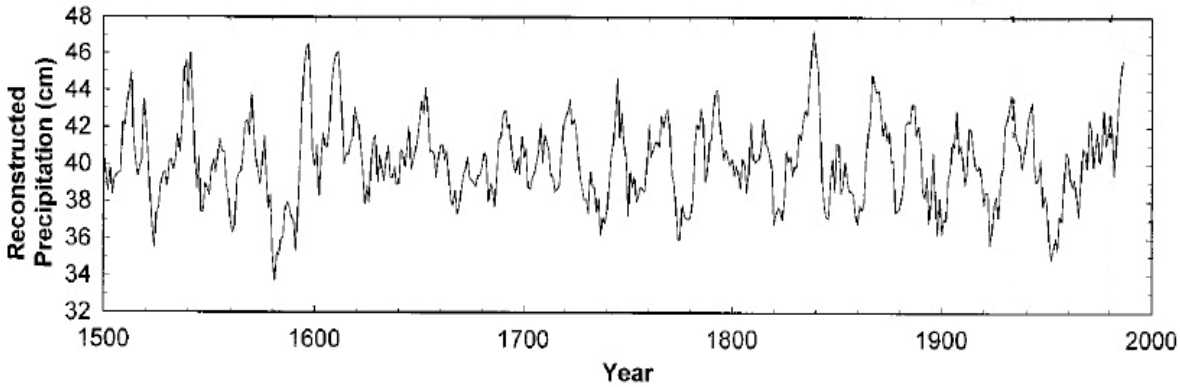

Fig. 2-3: Annual precipitation (5 year running average), reconstructed from dendrochronological records for the Jemenez Mountains, New Mexico, USA (Allen and Breshears, 1998).

Although the drought of the 1950s was thought to be one of the most severe of the past 500 years (Allen and Breshears, 1998), investigations about the most recent drought of the early 2000s (Breshears et al., 2005) have shown that this drought was less dry but warmer (Fig. 2-4), leading – mostly by drought-induced bark beetle infestation – to a rapid die-off of the overstory tree species *Pinus edulis* with an extent of 12'000 km² or more.

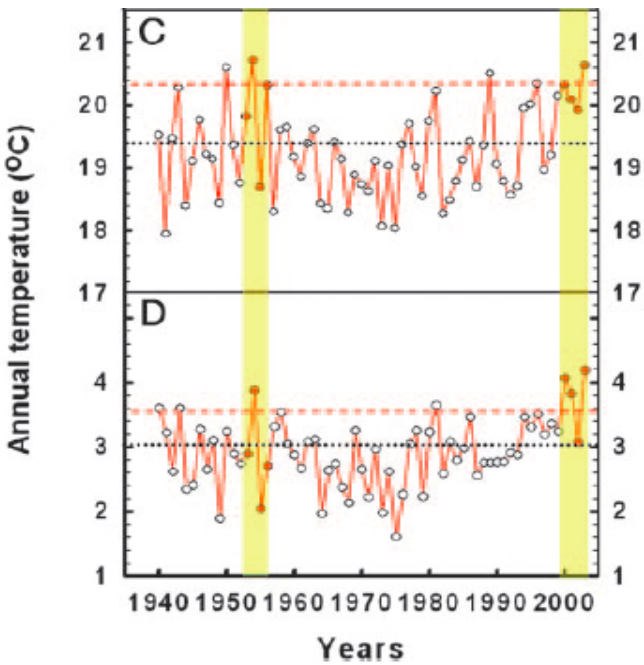

Fig. 2-4: Max (C) and min (D) temperature data from 22 meteorological stations in or near pinyon-juniper woodlands in Arizona, New Mexico, Utah and Colorado (USA). Black line: Long-term mean; Red line: 10th or 90th percentile, differentiating driest or hottest years, are shown. Shaded bands: Four consecutive driest years of the 1950s drought and of the recent drought (2000–2003) (Breshears et al., 2005).

Pinus edulis (Pinyon, Colorado pinyon, Two-needle pinyon) is a small, drought-hardy, long-lived tree of the pinyon pine group and is widespread in the naturally semi-arid and water-stressed environments of the southwestern United States (Santos and Whitham, 2010; Burns and Honkala, 1990). Pinyon is sensitive to climate variation, has evolved in the presence of bark beetles and dominates pinyon-juniper woodlands, covering about 14.9 million ha in Arizona, Colorado, New Mexico, and Utah (Burns and Honkala, 1990). Within these woodlands, the bark beetle *Ips confusus* (Pinyon ips) is a major disturbance agent (Santos and Whitham, 2010) and has the capacity to cause landscape-scale tree mortality (Bentz et al., 2010). The ecological implications of the die-off of *Pinus edulis* include large changes in carbon stores and dynamics, in near-ground solar radiation, runoff, erosion and large changes in the structure and composition of the landscape and its interactions with the atmosphere (Breshears et al., 2005).

Hence, the southwestern USA with its overall climatic conditions, the climate-sensitive tree species *Pinus edulis*, the presence of the Pinyon ips, the recent droughts with the well-documented regional vegetation die-off and the predicted more frequent and severe droughts (Seager et al., 2007) offer a valuable and representative study region for investigating drought-related forest mortality.

2.2 Scope and structure of this master thesis

To estimate the various negative impacts of "global change-type droughts" (such as the drought of the 2000s in the southwestern USA) on forests, it is important to better understand the effects of droughts on tree mortality, to define explanatory variables that can predict tree mortality and by that to improve models of future vegetation dynamics.

Despite the large negative implications of die-off of overstory trees and despite recent strong interest due to increased drought-induced tree mortality, the exact mechanisms leading to individual tree death are still relatively poorly understood. Multiple, interacting factors are operating on a variety of time scales and may cause eventual tree death (Manion, 1981), which make it difficult to clearly understand the underlying mechanisms. However, tree growth

characteristics like ring widths or basal area increments have proven to be useful for addressing mortality issues and long-term low growth rates seem to be a commonly accepted trait of dying trees. With a combination of growth level and growth trend variables, Bigler (2003) achieved good results in predicting probability of tree mortality. Wunder (2007) proposed the relative basal area increment as the best predictor variable for tree mortality. However, under influence of drought and drought-related insect pressure, growth variables may lose their predictive power for tree mortality. With samples of *Pinus edulis* from a large drought-induced die off by subsequent bark beetle infestations at three sites in New Mexico (USA), Macalady and Bugmann (in prep.) investigated growth-mortality relationships in space and time, but growth patterns were not always good predictors for tree mortality. This may indicate that the effects of drought and subsequent drought-induced insect pressure act too fast to be reflected in long-term growth patterns and may primarily influence other tree physiological mechanisms like the defensive system. The effects of drought-induced water stress and insect pressure have been outlined in several studies. Breshears et al. (2005) suggest that drought-induced water stress was tightly tied to infestations by bark beetles in the southwestern USA. After up to 15 months of depleted soil water, cessation of transpiration and photosynthesis in *Pinus edulis* made the trees vulnerable to the drought-associated infestations by bark beetles, leading to a regional die-off extending 12'000 km^2. Negron et al. (2009) conducted a study of a drought-affected landscape in Arizona (USA) and showed that extensive mortality of *Pinus ponderosa* was associated with increased bark beetle populations. Kane and Kolb (2010) compared growth and defense characteristics of Ponderosa pines after drought-associated bark beetle attacks and suggested that carbon allocation to defensive structures like resin ducts could be a more important determinant of tree mortality than allocation to radial growth.

Growth and defense are important carbon sinks, because plants must grow fast enough to compete and thus eventually survive, and yet build up and maintain their defensive system to survive in the presence of herbivores and pathogens (Herms and Mattson, 1992). Conifers have evolved resin-based defense mechanisms to deter insect pests and their symbiotic fungal pathogens (Phillips and Croteau, 1999). The vertical and radial resin ducts with their resin-secreting epithelial cells are interconnected and can form a three-dimensional defense system. As resin ducts are responsible for the production, storage and distribution of oleoresin (Strom et al., 2002; Hodges et al., 1979), they can be important proxy measures of tree investment in defense. Vertical resin ducts can be measured retrospectively with annual resolution using standard dendrochronological techniques. Thus, investigations of vertical resin ducts could be important for better understanding mechanisms of tree mortality under drought.

The *scope* of this master thesis is to investigate the effects of a severe drought with subsequent bark beetle attacks on the resin-based defensive system of *Pinus edulis* (Pinyon pine). With four consecutive driest years between 2000 and 2003, the drought affected the whole southwestern USA, leading to a large die-off of Pinyon pines. This research represents the first known study to compare resin duct characteristics between live and dead Pinyon trees following severe drought and bark beetle attacks. The master thesis is *structured* as follows:

- To present the investigated topics in a broader context and to build up the necessary knowledge for conducting the study, a literature review is presented in chapter 3. Derived from this review, specific research questions are presented in chapter 4.
- To answer these research questions, paired samples of live and dead Pinyon pine trees from three different sites in New Mexico (USA) are used to measure characteristics of xylem based vertical resin ducts. These characteristics, their relationships, temporal variations and differences between live and dead trees are investigated. Based on these data, resin duct variables with the greatest power to predict tree mortality are tested. While chapter 5 presents the applied material and methods, chapter 6 shows the obtained results. The discussion is presented in chapter 7 and conclusions are drawn in chapter 8.

The results of this master thesis could lead to a better understanding of specific mechanisms of tree mortality under drought and insect pressure, could complement and enhance the findings of Kane and Kolb (2010) about carbon allocation to defensive structures and could add explanatory power to the investigation of growth-mortality relationships by Macalady and Bugmann (in prep.).

3 Literature review

This master thesis deals with drought related tree mortality and the effects of severe droughts with subsequent bark beetle attacks on the defensive system of Pinyon pines (*Pinus edulis*). As an integral part of the thesis, the following literature review has been established to present the investigated topics in a broader context and to build up the necessary knowledge for conducting the study. The review aims to answer the questions "What are the leading theories of conifer mortality under drought" (chapter 3.1) and "What are the processes of bark beetle attacks under drought and how is the defensive system of pines constituted" (chapter 3.2). The review was conducted by a search for published accounts, using mainly the ISI Web of Science. Different combinations of key words were used in these searches: *tree, forest, mortality, mechanisms, die-off, dieback* and *drought* for the leading theories; *defense, defensive system, response, resistance, susceptibility, insects, bark beetles, pines, mortality, die-off, dieback* and *drought* for processes of attack and the defensive system. These searches uncovered an extensive set of documents. From this set, the most relevant and promising documents were chosen; this was done from a subjective viewpoint of the author of this master thesis. Exclusions included the following criteria: examples of fire-driven mortality, studies that documented only seedling or juvenile tree mortality and examples with other biotic agents than wood-boring insects with associated fungi.

3.1 Leading theories of tree mortality under drought

Leading theories of tree mortality under drought are based on two underlying basic concepts: The concept of growth-differentiation balance (Loomis, 1953, 1932) and the decline disease theory (Manion, 1981). Because of their general importance and influence on tree mortality, these concepts are preliminarily introduced below.

Growth-differentiation balance

Loomis (1932) divided the development of plants into three overlapping phases: Cell division, cell enlargement and cell differentiation. While the first two are elements of growth involving increases in plant size, the third is a change in morphology as a result of pre-existing chemical conditions in cells and tissues involved (Lorio, 1986). Examples of differentiation processes are: Thickening and lignification of secondary cell walls, thickening of the leaf cuticle, and the production of cellular inclusions such as resin (Loomis, 1953). The two elements of growth depend upon many factors, including light, water, temperature and available assimilates and nutrients. According to Lorio (1986), growth processes dominate over differentiation as long as all factors are favorable for growth. Differentiation depends mainly upon temperature and assimilates and is dominant only when other conditions are below optimum for growth.

This concept is helpful in understanding partitioning of resources: The allocation of resources by plants to differentiation processes decreases growth by diverting resources. Plants have limited resources, all requirements cannot be met simultaneously, and trade-offs with allocation priorities occur among growth, maintenance, storage, reproduction and defense (Fig. 3-1). These trade-offs reveal the dilemma of plants: They must grow fast enough to compete, and yet maintain the physiological adaptations necessary for survival in the presence of herbivores and pathogens (Herms and Mattson, 1992).

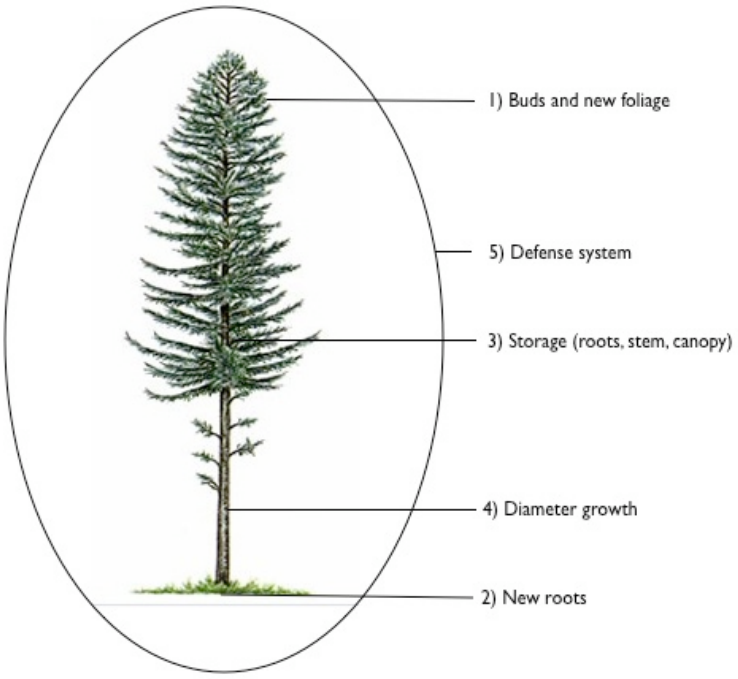

Fig. 3-1: Possible allocation priorities in Lodgepole pine under stressed conditions, priorities are numbered from 1 (highest) to 5 (based on Waring and Pitman, 1985, design adapted).

Decline disease theory

This general conceptual model for stress-induced forest decline provides a framework to relate the deterioration of a tree's vigor until tree death to a sequence of interchangeable but sequentially occurring environmental factors (Fig. 3-2). Based on Manion (1981), these factors are briefly outlined below:

Predisposing factors impact a tree during years or decades and are often expressed as reduced growth rates. They increase a trees susceptibility to short-term inciting stresses and include stand competition, air pollutants or general aridity.

Inciting factors affect the physiological functioning of a tree, and reduce its vigor and potential for pathogen defense, resulting in rapid growth decline. They include drought periods or insect larval feeding and defoliation.

Contributing factors act over the short or long term and often decide a tree's fate. They include secondary opportunistic insects, phytopathogens or additional climatic events, which ultimately may kill stressed trees.

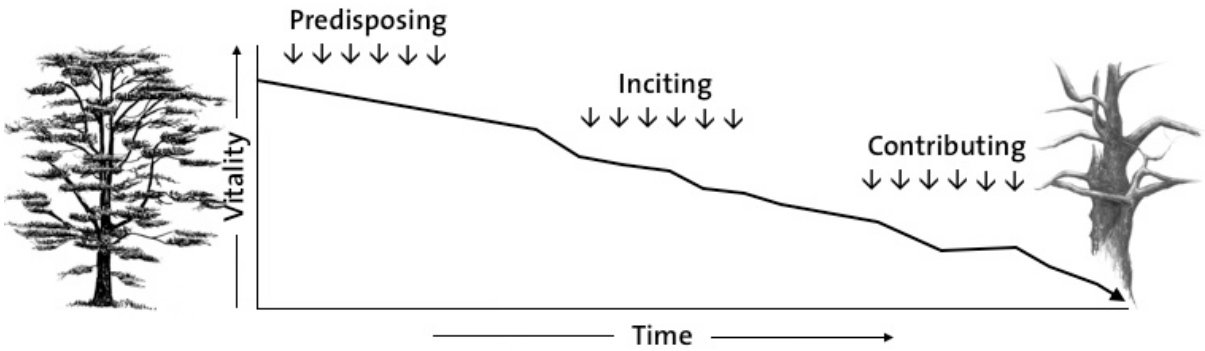

Fig. 3-2: A three-step process leads to deterioration of a tree's vigor and to tree death [based on Manion, 1981, design adapted, drawings by Manfred Müller-Berg (left) and Fabian Hofmann (right)].

The theory shows that it is often not a single environmental factor causing death of trees. Tree mortality commonly involves multiple, interacting factors, which makes it difficult to clearly understand the mechanisms underlying tree survival and mortality during droughts (Allen et al., 2010).

3.1.1 Key hypotheses of drought-related conifer mortality

From all the selected documents, the most encompassing and convincing hypotheses on mechanisms of drought-related mortality are from McDowell et al. (2008). Hence, the following mechanisms are mainly based on their work and are explained or enhanced with contributions of other authors. It is noteworthy that these mechanisms are especially appropriate to explain mortality due to "warm droughts" (e.g. the droughts in the southwestern USA) and may not be able to provide the same level of explanation to "cold droughts" (e.g. droughts in the boreal zone), because drought regimes, general water availability and temperature influence can be different.

McDowell et al. (2008) propose a general framework based on tree physiological mechanisms, in which they focus on three mechanisms of mortality: *hydraulic failure*, *carbon starvation* and *biotic agent demographics*. The fact that several mortality mechanisms are proposed, points to the underlying concept of Manion (1981). The basic concepts of Loomis (1932, 1953) are hidden in the carbon starvation hypothesis.

3.1.1.1 Hydraulic-failure mechanism

This mechanism predicts that reduced soil water supply coupled with high evaporative demand causes xylem conduits to cavitate, stopping the flow of water, desiccating plant tissue, leading to cellular death and finally to whole-plant mortality.

Hydraulic failure occurs when evaporation (E) exceeds the critical xylem water potential (Ψ_{crit}), which is species specific and is thought to be a function of interconduit pit structure (McDowell et al., 2008). E has an upper limit (E_{crit}), because increasing tension will result in embolized conduits (entry of air through pit pores) and will thereby cause cavitation. Hydraulic failure also occurs within soils and is functionally similar to xylem cavitation. Hydraulic failure during drought can therefore be predicted by plotting the decline in E_{crit} as a function of soil water potential (Fig. 3-3). On the extraction limit, the soil water potential reaches Ψ_{crit}, E_{crit} is zero and no further water can be extracted. The realized transpiration and thus the actual water use must fall within this envelope; if the transpiration threshold is exceeded, hydraulic failure occurs. The difference between the critical and realized E is a species-specific hydraulic margin of safety.

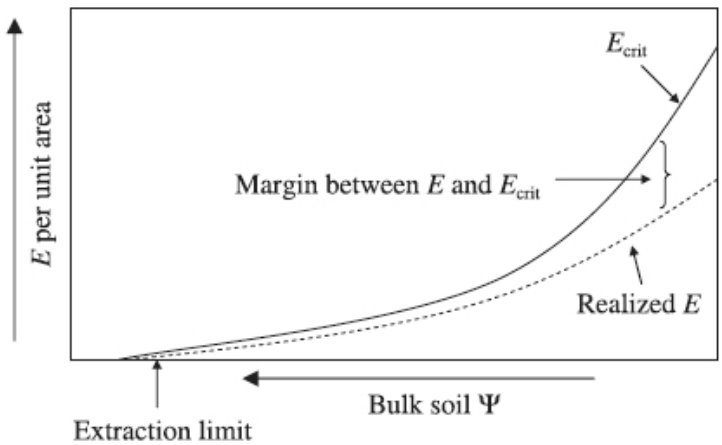

Fig. 3-3: Modeled transpiration per unit leaf area as a function of soil water potential (McDowell et al., 2008).

To avoid hydraulic failure, trees regulate their stomata. This stomatal regulation of water status ranges from isohydry to anisohydry, with many possible forms between those poles. *Isohydric* trees close their stomata near their Ψ_{crit} to minimize further transpiration and to protect xylem from cavitation. This strategy may prevent hydraulic failure, but can cause eventual carbon starvation, as stomatal closure stops the assimilation processes. *Anisohydric* trees can tolerate lower negative values of Ψ_{crit}, the stomatal closure is later and less severe, allowing assimilation processes and therefore carbon gain to continue. This strategy may prevent carbon starvation, but can cause eventual hydraulic failure, which might directly lead to the tree death. McDowell et al. (2008) are clarifying that their hydraulic framework is based on the premise that whole-plant hydraulic failure will cause death and that this premise may be false in cases of resprouting, xylem refilling, progressive loss of crown or desiccation-tolerant cells. By studying the effects of irrigation on drought-exposed Scots pines, Dobbertin et al. (2010) pointed out the unexpected result that mature trees with high crown transparency and apparently low vigor recover when water was added. They responded with an increase in growth of needles, shoots and stem of the same magnitude as vigorous trees. This indicates that partial hydraulic failure may not be lethal to some tree species. While refilling of cavitated elements has been observed in pines, resprouting and desiccation-tolerance have not (McDowell et al., 2008).

Anisohydric versus isohydric regulation of water status may be a critical factor in the regulation of survival and mortality during drought.

3.1.1.2 Carbon starvation

Stomatal closure to prevent hydraulic failure stops the assimilation processes. Essential metabolic processes continue to deplete carbon stores, leading eventually to starvation or an inability to maintain structural and chemical defenses against attack from biotic agents (McDowell et al., 2008).

The link between hydraulic failure and carbon starvation is obvious: Stomatal regulation of the gas exchange of a tree physiologically links tree water relations to the carbon balance by trading a maximized CO_2 uptake for assimilation against a non-damaging loss of water through transpiration (Zweifel et al., 2009). Intense multi-year droughts represent very low resource levels and severe water deficit. In such situations, both growth and secondary metabolism are limited (Lorio, 1986). Essential primary metabolic processes and baseline maintenance receive priority use of limited carbon, and carbon-based secondary metabolism and herbivore resistance generally decline (Fig. 3-4).

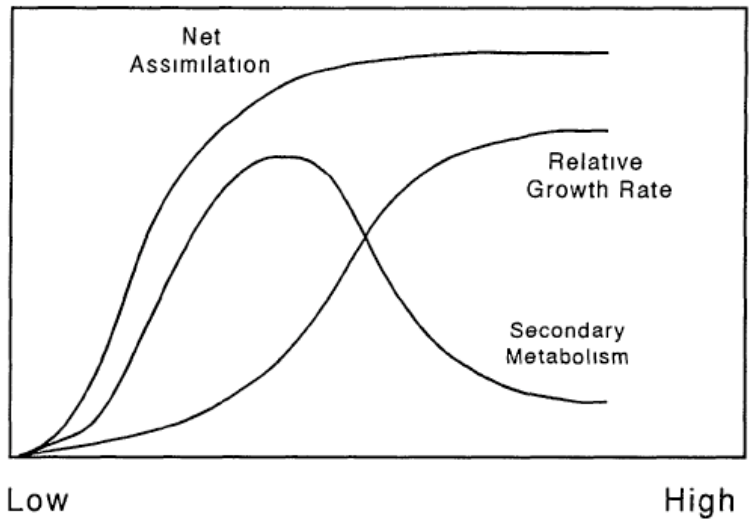

Fig. 3-4: Norms of reaction for net assimilation rate, relative growth rate and secondary metabolism in response to changing resource availability (Herms and Mattson, 1992).

This effect can also be explained by the cellular metabolism limitation (Allen et al., 2010): Low tissue water potentials during droughts can constrain cell metabolism, thereby preventing the production and transport of carbohydrates and secondary metabolites necessary for plant defense against biotic attack. In their mortality insights from a decade of plant water potential measurements, Breshears et al. (2009) suggest that Pinyon pine mortality was driven by protracted water stress, leading to carbon starvation and associated increases in susceptibility to drought-related bark beetle attacks. These remarks may demonstrate the two main effects of drought-related carbon starvation proposed by McDowell et al. (2008): Outright starvation or the inability to maintain structural and chemical defenses against attack from biotic agents.

Weakening of trees by drought and above-average temperatures has also been studied by Dobbertin et al. (2007): Warmer years coincided with periods of drought that weakened trees as indicated in a tree-ring-based mortality index and observed mortality rates on monitoring plots. Low carbon pools as a consequence of severe water stress have also been discussed in a study of Bigler et al. (2006) of Scots pine stands in a dry Swiss valley. The length of the induced defense reaction was reduced and the resin content lowered, which facilitates insect infestations.

According to McDowell et al. (2008), droughts may reduce photosynthesis by other mechanisms than stomatal closure, such as loss of leaf turgor and leaf shedding. Dobbertin et al. (2010) tested the effect of irrigation of Scots pines on dry sites. Irrigation significantly increased needle length, needle mass, needle area and shoot length. The drought-related deterioration of photosynthesis hampers the gain of carbon and may accentuate carbon scarcity. These findings are supported by the study of Bigler et al. (2006), who also emphasize the importance of multi-year droughts, which increase needle loss, decrease needle and shoot length over several years and lead to reduced growth and higher mortality.

3.1.1.3 Biotic agent demographics

Extended warmth during droughts can drive increased population abundance in mortality agents, allowing them to overwhelm their tree hosts (Allen et al., 2010).

Potential demographic changes include increased intrinsic population growth rate, increased number of pathogen generations per year as a result of longer growing seasons or decreased winter mortality because of warmer winter minimum temperatures and changes in the synchrony of key development phases (McDowell et al., 2008).

In their evaluation of the impacts of a recent drought on regional scale tree-mortality, Breshears et al. (2005) pointed out the amplifying effects of warmer temperatures and longer growing seasons on growth rates and population dynamics of bark beetles, which were the proximal cause of mortality for most trees. In a study of long-term data indicating increasing drought-induced mortality in Scots pine forests, Dobbertin et al. (2007) revealed that beetle-related mortality coincided with periods of above-average temperatures. It seemed very likely that higher temperatures have favored beetle development and thus increased population size and infestation pressure, eventually contributing to subsequent tree mortality. These findings are supported by the study of Bigler et al. (2006), where significantly rising temperatures are likely to have favored insect development rates and increased their populations. Because Scots pine is a host for a large number of potentially harmful insect species, the risk of insect outbreaks is high, especially after drought periods.

Besides the influence of those abiotic factors, high abundance of biotic mortality agents also results from other effects: 1) Influence of anthropogenic activities may alter tree stand density, heterogeneity, age and proximity, enabling the beetles to surpass a series of thresholds and exert widespread disturbance (Raffa et al., 2008). 2) Biological interactions with other organisms within the community such as key predators, which produce delayed density-

dependence (Ayres and Lombardero, 2000). The direction and magnitude of the effects of droughts and increased abundance of mortality agents on natural enemies and mutualists are largely unknown (McDowell et al., 2008). 3) Biotic attacks may also be facilitated by changes in the release of volatile attractants. The emission of ethanol and other volatiles is believed to increase during drought and may be used by insects to locate stressed plants. According to McDowell et al. (2008), the changes in the production and emission are aimed to preserve cellular function and perhaps as a byproduct of increased tissue temperatures due to reduced transpiration.

3.1.1.4 Conclusions for Pinyon pine

McDowell et al. (2008) present the Pinyon pine as a good example of isohydric regulation. Stomatal closure keeps xylem water potentials above -2.5 MPa during drought, while anisohydric plants like Juniper with xylem that is more resistant to negative water potentials can tolerate lower negative values (Fig. 3-5).

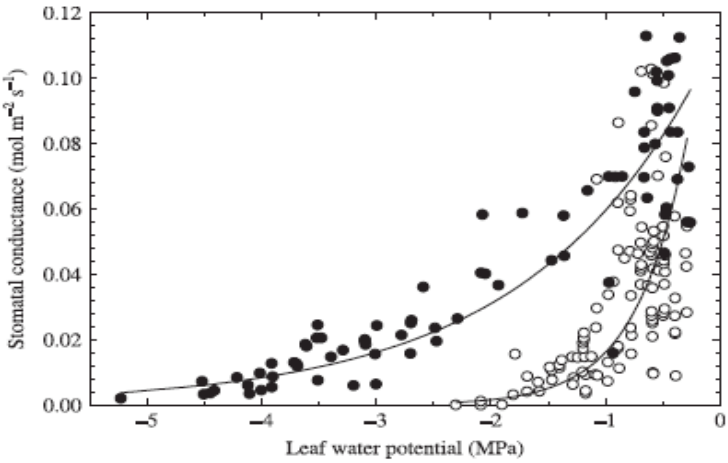

Fig. 3-5: Stomatal conductance versus leaf water potential for Pinyon (open circles) and for Juniper (closed circles) at a site in New Mexico (McDowell et al., 2008).

The isohydric behavior of Pinyon prevents cavitation and therefore hydraulic failure is unlikely to happen. Under drought and drought-associated beetle pressure, this in turn would imply that Pinyon is prone to carbon starvation: The trees either starve outright or they succumb to beetle attacks. The evaluation of Pines as isohydric plants is supported by several other findings:

Eilmann et al. (2009) studied drought-induced adaptations of the xylem in Scots pine and Pubescent oak. Under drought, Scots pine reduces radial increment and therefore the conducting area is reduced. As compensation, decreasing the cell-wall thickness increases the lumen diameters of the conduits. This enables the pine to build a more effective water conducting system with lower construction costs in terms of carbon investment. The more effective water system comes at the expense of safety, because larger conduits with thinner cell walls are more vulnerable to cavitation. To prevent cavitation, pines are therefore forced to close their stomata relatively early, making them prone to carbon starvation.

Physiological studies in a dry Swiss valley confirm that Scots pine closes its stomata relatively early during dry and warm days and when water availability is low (Dobbertin et al., 2007). With low carbon pools as a consequence of severe water stress, pines reduce the length of the induced defense reaction and lower their resin content, making them vulnerable to beetle infestations.

Rigling et al. (2006) discovered the highest pine mortality after dry years. Drought was not the proximal cause of mortality by hydraulic failure, but acted as inciting factor by significantly

reducing assimilation and therefore preventing the accumulation of reserves. In this situation, further contributing factors like infestations by beetles, mistletoes, nematodes or fungi can lead to death. Multi-year droughts amplify these effects.

3.1.1.5 Evaluation of the general framework

The general framework of McDowell et al. (2008) provides an appealing and useful approach to investigate mechanisms of tree survival and mortality under drought. However, the three mechanisms of mortality (hydraulic failure, carbon starvation and biotic agent demographics) have to be interpreted as theoretical and not mutually exclusive states with many possible combinations between those poles. Dependent on pre-drought conditions, drought intensity, drought duration and further factors at the tree and stand level, an interrelated combination of factors or even framework-external factors can eventually lead to death of an individual tree. Sala et al. (2010) add more specific criticism with their statement that the carbon starvation hypothesis rests on untested assumptions and ignores other possible mechanisms like failure of carbon metabolism and transport. Even McDowell (McDowell and Sevanto, 2010) agrees on the general conclusion of Sala et al. (2010) that "the physiological mechanisms of drought-induced tree mortality are far from being resolved". They highlight the importance of furthering the understanding of vegetation mortality and suggest that further research should encompass – among other issues - the availability of stored carbohydrates, the role of starch and sugar in survival mechanisms and the interaction of hydraulics, metabolism and phloem transport. The amplifying effects of droughts on biotic agent demographics are also disputable. Carnicer et al. (2011) state that combined effects of forest drought can severely increase the mortality rates of insect herbivores and may even truncate multiyear insect outbreak dynamics.

For Pinyon, complex interactions among the three mechanisms of mortality, depending mostly on drought severity and duration, could bring hydraulic failure back into play. An investigation of growth-mortality relationships of live and dead Pinyon trees (Macalady and Bugmann, in prep.) shows diverging growth patterns after a severe drought period in the 1950s. This inciting factor caused different growth levels, weakened trees permanently and finally led to their death in the recent drought period in the early 2000s (Fig. 3-6). The trees that eventually died showed a certain inability to recover during the more favorable climatic conditions after the 1950s drought. A speculative but possible explanation could be that these trees, forced by the severe multiyear drought in the 1950s, finally had to re-open their stomata to prevent immediate death by starvation. Open stomata caused permanent and irreparable partial damage to their hydraulic system. With their so caused partially damaged hydraulic system, the trees were not able to recover and thus permanently weakened succumbed to the recent severe drought.

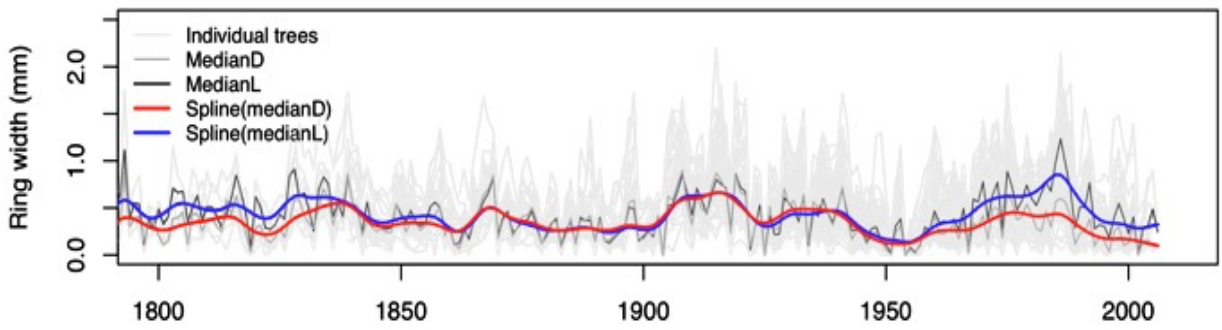

Fig. 3-6: Ring width-chronology of live and dead Pinyon pines from New Mexico (USA), showing diverging growth patterns before 1850 and after a severe drought in the 1950s (Macalady and Bugmann, in prep.).

Evidence that hydraulic failure is a relevant mechanism of pine mortality under drought can be found in the study of Martinez and Pinol (2002): Low leaf specific conductivity caused higher water potential gradients and therefore higher levels of embolism, resulting in drought-

induced mortality of *Pinus sylvestris* in eastern Spain. It is noteworthy that these processes occurred in the absence of aggressive bark beetles. However, carbon starvation may also be responsible for the mortality pattern observable in Fig. 3-6: According to Galiano et al. (2011), severe drought events can induce long-term physiological disorders associated with canopy defoliation and depletion of carbon reserves, leading to deferred recovery of surviving trees and eventually to delayed tree death.

All these possible explanations demonstrate that the understanding of the exact mechanisms of tree mortality is still limited and that more attention needs to be given to the ecological and tree physiological mechanisms and processes leading to individual tree death.

3.2 Processes and mechanisms of bark beetle attacks under drought and defensive system of pines

This master thesis deals with the effects of severe drought with subsequent bark beetle attacks on the resin-based defensive system of *Pinus edulis*. Hence, the following part of the literature review first outlines the processes and mechanisms of bark beetle attacks under drought and second presents the defensive system of pines.

3.2.1 Processes and mechanisms of bark beetle attacks under drought

Of the more than 5'800 described bark beetle species, probably less than a dozen species are aggressive, i.e. attacking and killing living trees (Franceschi et al., 2005). Still, tree-killing bark beetles are the most destructive agents in conifer forests, causing major economic damages and ecological changes. Conifers and bark beetles have co-existed since the early Mesozoic, and the co-evolution suggests an arms race that includes the usurpation of part of the defense of pine trees (Franceschi et al., 2005; Seybold et al., 2000). Lieutier (2002) proposes two basic colonization strategies for all bark beetles attacking living trees: 1) Exhausting tree defenses and killing the tree, referred as the cooperative strategy; 2) Avoiding / tolerating tree defenses, referred as the solitary strategy with beetle life cycles that can take place without killing the tree. The situation of *Pinus edulis* in the southwestern USA is clearly related to the first strategy, thus the following investigations are based on this strategy. Within this strategy, a beetle attack mainly develops in the following steps:

1) Host selection

2) Colonization and development

3) Dispersal

1) Host selection

Pioneer beetles initially attack the tree and enable a mass attack by producing population-aggregating pheromones. These pheromones consist of terpenoids that are biosynthesized from tree resin components (Wermelinger, 2004). Severe drought conditions may influence these processes as follows:
1) McDowell et al. (2008) hypothesize that due to reduced transpiration tree tissue temperatures are increased and – to preserve cellular function – the emission of ethanol and other volatiles increases during drought. This can enhance the attraction for the pioneer beetles and can facilitate the selection process of susceptible hosts.
2) Extended warmth during droughts can favor beetle development, which leads to an increased regular background population size and results in higher infestation pressure.

2) Colonization and development

It may be appropriate to assign two main beetle mechanisms of attack and development (Lieutier, 2002) to the colonization phase: Exhaustion of host defenses and tissue colonization, development and tree death.

For successful *exhaustion of host defenses*, rapidity and – depending on the tree vigor – a minimum number of simultaneous attacks are necessary (Lieutier, 2002). The mass attack must be fast and numerous enough to exhaust the host defenses and then to invade and successfully colonize non-resistant tree tissues. Not only the constitutive tree defense must be exhausted and overcome, but also the inducible defense system. Rapid attacks are important to overcome the trees defenses; under favorable climatic conditions high populations of aggressive bark beetles can completely terminate their aggregation within one week and sometimes less (Payne, 1980). Most conifer bark beetles vector some species of moderately pathogenic blue-stain fungi from the genus *Ophiostoma spp.* or *Ceratocystis spp.* (Wingfield et al., 1993). The fungi are suspected to be important in tree killing, and an attacked tree will face a beetle-fungus complex that can act synergistically to overwhelm its defenses (Franceschi et al., 2005). It has been suggested that the inoculation with beetle associated blue stain fungi helps to exhaust the tree defenses, mainly by two effects (Franceschi et al., 2005; Lieutier, 2002): 1) The mere presence of the fungi additionally stimulates the tree defenses and leads to a more rapid exhaustion; 2) The fungi produce toxins that can alter physiological processes in the tree, thus weakening its resistance. However, the mechanisms of beetle-fungi-interactions are still far from being resolved, and Six and Wingfield (2011) present indirect evidence that question this classic paradigm of beetle-fungi-mutualism. They suggest that, rather than playing a supporting role for the host bark beetle, phytopathogenicity performs an important role for the fungi in mediating competitive interactions with other fungi and supporting survival and efficient resource capture in living, defensive trees.
Severe drought conditions may influence these processes as follows:
1) As stated in the host selection phase, extended warmth during droughts may have resulted in higher infestation pressure, making it more likely to cross the threshold of a successful beetle attack density.
2) Formation and maintenance of the defense systems are energy-demanding processes, and research has shown that sugar and starch concentrations in the phloem - used as energy sources for the induced response - decrease rapidly (Franceschi et al., 2005). As stated before, severe multi-year droughts can produce high water stress, leading to cessation of transpiration and photosynthesis and thus resulting in low tree resource levels. These low resource levels may not only negatively affect the quantity and effectiveness of the preformed defense system, but also of the induced response. Weaker defense and resistance make the trees more susceptible and more likely to succumb to drought-associated beetle attacks.

After successfully having exhausted host defenses, beetles and fungi are able to *colonize tree tissues and to develop*. Beetle activities including mating, gallery construction, oviposition and brood development destroy cambium, phloem translocation and sapwood surface (Parmeter et al., 1989). As suggested by Hobson et al. (1994), phloem destruction could also lead to sapwood occlusion, possibly by inducing death of ray parenchyma cells and subsequent cavitation of adjacent tracheids. Beetle-inoculated fungal penetration of the sapwood leads to occlusion and thus disrupted water conduction, mainly through aspiration of tracheid tori and release of fungal toxins (Paine et al., 1997). An instantaneous effect of disrupted water conduction may be fading foliage. Eventual tree death is suggested as a result of simultaneous actions and interactions of the beetle-fungus complex (Berryman, 1972).

3) Dispersal

During the dispersal phase, the next generation emerges and flies to a new host. Severe drought conditions may influence these processes mainly by the amplifying effects of warmer temperatures and longer growing seasons. Several authors (e.g. Allen et al., 2010; McDowell et al., 2008; Breshears et al., 2005; Wermelinger, 2004) highlighted these effects on reproduction,

population growth rate, number of generations per year, over-winter mortality and synchrony of key development phases.

3.2.2 Defensive system of conifers

Conifers are a successful group of plants, containing long-lived organisms. Their potent defense mechanisms are part of their success, though millions of trees are killed each year by a wide range of organisms. The main defense systems consists of the pre-set *constitutive defense* and the *inducible defense*. As a part of this literature review, the following investigations aim to answer the question "How is the defensive system of pines constituted". Following the main topic of this master thesis, special emphasis has thereby been put on the elements of mature trees of the genus *Pinus* and on resin elements.

3.2.2.1 Constitutive defense system

Mechanical and chemical defenses complement each other in this first tier of defense, which is formed and maintained in the absence of attack by organisms (preformed defenses) and whose elements are mainly located in the periderm, cortex, secondary phloem and secondary xylem (Fig. 3-7). The cortex is only important during early growth years, its role is taken over by the secondary phloem and hence the cortex will not be further investigated here.

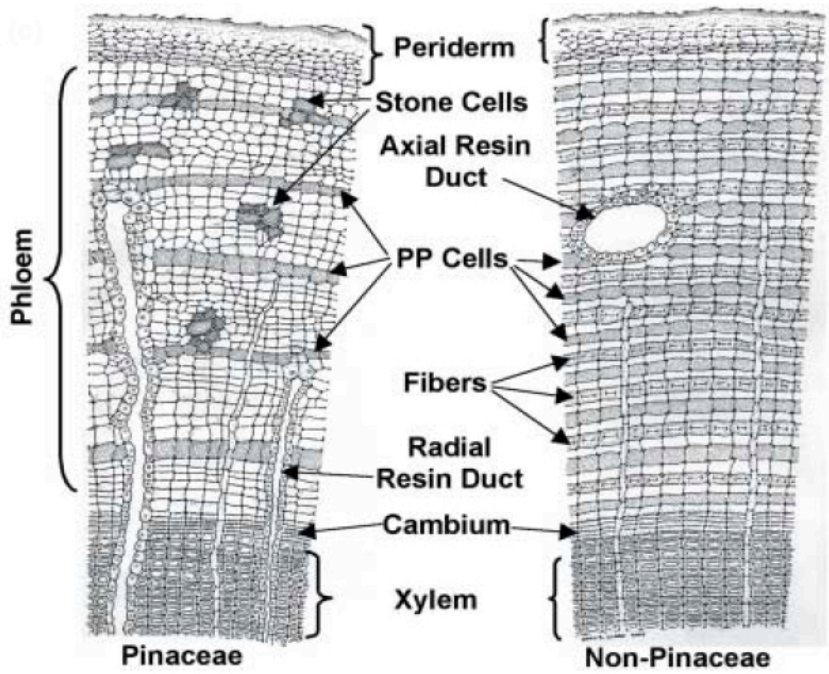

Fig. 3-7: Drawings of two different types of conifer bark, showing multiple concentric layers of defensive elements: Pinaceae with stone cells, Non-Pinaceae with fiber rows (Franceschi et al., 2005).

The first line of defense

The first line of defense is provided by the periderm, consisting of the phellem or cork tissue outwards and the phelloderm inwards. The mostly dead cells of the phellem have lignified or suberized walls, may contain large amounts of phenolic materials and are partly encrusted with calcium oxalate crystals. These mechanical and chemical elements form a multifunctional barrier to the external environment. However, lenticels in the periderm allow gas exchange and may therefore be potential entry points for invasive organisms; moreover, bark beetles may sense chemical signals that emerge through these lenticels (Franceschi et al., 2005).

The second line of defense

The second line of defense is the secondary phloem with phenolic cells, sclerenchyma, calcium oxalate crystals and radial resin ducts. Phenolic cells are specialized for synthesis and accumulation of soluble phenols and more complex polyphenolic compounds (Lieutier, 2002). While the compounds of these phenolic cells serve as antifeedants and antifungal agents (Beckman, 2000), their cell walls are thickened with plasmodesmata that allow for axial and tangential exchange of information, and possibly for defense signaling (Krekling et al., 2000). Sclerenchymal elements are the stone cells (sclereids). These cells with lignified secondary wall thickenings are structural and defensive elements, deterring bark-boring organisms (Franceschi et al., 2005; Howard, 1970). Calcium oxalate crystals are deposited in the vacuoles of modified phenolic cells (Hudgins et al., 2003). This abundant by-product of the metabolism (Howard, 1970) with its tough physical nature of the crystals is believed to deter bark-boring or –chewing animals (Franceschi et al., 2005). Radial resin ducts occur in the secondary phloem and xylem and form a part of the resin- producing and –storing structures, a major constitutive defense of particular importance (see below).

The third line of defense

The third and major line of defense is the secondary xylem with vertical and radial ducts containing resin (Fig. 3-8). Resin (oleoresin) is a complex mixture of monoterpenes, sesquiterpenes and diterpenoid acids. They contain a range of insect and microbial toxins and other biologically active agents. Besides this toxic impact, resin can act mechanically: 1) The pressurized resin flow flushes out invading organisms; 2) The volatile components of oleoresin evaporate upon exposure to the atmosphere, leaving a semi-crystalline mass of resin acids that polymerizes oxidatively and forms a hardened barrier that seals the wound and traps insects and pathogens in the matrix (Phillips and Croteau, 1999).

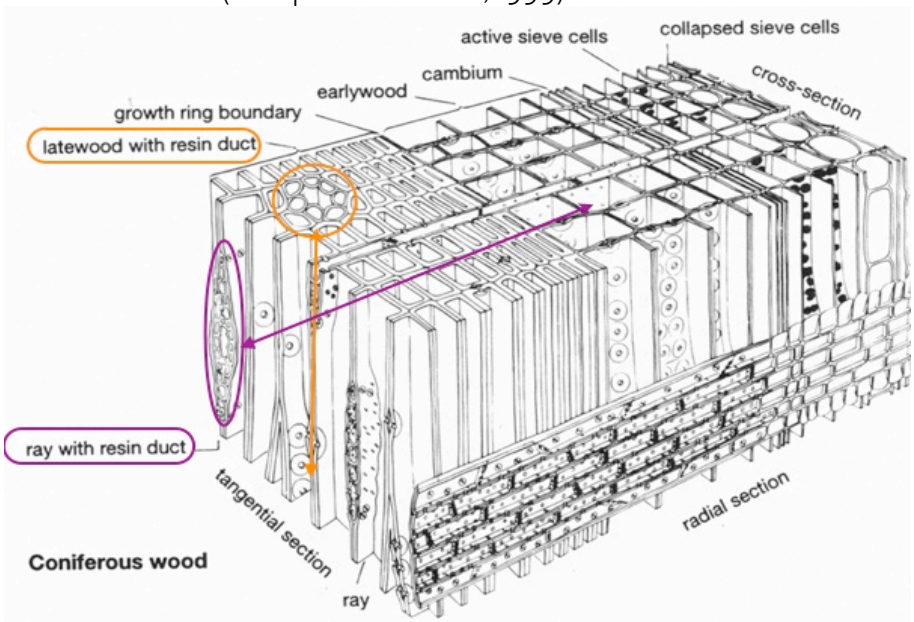

Fig. 3-8: Block of conifer wood: Latewood with vertical resin duct (orange) and radial resin duct (violet) (based on Grosser, 1977, adapted).

Vertical resin ducts are the most abundant, represent the most important reservoir of preformed resin and occur in the sapwood only (Lieutier, 2002). Xylem resin ducts in *Pinus* occur mainly in the outer region of earlywood and first-formed latewood (Wu and Hu, 1997) and are formed schizogeneously by the young cambium at the beginning of its activity [(Li et al., 2009; Bosshard, 1974; Werker and Fahn, 1969); (Fig. 3-9)], when intracellular space is formed by separation of parenchyma cells, enlarged and lined by the resin secreting epithelial cells. Different phytohormones are believed to play a key role in formation of resin ducts: Indole-3-acetic acid, 1-naphtyl acetic acid and auxin induced ethylene (Wimmer and Grabner, 1997).

Especially ethylene seems to be important for linking exogenous stress factors like temperature extremes or drought and increased resin duct production, but further investigation is needed to understand the mechanisms behind the effects of environmental variations, genetics and resin duct formation. However, it has been suggested that the stimulus for duct formation first affects the ray initials and is then conducted horizontally inwards by the rays to the xylem mother cells of the axial system, where the stimulus spreads vertically, causing the axial components to change into duct cells (Evert, 2006).

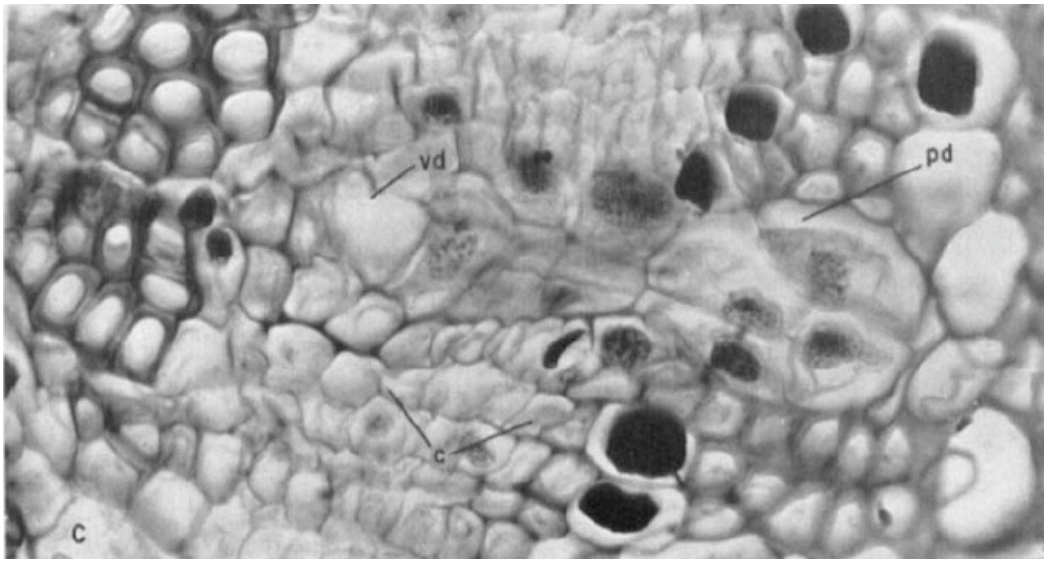

Fig. 3-9: Early stage of development of vertical resin duct in the secondary xylem (vd) and of radial resin duct in the phloem (pd) from the cambial zone (c) (Werker and Fahn, 1969).

The structure of a vertical resin duct is composed of the lumen, the resin secreting living and thin-walled epithelial cells, the thicker walled sheath cells and the surrounding parenchymal cells (Fig. 3-10). In *Pinus*, the epithelial cells remain active for several years and produce abundant resin; the height of the vertical ducts is variable, 10- to 23-years old Pinus taeda trees showed lengths from 20 to 510 mm (Evert, 2006).

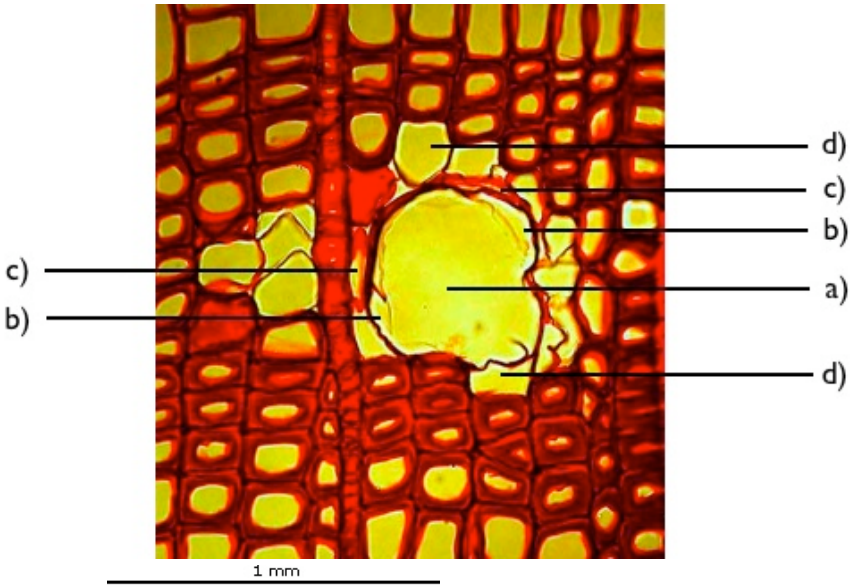

Fig. 3-10: Transversal section of Pinus sylvestris with a vertical resin duct structure, embedded in the tracheids of the early latewood. a) = Lumen; b) = Epithelial cells; c) = Sheath cells; d) = Parenchymal cells (Schoch et al., 2004, adapted).

The vertical resin ducts show three main stages (Fig. 3-11): 1) The epithelial cells are filled with resin and close the empty lumen; 2) Transition stage; 3) The epithelial cells have depleted their resin into the lumen.

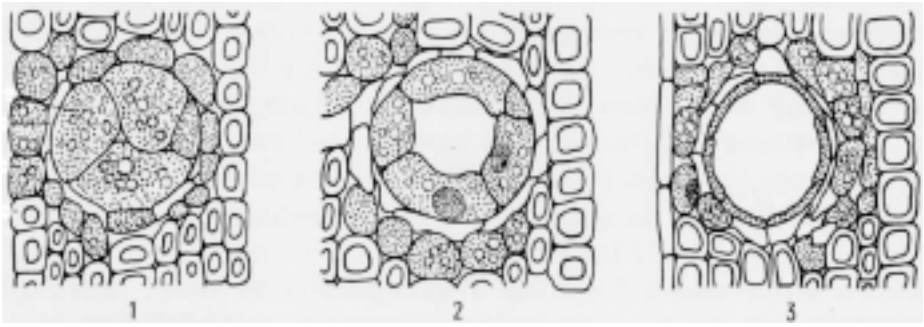

Fig. 3-11: The three main stages of vertical resin ducts (Bosshard, 1974).

The resin in the epithelial cells is accumulated under growing pressure, allowing the hydrophobic resin to pass the water saturated epithelial cell wall and accumulate in the lumen. In case of a wound, the epithelial cells can swell by absorbing water from the surrounding tissue; the resulting pressure pushes the resin in the lumen to the wounded site (based on Bosshard, 1974).

Vertical and radial resin ducts are connected and were believed to form a three-dimensional defense network, but this concept may not be true for all conifer woods. In *Pinus halepensis*, connections exist only between radial and axial ducts situated on the same radial plane, resulting in many two-dimensional networks on different radial planes (Evert, 2006; Werker and Fahn, 1969). While there is contiguity but usually no communication between the radial ducts of the phloem of Lodgepole pines and those of the xylem, connection exist between vertical and radial ducts in the xylem (Lieutier, 2002).

Besides the resin ducts, further defense functions of the secondary xylem are the development of phenol-impregnated heartwood and the formation of reaction zones to stop the spread of heart rot. It appears that radial rays are the only means of transport and translocation of phenolics to the border between sapwood and heartwood (Franceschi et al., 2005).

3.2.2.2 Inducible defense system

Inducible defenses are the second tier of defense, enhancing the overall defense capacity by a combination of synthesis of chemical and biochemical compounds and structural changes.

Inducible chemical defenses

Chemical defenses are very diverse, the broad-spectrum compounds can slow down an attack, while recognition mechanisms identify the organisms and activate specific compounds (Franceschi et al., 2005).

The preformed resin flow dries up approximately 1 to 3 days after wounding (Lieutier, 2002). In two days, a new resin flow appears and is increasing over the following days, reaching two to three times the values of the preformed flow (Fig. 3-12). It is suggested that this enhanced resin flow is achieved by activating the constitutive ducts to produce more resin and by resin translocation within the tree (Franceschi et al., 2005; Lieutier, 2002). Additionally, new traumatic resin ducts can be formed by a reprogramming of the cambial zone xylem mother cells within two to three weeks after attack; there is ample evidence that the octadecanoid pathway with the phytohormone jasmonate is involved in the formation of these traumatic ducts (Franceschi et al., 2005).

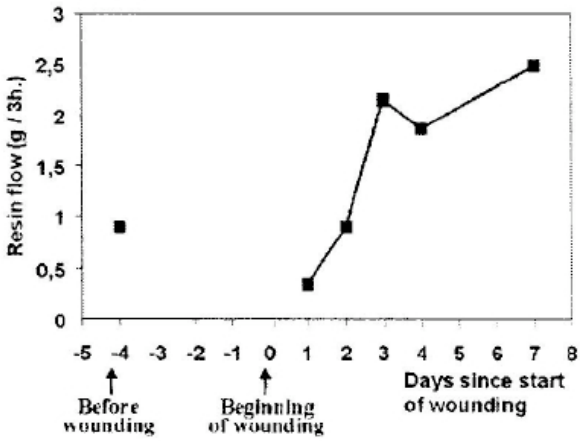

Fig. 3-12: Resin flow induced by wounding in Pinus taeda (Lieutier, 2002).

Other inducible chemical defenses are phenolics, tannins and protein-based compounds, which act as antifungal agents, antifeedants and inhibitors of advancement (Franceschi et al., 2005).

Hypersensitive reaction

The so called hypersensitive reaction is believed to play a fundamental role in the resistance of many conifer species to bark beetles and their associated fungi. The reaction in both the phloem and sapwood develops around each point of aggression and is visible as a longitudinal resin impregnated zone associated with extended cell necrosis (Lieutier, 2002). Oxygen compounds invade intercellular spaces and sieve cells, resulting in rapid cell death that is intended to kill and contain invading organisms (Franceschi et al., 2005; Lieutier 2002). This scorched earth defense sacrifices a small volume of tissue in an attempt at rapid containment of hostile organisms (Berryman, 1972). Studies have revealed that the hypersensitive reaction is a wound reaction induced by the mechanical stress caused by the tunneling activity of the beetle, and that this reaction can be stimulated and amplified, but not induced by the fungi introduced by the beetle in its gallery (Lieutier, 2002). The direction of the beetle gallery is important for the reaction. Due to the anatomical structure of the tree, the hypersensitive reaction develops mainly in the longitudinal direction, and is consequently strongly stimulated by longitudinal, but not by vertical beetle galleries (Lieutier, 2002). The hypersensitive reaction is completed by the formation of wound periderm, a long-term induced response and thus a delayed resistance (see below).

Delayed resistance

Delayed resistance is a long-term inducible response involving complex cell division and differentiation. According to Lieutier (2002), two kinds of resistance mechanisms are associated with bark beetle attacks: The wound periderm and the induced protection phenomenon.

The formerly described hypersensitive reaction can be completed by the formation of callus tissue that subsequently can become lignified, suberized or impregnated with phenolics to form part of the wound periderm. In a compartmentalization process, this reaction leads to the isolation of the dead tissues from the rest of the tree, walls off invading organisms and provides protection against further intrusion by re-establishing a continuous surface barrier (Franceschi et al., 2005; Lieutier, 2002).

The phenomenon of induced protection has been recently described in *Picea abies*: When the boles were pre-treated with mass inoculations of fungus at below the lethal density, they became resistant to mass inoculations made three weeks later in the same zone of the tree with the same fungus but at a deadly dosage (Lieutier, 2002). Formation of traumatic resin ducts around the wounds may be at least partly related to this induced protection.

4 Research questions

As stated in the literature review, a general framework of tree mortality under drought (McDowell et al., 2008) suggests the three main mechanisms hydraulic failure, biotic agent demographics and carbon starvation. The mechanism of carbon starvation suggests that under drought isohydric species have to close their stomata to prevent hydraulic failure. With closed stomata the assimilation processes stop, but primary metabolic processes continue to deplete stored carbon. This would then lead to outright starvation or to an inability to form and maintain structural and chemical defenses, making the trees vulnerable to drought-induced beetle attacks. Although Sala et al. (2010) question the mechanism of carbon depletion and in return suggest a failure of carbon metabolism and translocation, the proposed effect on the defensive system would be the same. Investigations of the defensive system of pines have revealed that the resin-based system with vertical and radial resin ducts is a major part of both the constitutive and the inducible defense. Hence, comparing characteristics of vertical resin ducts between trees that survived and trees that succumbed to a bark beetle-accompanied severe drought are suitable to investigate if drought has effects on the defensive system of pines. By investigating these characteristics, the understanding of tree defense structures and processes as determinants of tree mortality under drought can be improved. Due to temporal constraints, it is not possible to investigate if hydraulic failure and/or biotic agent demographics are relevant mortality mechanisms at the study sites. Although it is suggested that the isohydric behavior of Pinyon prevents excessive cavitation and thus hydraulic failure (McDowell et al., 2008), other studies (e.g. Martinez and Pinol, 2002) show that hydraulic failure may be relevant. Besides that, the potential amplifying effects of droughts on population dynamics of biotic agents like bark beetles also need research attention.

The following study (chapters 5 to 8) investigates the effects of a severe drought with subsequent bark beetle attacks on the resin-based defensive system of *Pinus edulis* at three sites in New Mexico, USA. This research represents the first known study to compare resin duct characteristics of Pinyon pine trees. The research questions are as follows:

1) **Do significant differences of resin duct variables exist between live and dead trees?**

   To answer this question, paired t-tests are used to statistically compare measures of vertical resin ducts between live and dead trees. It is expected that drought and drought-induced insect pressure have effects on the resin-based defensive system of Pinus edulis and that trees that survived invested more resources in that system.

2) **Are resin duct variables good predictors of tree mortality, and to which extent can the prediction models be generalized for Pinyon pine populations under drought and insect pressure across New Mexico, USA?**

   To answer this question, logistic regression with resin duct properties as independent variables is used to model probability of Pinyon mortality and the models are externally validated. If significant differences of resin duct variables exist between live and dead trees, it is expected that these variables are good predictors of tree mortality at the study sites.

3) **General relationships: What are the relationships among resin duct variables? What are the relationships between resin duct variables and growth and tree size? Do the resin duct variables show temporal variation?**

   To investigate the relationships, Pearson correlation analysis is conducted. Mean value chronologies of resin duct properties are established to show temporal variation. It is expected that a trade-off between growth and defense exist and that resin duct variables are influenced by environmental factors and thus show considerable variation over time.

Besides showing characteristics and relationships of vertical resin ducts in Pinyon pine trees, the results should reveal if:

- Pinyon trees that survive drought and subsequent bark beetle attack invest more carbon in resin defense than trees that die and that this carbon investment in defense is a more important determinant of Pinyon mortality than investment in growth.
- Vertical resin ducts as a proxy for the defensive system are good predictor variables for Pinyon mortality under drought and insect pressure.
- Evidence can be found that the above-mentioned effect of carbon starvation and/or phloem transport failure is a relevant mechanism for Pinyon mortality at the investigated study sites.

5 Material and methods

5.1 Study sites and sampling procedure

This study was conducted with pairs of live and dead *Pinus edulis*, sampled 2008-2009 within pinyon-juniper woodlands at three sites in New Mexico, USA (Fig. 5-1). The pairs were sampled by Macalady and Bugmann for their investigation of growth-mortality relationships and were now reused to conduct this study (Table 5-1).

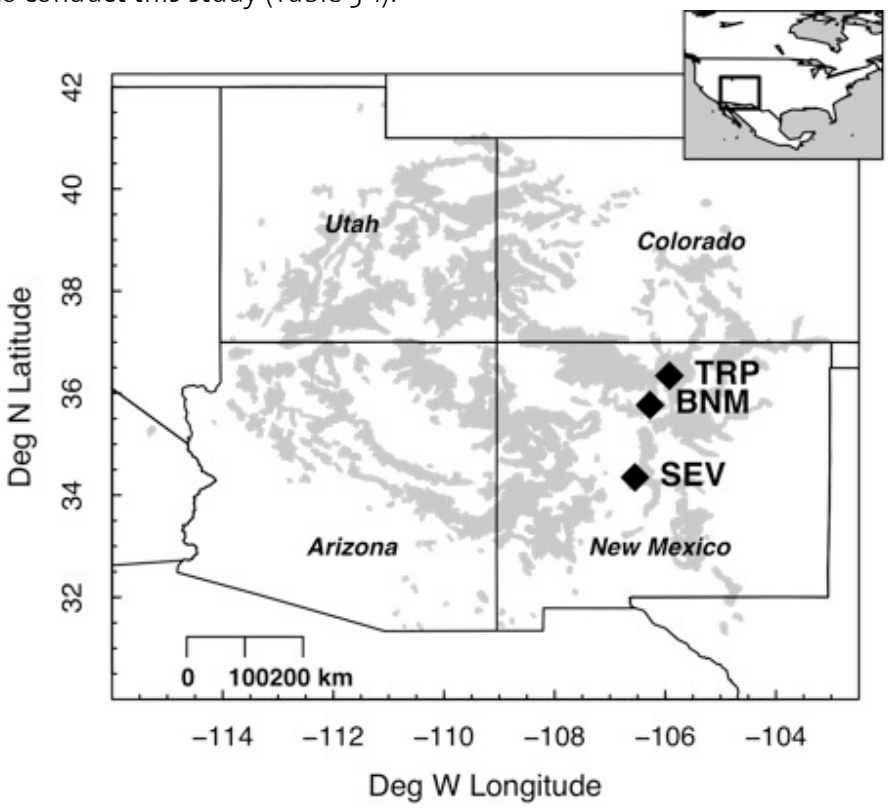

Fig. 5-1: Distribution range of Pinyon in the southwestern USA (grey) and study sites in New Mexico (Macalady and Bugmann, in prep.).

Table 5-1: Investigation of the severe drought in the early 2000s: Sites, stand level mortality of Pinyon and number of live and dead trees used in this study.

Sites	Site acronym	Stand level mortality	n live trees	n dead trees
Carson National Forest, Tres Piedras	TRP	75%	20	20
Bandelier National Monument	BNM	100%	0	20
Sevilleta National Wildlife Refuge	SEV	26%	20	20
Total			40	60

The sites represent a latitudinal gradient in the distribution of *Pinus edulis* in the southwestern USA with populations that could have different genetic responses to drought and insect outbreaks. Tree mortality at these sites ranged from 26 to 100% and was attributed to a recent severe drought, which started in the late 1990s and reached its four consecutive driest years between 2000 and 2003. At all sites, the proximal cause of tree death is believed to be bark beetle attacks (*Ips* spp.), of which considerable and widespread evidence was found at the sites (e.g. galleries, exit holes and the fungal associate *Ophiostoma* spp.). The dead tree in each pair was selected based on close location and similar size to the live tree, which controls for similar conditions of soil, aspect and slope. At breast height, two cores were taken per live tree, using a 5 mm increment borer. From each dead tree, a stem disc was taken and two measurement radii

were selected on each stem disc. The resulting two samples per tree (four samples per tree pair) were cross-dated by Macalady and Bugmann (in prep.) using standard dendrochronological techniques.

Carson National Forest, Tres Piedras, New Mexico, USA (TRP)

20 live and 20 dead trees were used, encompassing 80 samples. The site elevation of 2'090 m represents the high end of the local distribution of Pinyon pine. Recent tree mortality was 75%, evidence of bark beetle signs and/or symptoms could be found on all samples of dead trees. On the assumption that the last formed ring corresponded to the year of death of the dead tree, all dead trees died between 2001 and 2005. At this site, growth patterns were not good predictors for tree mortality (Macalady and Bugmann, in prep.), with the best logistic-regression based models correctly classifying < 60% of the trees.

Bandelier National Monument, New Mexico, USA (BNM)

20 dead trees were used, encompassing 40 samples. The site elevation of 1'935 m represents the middle to high end of the local distribution of Pinyon pine. Recent tree mortality was 100%, evidence of bark beetle signs and/or symptoms could be found on all samples of dead trees. On the assumption that the last formed ring corresponded to the year of death of the dead tree, all dead trees died in the year 2001. At this site, growth patterns were not good predictors for tree mortality (Macalady and Bugmann, in prep.), with the best logistic-regression based models correctly classifying < 60% of the trees.

Sevilleta National Wildlife Refuge, New Mexico, USA (SEV)

20 live and 20 dead trees were used, encompassing 80 samples. The site elevation of 2'050 m represents the middle to high end of the local distribution of Pinyon pine. Recent tree mortality was 26%, evidence of bark beetle signs and/or symptoms could be found on all samples of dead trees. On the assumption that the last formed ring corresponded to the year of death of the dead tree, all dead trees died between 1997 and 2005, with 80% dying between 2001 and 2005. At this site, growth patterns were good predictors for tree mortality (Macalady and Bugmann, in prep.), and logistic regression based models correctly classify > 80% of the trees.

5.2 Measurements of defense and growth

The already mounted, belt sanded and cross-dated samples were manually re-sanded with 30 and 15 micron sandpaper, respectively. A high-resolution image (optical resolution of 2'400 dpi) was created by scanning each sample with a distortion-free scanner (Color Scanner Epson Expression 10000XL). The resulting TIFF-images (Fig. 5-2) were entered into the public domain image-processing program ImageJ. Defense measures of the two trees in each pair were made within a constant tangential window of 4.5 mm width, spanning 20 years of growth including the last ring formed in dead trees. Due to the assumed and also empirically shown (Blanche et al., 1992; Schopmeyer et al., 1954) positive correlation between resin duct size or abundance and resin flow, annual measures of defense structures included resin duct production (#) and resin duct area (mm^2); additionally the area of the annual radial increment section within the tangential window was measured (mm^2). Derived from these data, the variables resin duct density (# mm^{-2}) and relative resin duct area (%) were calculated. To reveal different temporal patterns, mean values of resin duct properties were calculated annually and for 3, 5, 7, 10, 15 and 20 year growth periods, beginning and including the year of death of the dead tree. The whole resin duct structures as a proxy for tree investment in defense were measured, i.e. no distinction between the resin canal, the resin secreting epithelial cells and the surrounding parenchymal tissue was made.

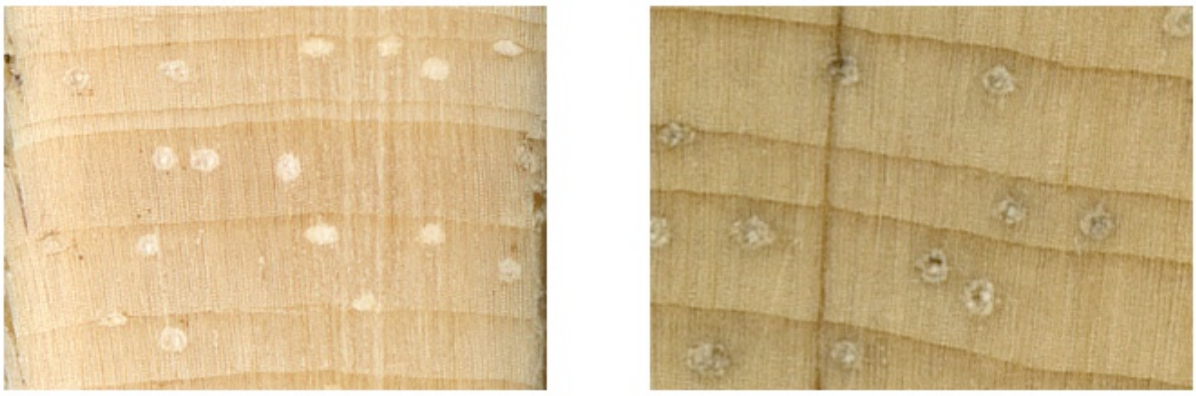

Fig. 5-2: Scanned images from a live (left) and a dead (right) tree sample, within a constant tangential window of 4.5 mm width.

Resin duct production (*RD production*), representing a relative measure of carbon investment in vertical resin ducts, was measured within ImageJ as a count of resin ducts per annual ring. Individual resin duct areas were measured within ImageJ with the freehand selection tool and resin duct size was defined as the average area of all resin ducts counted in each annual ring (*RD mean area*). The area of the annual radial increment section (*RIA*) as a growth-variable was measured within ImageJ with the polygon selection tool. Resin duct density (*RD density*), representing a measure of carbon investment in vertical resin duct relative to radial growth, was calculated by dividing the annual resin duct production by the corresponding annual area of the increment section. Relative resin duct area (*RD relative area*) is the proportion of resin duct area relative to the corresponding area of radial growth, and was calculated for each year by multiplying the average resin duct area by the resin duct production, dividing the result by the annual area of the increment section, and then multiplying the quotient by 100.

In the data files, values of zero occur in two cases: First in the case of missing rings (values of zero for resin duct production, resin duct area and increment area) and second in the case of no resin ducts within a present increment area (values of zero for resin duct production and resin duct area, but a value of x for the increment area). For the calculation of the mean values, the zero values have been considered as stated in Table 5-2.

Table 5-2: Consideration of values of zero in the mean value calculations.

Variables / Cases with values of zero	Missing ring	No resin ducts in increment section
RD production	Zeros are considered in the mean value calculations	Zeros are considered in the mean value calculations
RD relative area	Calculation is "zero/zero" which equals NA, NAs are not considered	Calculation is "zero/x" which equals zero, zeros are considered
RD density	Calculation is "zero/zero" which equals NA, NAs are not considered	Calculation is "zero/x" which equals zero, zeros are considered
RD mean area	Zeros are converted into NAs, NAs are not considered	Zeros are converted into NAs, NAs are not considered

Preparation, scanning and measuring of two samples per tree are time consuming. With four samples per pair, 20 pairs per site, 20 years of measurement and a mean value of 3.5 resin ducts per year, one measures around 6'000 resin ducts and 1'500 increment sections per site. To decide whether two samples per tree are needed or just one sample could be used, a regular and paired t-test has been performed with the measurements of the first site (TRP). The tests have been performed for two samples per tree and for just one sample per tree (a-cores and b-cores, respectively). Though the main results did not change significantly, the sample depth in

the paired t-test dropped, meaning that due to years without measurements (missing rings etc.) either in the sample of the live and the dead tree, many corresponding years could not be paired and tested. Therefore the measurements were continued with two samples per tree.

In general, the method outlined above for measuring vertical resin duct characteristics was appropriate to achieve the defined goals. However, the following advantages and disadvantages could be identified:
Advantages: No chemical treatment and just low-tech laboratory equipment (scanner and computer with software) are needed. The work process is relatively fast. ImageJ supports the measuring process with suitable features, enables precise work and is freeware.
Disadvantages: The main disadvantage is the obtained optical resolution of the scanned pictures. The detection of some resin ducts and the distinction from the surrounding tissues can be hard, especially in the earlywood. No distinctions between the different elements of the resin ducts are possible (e.g. resin canal, epithelial cells, parenchymal cells).

5.2.1 Further methods for measuring resin duct parameters

Throughout the literature, various methods for measuring vertical resin ducts can be found. The following section provides a brief overview over the main measurement procedures with their advantages and disadvantages. The overview is not intended to be exhaustive, intermediate forms and/or combinations are possible.

Examine the cores manually with the aid of a stereo binocular (based on Rigling et al., 2003)

In a constant tangential window of pre-defined width (e.g. 4 to 5 mm), the resin ducts of each ring are counted with the aid of a stereo binocular. The resin duct density is calculated by relating the number of resin ducts to the area, obtained by multiplying the given tangential window by the tree-ring width of the corresponding year. The width of the resin ducts could be measured with the scaled crosshair of the stereo binocular or with the encoder of the measuring bench.
Advantages: Just standard technical lab equipment (stereo binocular with a scaled crosshair) and no chemical sample treatment is needed, the method is simple.
Disadvantages: Time-consuming manual measuring and following calculation processes. The measurements with the scaled crosshair can be burdensome and imprecise, no direct and accurate area measurements (resin duct area, area of the increment section) are possible.

Examine the cores manually with the aid of micro-pictures and image analysis software (based on Wimmer and Grabner, 1997)

A light-microscope (e.g. Nikon SMZ-U stereoscopic microscope, zoom 1:10) with a camera (e.g. Olympus e-volt 330) is connected to a computer with image analysis software (e.g. ImageJ). Micro-pictures from the cores and from parts of the stem discs are taken and stitched together with the aid of software (e.g. PTGui or CS Photoshop). Using these pictures in the image analysis software, the resin ducts can be counted and the area of each resin duct is measured. The area of the corresponding increment section can also be measured directly with features within the software. Depending on the resolution, distinctions between the different components of resin ducts could be made.
Advantages: No chemical treatment of the cores is needed. The high resolution of the micro-pictures and the image analysis software enables accurate measurements and support measuring and calculation processes. Suitable software can be found as freeware.
Disadvantages: More technical equipment is needed (light microscope, camera, computer with software). Depending on the chosen magnification, many micro-pictures will be needed for one core and have to be stitched together, which is time consuming and work intensive. Freeware might have limited functions and documentation and requires experience in handling.

Examine thin sections of the cores manually with the aid of a scanner and image analysis software (based on Eilmann, 2009 and Rosner and Hannrup, 2004; adapted)

Thin sections of the cores are cut on a sliding microtome. To obtain a good contrast, the thin sections are chemically prepared (e.g. safranin, astra blue) and afterwards scanned with a distortion-free color scanner. Using these pictures in the image analysis software, the resin ducts can be counted and the area of each resin duct can be measured. The area of the corresponding increment section can also be measured directly with features within the software. Depending on sample preparation and resolution, distinctions between the different components of resin ducts could be made.
Advantages: The high resolution of the scanned thin sections and the image analysis software enable accurate measurements and support measuring and calculation processes. Suitable software can be found as freeware.
Disadvantages: Requires surfaces with clear contrasts, obtained by time and/or work intensive sample preparation (e.g. establishing core slides, chemical preparations).

Examine the cores automatically with the aid of a scanner and image analysis system (based on Chen, 2008)

For obtaining a good contrast, the cores or slides of the cores are polished or chemically prepared (e.g. safranin, astra blue) and afterwards scanned with a distortion-free color scanner. The resin ducts are counted and measured automatically with image analysis software (e.g. Image Pro Plus, WinCell, Roxas), using automatic detection algorithms. Tests by Chen (2008) showed an overall automated resin canal detection rate of 68%, but the accuracy drops when the measurements are extended to areas. Correction tools should allow users to review and correct the automatic decisions.
Advantages: Professional software with many functions automatically supports measuring processes and enables precise work; the automated work process can be fast and enables fast measurements of whole cores from bark to pitch to establish long time series.
Disadvantages: Requires surfaces with clear contrasts, obtained by time and/or work intensive sample preparation (e.g. polishing the surface, establishing core slides, chemical preparations). The automatic detection and measurement rates can be low and require time and work intense manual corrections. Complex image analysis systems can be expensive and need experienced skills.

5.3 Statistical analysis

A descriptive table with overall mean values (n = 100 trees with values from 40 live trees and 60 dead trees from all three sites) was established to show the *main mean characteristics and resin duct properties* of the sampled Pinyon pine trees.

Relationships among resin duct properties, between resin ducts properties and tree size and measures of annual radial growth (using the area of the annual radial increment section RIA) were investigated using Pearson correlation analysis (n = 100 trees with values from 40 live trees and 60 dead trees from all three sites). Pearson's correlation is the most widely used computational index to measure bivariate association (Rodgers and Nicewander, 1988). In addition to the investigated relationships, Tukey's biweight robust mean was used to establish *mean value chronologies* of resin duct properties. Simple robust estimates were used to estimate the population mean in the presence of outliers (Singh and Nocerino, 2001).

Paired t-tests were used to statistically *compare measures of defense between live and dead trees* at the TRP- and SEV-site. A Lilliefors test as a specific adaptation of the general Kolmogorov-Smirnov test was performed to test for normality. The tests showed enough evidence (i.e. > 2/3 of the test cases) to accept the assumption of normal distribution and therefore to use the paired t-tests and not the Mann-Whitney Wilcoxon-tests. Since BNM

contains just samples from dead trees (100 % stand level mortality), no comparison between live and dead trees was possible.

Probability of tree mortality was modeled using logistic regression. The logistic regression model has become the standard method for the prediction of a binary response (Hosmer and Lemeshow, 1989), such as the tree status "alive" or "dead" from continuous resin duct variables. The general expression for logistic regression is as follows:

$$\Pr(Y = 1|\mathbf{X}) = \frac{e^{\mathbf{X}\beta}}{1 + e^{\mathbf{X}\beta}}$$

Pr(Y = 1|X) is the survival probability of an individual tree expressed as a function of a matrix X of independent variables (in this study resin duct properties). Y is the dependent variable (Y = 1 if the tree is alive, Y = 0 if the tree is dead) and ß is a vector containing the regression coefficients. A series of single variable logistic regression models was fitted using the following data and sites:

- Combined samples from TRP and SEV with 80 trees (40 living, 40 dead)
- Samples from TRP with 40 trees (20 living, 20 dead)
- Samples from SEV with 40 trees (20 living, 20 dead)

Since BNM contains just samples from dead trees, these data were not used for model fitting. Models were fitted by maximum log-likelihood estimation using the Akaike information criterion AIC. The findings of Akaike (1974) made it possible to combine estimation and model selection under a single theoretical framework (optimization), and this paradigm as an extension of the likelihood theory avoids many of the fundamental limitations and common misuses of null hypothesis testing (Anderson et al., 2000). The AIC is defined as follows:

$$\mathrm{AIC} = -2\log_e(\ell(\hat{\theta}|data)) + 2K$$

$\log_e \ell(\hat{\theta}|data)$ is the value of the maximized log-likelihood over the unknown parameters θ (given by the data and the model), and K is the number of parameters estimated in that approximating model. The AIC is computed for each of the approximating models and the model where AIC is minimized is selected as best for the empirical data at hand (Anderson et al., 2000). An additional metric was used to assess model suitability: The area under the Receiver Operating Curve (AuROC) was computed to provide a threshold-independent measure of model discrimination, where values greater than 0.8 suggest excellent discrimination (Hosmer and Lemeshow, 1989).

Two validation methods were used to determine the prediction accuracy of the models:

1) Internal validations were performed where data from TRP and SEV combined, from TRP and from SEV, respectively, were used. By calculating all single-variable models for defense measures, a series of models was fitted with random samples containing 60% of the trees and were then applied to the remaining 40% of the trees to predict their status. 200 re-samplings were used to perform this cross-validation.

2) External validations were performed to assess the extent to which the models can be generalized to Pinyon pine populations under drought and insect pressure across New Mexico, USA. Three external validations were performed:

- By fitting two single variable models (i.e. models with the two lowest AIC-values) for the combined samples from TRP and SEV, using the full data set and testing this model against the data set from BNM. BNM contains just samples from dead trees and these data were not used for the prior model fitting.

- By fitting two single variable models (i.e. models with the two lowest AIC-values) for TRP using the full data set and testing this model against the data sets from SEV and BNM. This validation has been performed to reveal possible site influences and differences.
- By fitting two single variable models (i.e. models with the two lowest AIC-values) for SEV using the full data set and testing this model against the data sets from TRP and BNM. This validation has been performed to reveal possible site influences and differences.

All analyses were performed using the R software (The R Foundation for Statistical Computing, GNU General Public License, Version 2.10.1, 2010).

6 Results

6.1 Descriptive results and relationships

Descriptive results as a general overview of the main mean sample characteristics are presented in chapter 6.1.1. Since this study represents the first known comparison of vertical resin ducts of *Pinus edulis*, relationships among these defense variables, between them and tree size and tree growth, respectively, are tested in chapters 6.1.2 to 6.1.4. Additionally, temporal variations are shown in chapter 6.1.5.

6.1.1 Descriptive results

Table 6-1 provides descriptive results as a general overview by showing sample characteristics (tree age and diameter at breast height DBH), growth characteristics (ring width RW, radial area increment RIA, basal area increment BAI) and resin duct (RD) measurements as mean values for all trees (n = 100) at all three sites.

Table 6-1: Mean sample characteristics, mean 20yr growth and mean 20 yr resin duct values of 100 trees.

Variables	Mean (Standard error)
Tree age (a)	160.1 (5.5)
DBH (cm)	17.0 (0.5)
20 yr RW (mm)	0.57 (0.038)
20 yr RIA mm^2	2.49 (0.16)
20 yr BAI (cm^2)	2.16 (0.15)
20 yr RD production (#)	3.50 (0.15)
20 yr RD mean area (mm^2)	0.027 (0.001)
20 yr RD density (# mm^{-2})	1.62 (0.05)
20 yr RD relative area (%)	4.1 (0.1)

While the RD production of the sampled Pinyon pine trees is roughly 9% lower than reported RD production of Ponderosa pine in northern Arizona (Kane and Kolb, 2010), RD density is roughly a factor 2.5 higher and RD relative area is roughly 36% greater. Compared to the mean density of 11 pine species of 4 to 5 ducts per square millimeter (Wu and Hu, 1997), the Pinyon-density is considerably lower, demonstrating a great variability even within pine species.

6.1.2 Relationships among defense

From all possible correlations among the measured resin ducts properties, the correlation between 20 yr RD density and 20 yr RD mean area (Fig. 6-1) and between 20 yr RD density and 20 yr relative area (Fig. 6-2) are significant. Though the figures show regression lines for live and dead trees, the indicated r- and p-values are for the whole dataset.

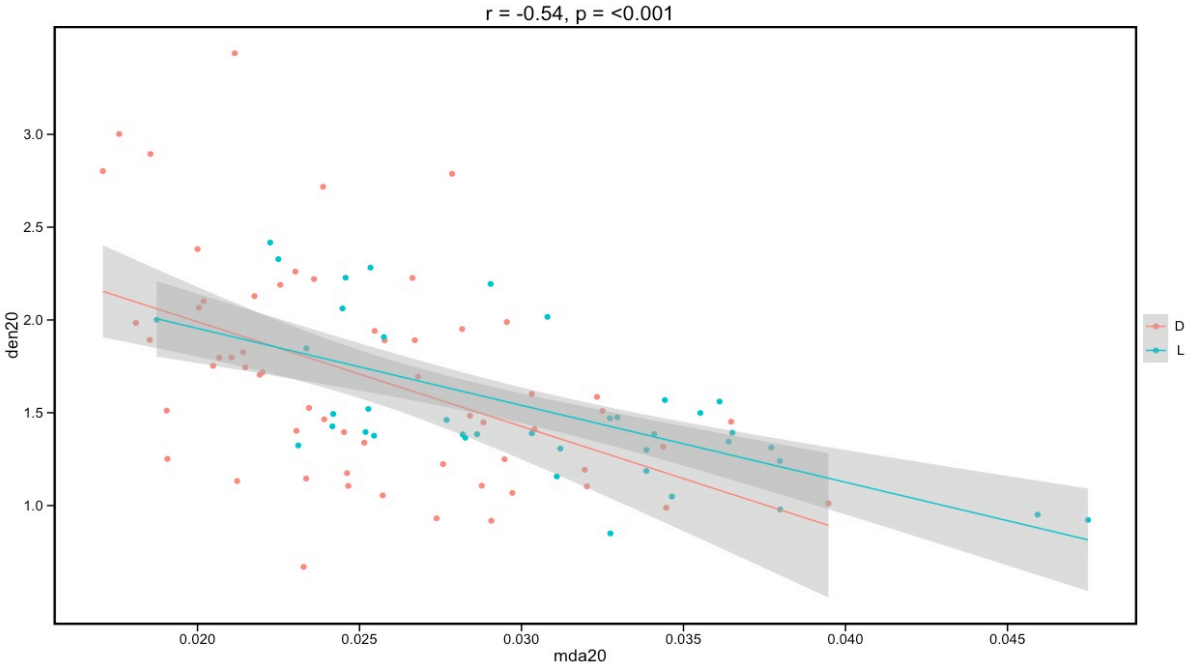

Fig. 6-1: Scatterplot with regression line and 95% confidence interval (grey shaded band) between 20 yr RD density (den20) and 20 yr RD mean area (mda20) for 40 live (blue) and 60 dead (red) trees from all three sites in New Mexico, USA.

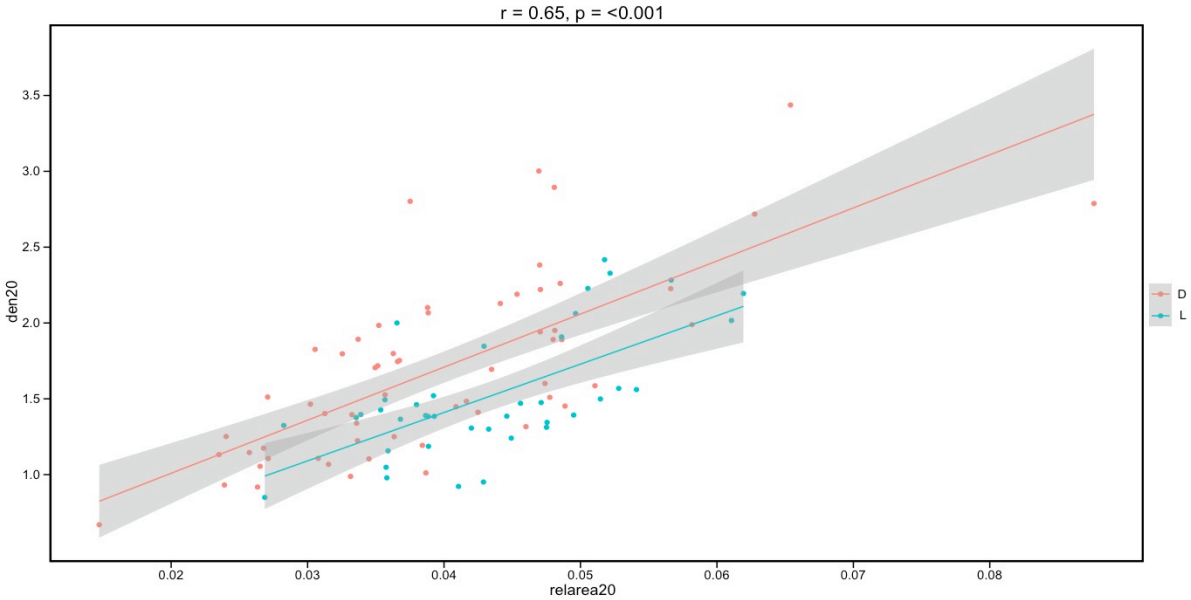

Fig. 6-2: Scatterplot with regression line and 95% confidence interval (grey shaded band) between 20 yr RD density (den20) and 20 yr RD relative area (relarea20) for 40 live (blue) and 60 dead (red) trees from all three sites in New Mexico, USA.

6.1.3 Relationships between tree size and defense

Two significant correlations could be found between tree size (diameter at breast height DBH) and the resin duct variables: While the correlation between DBH and 20 yr RD density is negative (Fig. 6-3), the correlation between DBH and RD mean area is positive (Fig. 6-4). Though the figures show regression lines for live and dead trees, the indicated r- and p-values are for the whole dataset.

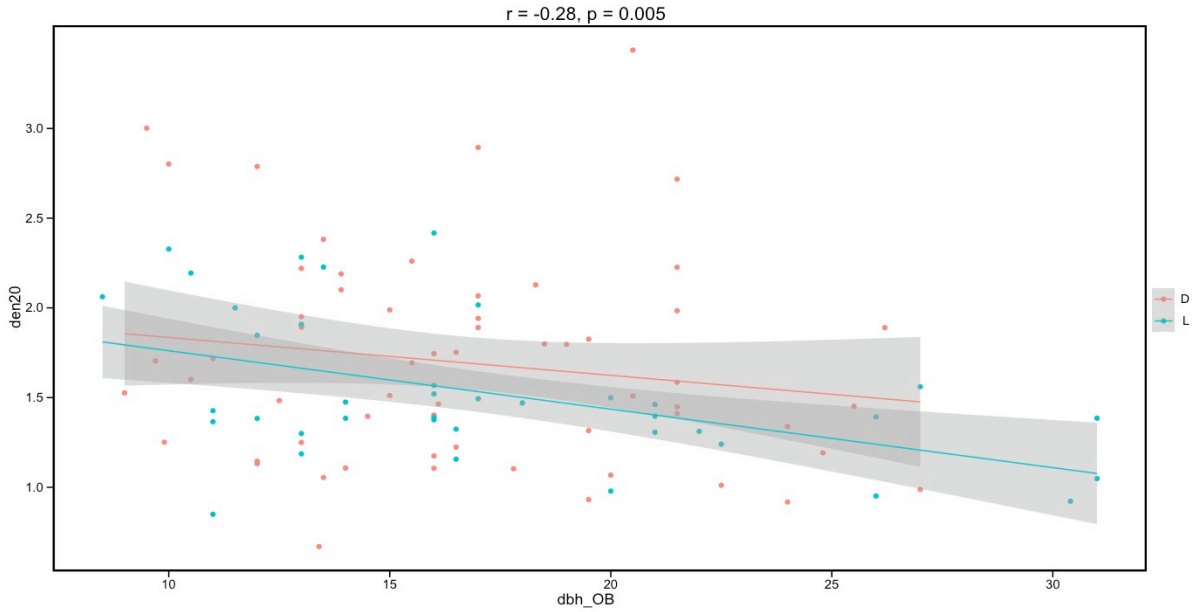

Fig. 6-3: Scatterplot with regression line and 95% confidence interval (grey shaded band) between 20 yr RD density (den20) and DBH for 40 live (blue) and 60 dead (red) trees from all three sites in New Mexico, USA.

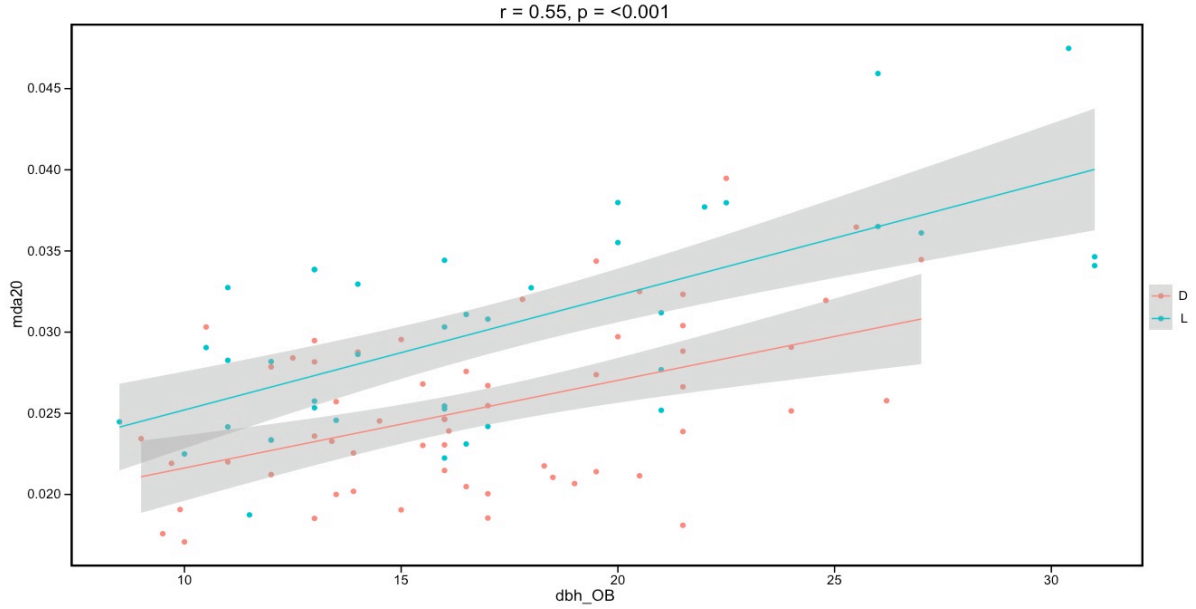

Fig. 6-4: Scatterplot with regression line and 95% confidence interval (grey shaded band) between 20 yr RD mean area (mda20) and DBH for 40 live (blue) and 60 dead (red) trees from all three sites in New Mexico, USA.

6.1.4 Relationships between growth and defense

Three out of four possible correlations between the radial increment area (RIA) as the growth variable and resin duct variables are significant. While the correlations between RIA and 20 yr RD production (Fig. 6-5) and 20 yr RD mean area (Fig. 6-6), respectively, are positive, the correlation between RIA and 20 yr RD density is negative (Fig. 6-7). Though the figures show regression lines for live and dead trees, the indicated r- and p-values are for the whole dataset.

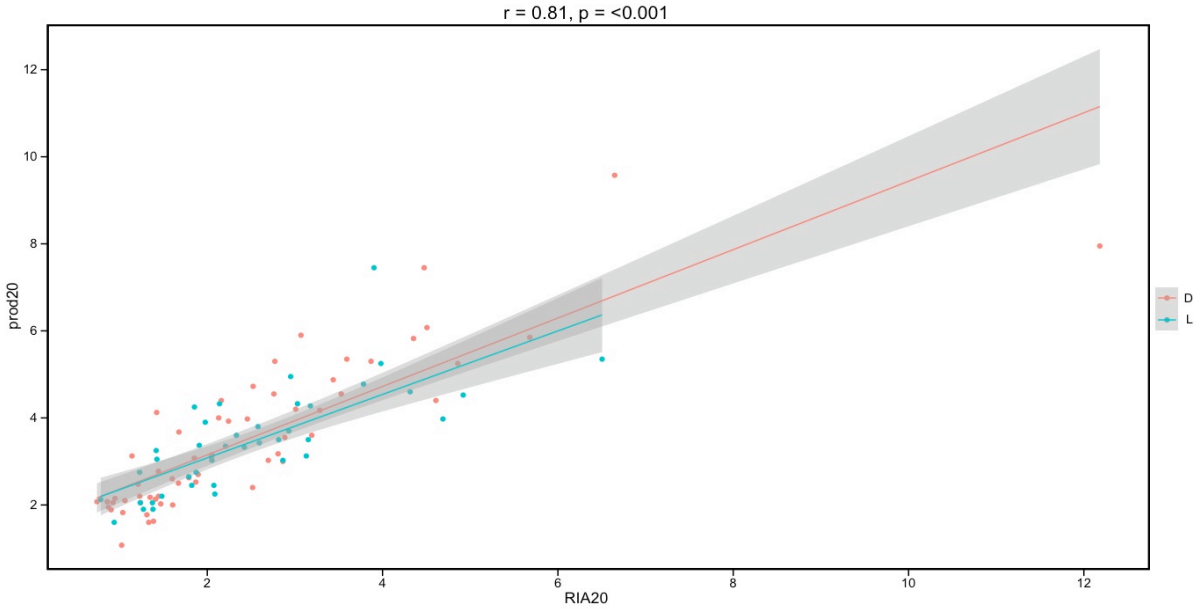

Fig. 6-5: Scatterplot with regression line and 95% confidence interval (grey shaded band) between 20 yr RD production (prod20) and 20 yr RIA for 40 live (blue) and 60 dead (red) trees from all three sites in New Mexico, USA.

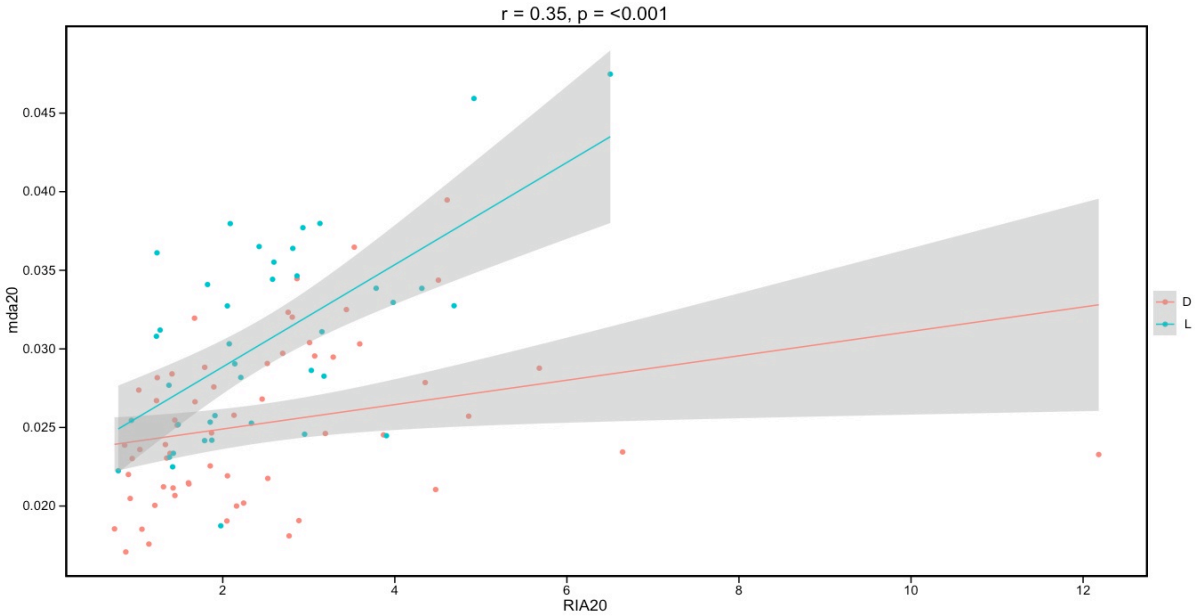

Fig. 6-6: Scatterplot with regression line and 95% confidence interval (grey shaded band) between 20 yr RD mean area (mda20) and 20 yr RIA for 40 live (blue) and 60 dead (red) trees from all three sites in New Mexico, USA.

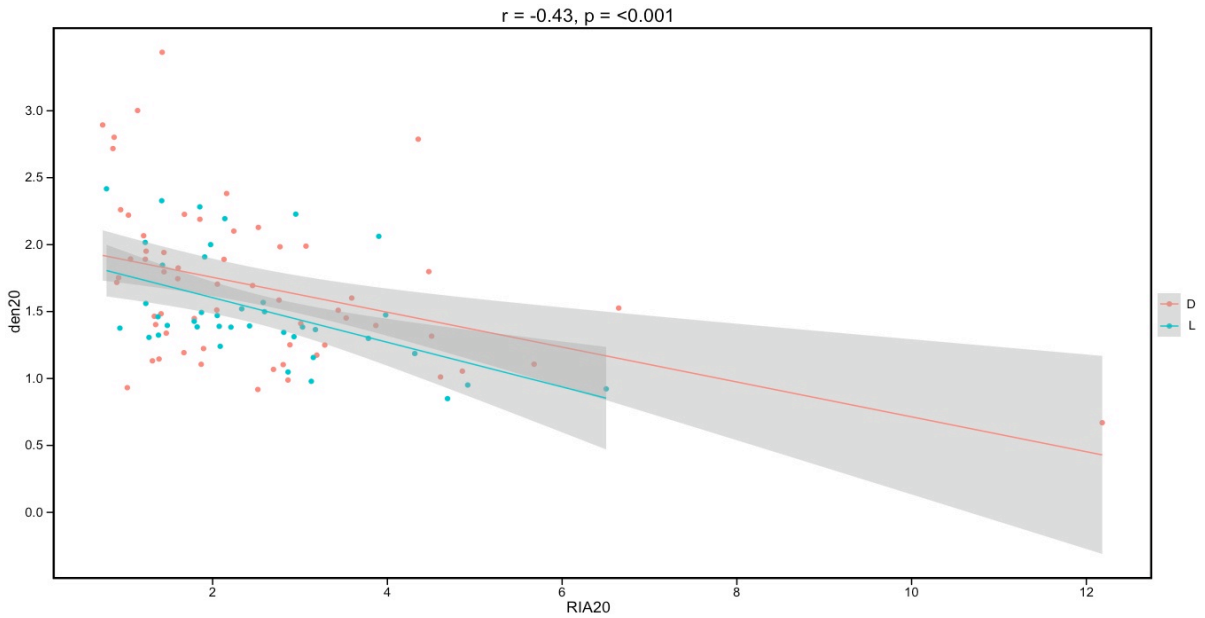

Fig. 6-7: Scatterplot with regression line and 95% confidence interval (grey shaded band) between 20 yr RD density (den20) and 20 yr RIA for 40 live (blue) and 60 dead (red) trees from all three sites in New Mexico, USA.

6.1.5 Temporal variations

Fig. 6-8 to Fig. 6-11 show Tukey's biweight robust mean value chronologies for all four resin duct variables for all trees (n = 100, with 40 live and 60 dead trees) from all three sites in New Mexico, USA.

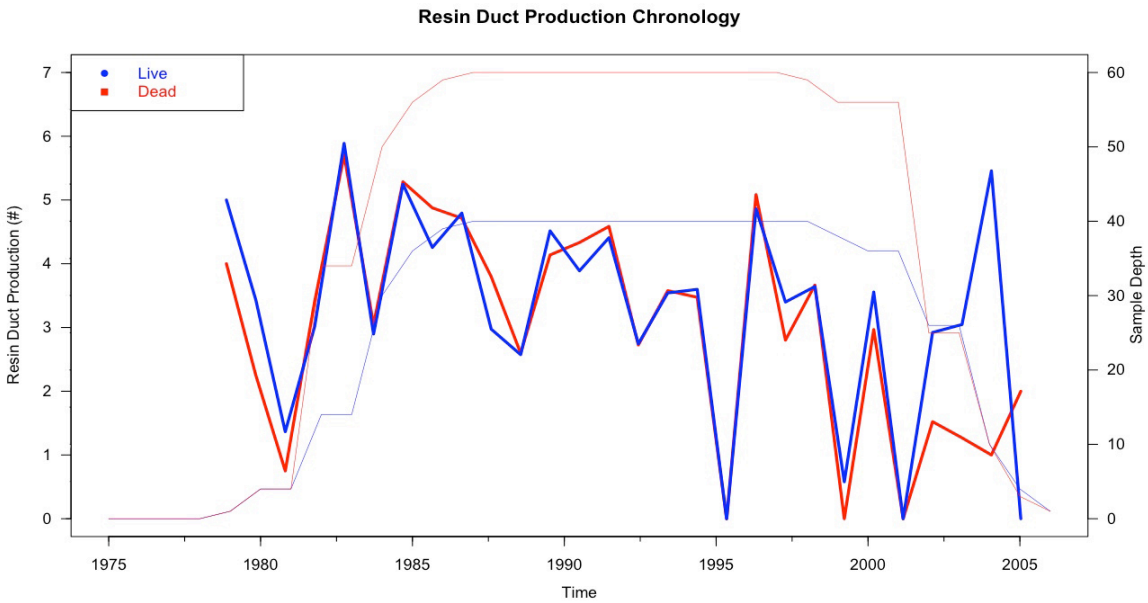

Fig. 6-8: Mean value chronologies (bold lines) and sample depth (thin lines) for RD production for 40 live (blue) and 60 dead (red) trees from all three sites in New Mexico, USA.

The two chronologies for RD production show high variation and develop mostly in phase until the year 2001. The years 2000 - 2003 have been reported as the four consecutive driest years of the recent drought (Breshears et al., 2005).

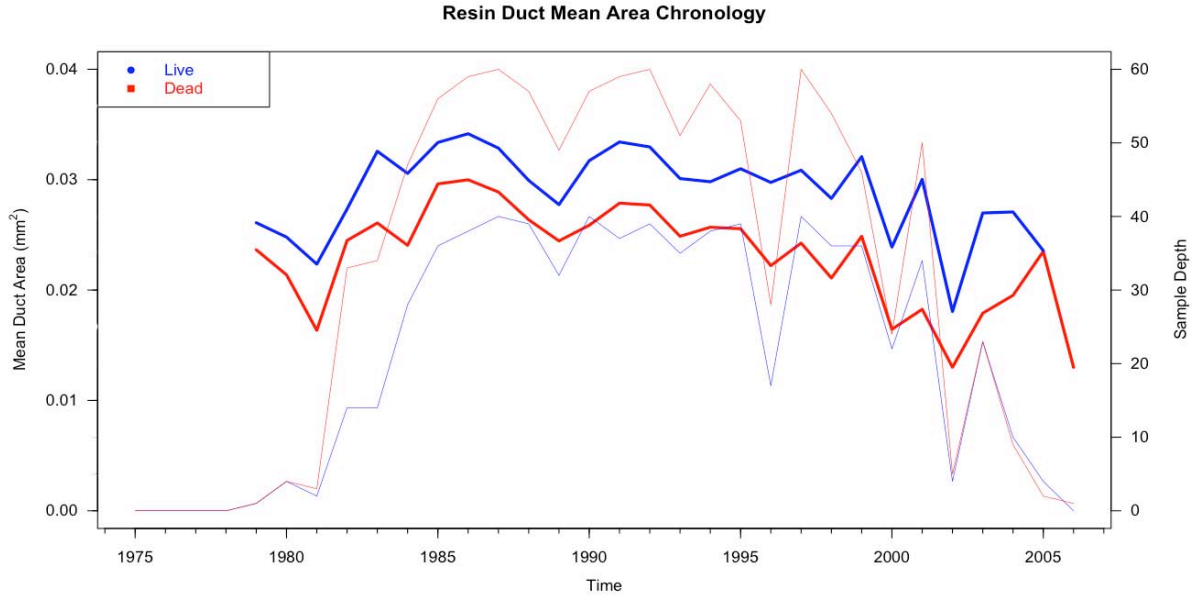

Fig. 6-9: Mean value chronologies (bold lines) and sample depth (thin lines) for RD mean area for 40 live (blue) and 60 dead (red) trees from all three sites in New Mexico, USA.

The two chronologies for RD mean area show moderate variation and develop mostly in phase, but the RD mean area of the dead trees develops constantly on lower value levels than that of the live trees. Both chronologies show a decreasing trend toward the recent drought period.

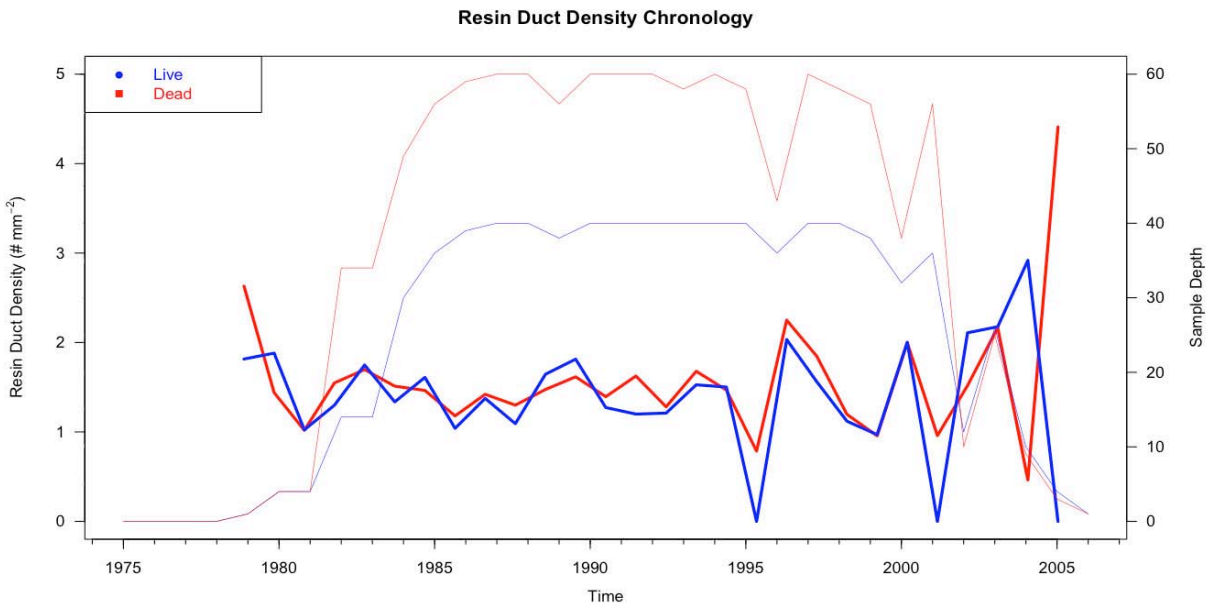

Fig. 6-10: Mean value chronologies (bold lines) and sample depth (thin lines) for RD density for 40 live (blue) and 60 dead (red) trees from all three sites in New Mexico, USA.

Until 1995, both chronologies for RD density show low variation and develop on comparable levels. From 1996 on, the variation and the differences between both chronologies increase. 1996 has been reported as a severe drought year.

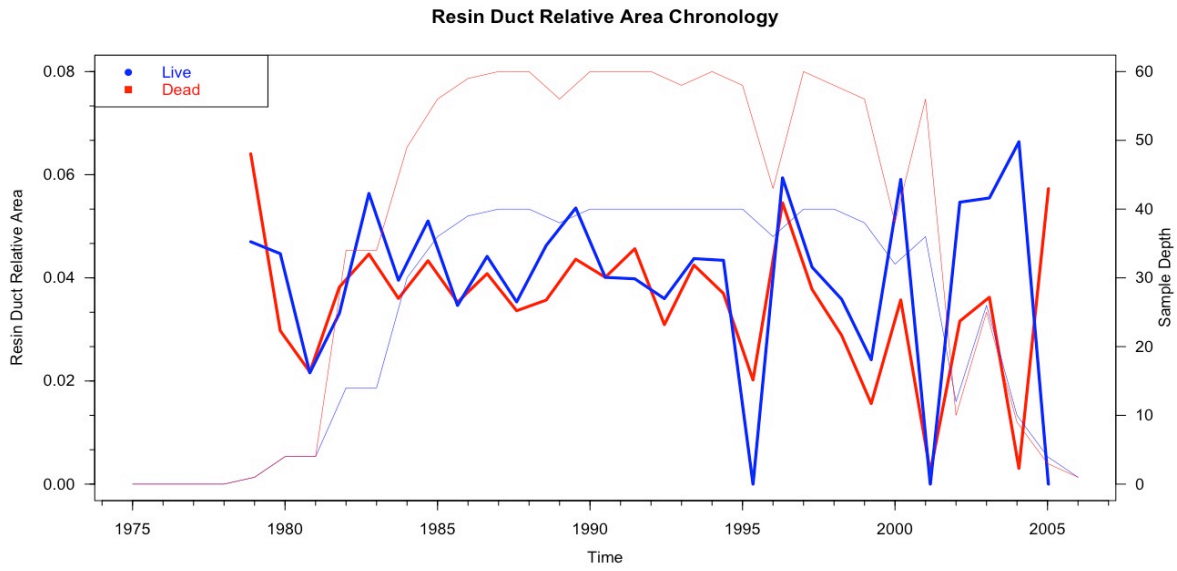

Fig. 6-11: Mean value chronologies (bold lines) and sample depth (thin lines) for RD relative area for 40 live (blue) and 60 dead (red) trees from all three sites in New Mexico, USA.

Both chronologies for RD relative area develop on comparable values and show medium variation until the drought year of 1996, after which a clearer separation with higher variation seems to occur, further emphasized by the onset of the recent drought period.

6.2 Comparisons between live and dead trees

Paired t-tests were used to statistically compare measures of defense between live and dead trees at TRP and SEV. Since BNM contains just samples from dead trees (100 % stand level mortality), no comparison between live and dead trees was possible.

6.2.1 TRP

Table 6-2: Mean 3, 5, 7, 10, 15 and 20 year resin duct measurements of 20 pairs of living and dead trees. Bold P-values represent significant differences as a result of a paired t-test.

Variables	Mean (Standard error)		P-value
	Live	Dead	
3 yr RD production (#)	2.375 (0.247)	1.517 (0.250)	**0.001**
5 yr	2.515 (0.235)	1.990 (0.238)	**0.010**
7 yr	2.757 (0.204)	2.350 (0.195)	**0.042**
10 yr	2.863 (0.216)	2.560 (0.214)	0.159
15 yr	3.258 (0.197)	3.093 (0.230)	0.452
20 yr	3.265 (0.214)	3.231 (0.246)	0.887
3 yr RD mean area (mm^2)	0.031 (0.002)	0.020 (0.001)	**< 0.001**
5 yr	0.032 (0.002)	0.022 (0.001)	**< 0.001**
7 yr	0.033(0.002)	0.023 (0.001)	**< 0.001**
10 yr	0.033 (0.001)	0.025 (0.001)	**< 0.001**
15 yr	0.034 (0.001)	0.027 (0.001)	**< 0.001**
20 yr	0.033 (0.001)	0.027 (0.001)	**0.001**
3 yr RD density (# mm^{-2})	1.866 (0.157)	1.441 (0.175)	0.102
5 yr	1.581 (0.130)	1.264 (0.096)	0.069
7 yr	1.559 (0.117)	1.325 (0.086)	0.153
10 yr	1.391 (0.089)	1.319 (0.089)	0.601
15 yr	1.431 (0.101)	1.499 (0.127)	0.674
20 yr	1.418 (0.095)	1.491 (0.104)	0.584
3 yr RD relative area (%)	5.4 (0.4)	2.7 (0.3)	**< 0.001**
5 yr	4.7 (0.3)	2.6 (0.2)	**< 0.001**
7 yr	4.8 (0.3)	3.0 (0.2)	**< 0.001**
10 yr	4.3 (0.2)	3.1 (0.2)	**< 0.001**
15 yr	4.5 (0.2)	3.9 (0.4)	**< 0.001**
20 yr	4.4 (0.2)	3.9 (0.3)	**0.001**

RD mean area and RD relative area differ highly significantly (P-value < 0.001) between live and dead trees for all growth periods. On average, live trees have 22% to 55% greater mean duct area and 13% to 100% greater relative resin duct area than dead trees, respectively. The greatest difference can be observed within and around the drought period, with decreasing, but still highly significant values further back in time. RD production differs significantly for the 3 yr, 5 yr and 7 yr growth periods with live trees having 17 to 57% more resin ducts than dead trees. No significant differences could be found for RD density.

6.2.2 SEV

Table 6-3: Mean 3, 5, 7, 10, 15 and 20 year resin duct measurements of 20 pairs of living and dead trees. Bold P-values represent significant differences as a result of a paired t-test.

Variables	Mean (Standard error)		P-value
	Live	Dead	
3 yr RD production (#)	3.212 (0.332)	1.892 (0.292)	**0.005**
5 yr	3.188 (0.340)	1.901 (0.334)	**0.011**
7 yr	2.979 (0.319)	1.951 (0.270)	**0.027**
10 yr	3.151 (0.321)	2.095 (0.262)	**0.020**
15 yr	3.314 (.306	2.290 (0.273)	**0.019**
20 yr	3.641 (0.305)	2.633 (0.271)	**0.015**
3 yr RD mean area (mm^2)	0.026 (0.001)	0.016 (0.001)	**< 0.001**
5 yr	0.026 (0.001)	0.017 (0.001)	**< 0.001**
7 yr	0.025 (0.001)	0.018 (0.001)	**< 0.001**
10 yr	0.026 (0.001)	0.019 (0.001)	**< 0.001**
15 yr	0.027 (0.001)	0.020 (0.001)	**< 0.001**
20 yr	0.028 (0.001)	0.022 (0.001)	**0.002**
3 yr RD density (# mm^{-2})	2.047 (0.182)	2.836 (0.497)	0.122
5 yr	1.952 (0.144)	2.504 (0.329)	0.159
7 yr	1.773 (0.100)	2.402 (0.274)	0.059
10 yr	1.795 (0.105)	2.286 (0.238)	0.094
15 yr	1.697 (0.096)	2.055 (0.194)	0.148
20 yr	1.620 (0.082)	1.917 (0.158)	0.143
3 yr RD relative area (%)	5.1 (0.4)	3.8 (0.5)	**0.038**
5 yr	4.9 (0.3)	3.7 (0.4)	**0.040**
7 yr	4.4 (0.2)	3.8 (0.3)	0.201
10 yr	4.5 (0.2)	3.8 (0.3)	0.144
15 yr	4.4 (0.2)	3.8 (0.3)	0.139
20 yr	4.3 (0.2)	3.8 (0.2)	0.162

RD mean area differs highly significantly between live and dead trees for all growth periods. On average, live trees have 27 to 63% greater mean duct area than dead trees. The greatest difference can be observed within and around the drought period, with decreasing, but still highly significant values further back in time. RD production differs significantly for all growth periods with live trees having 38% to 70% more resin ducts. RD relative area differs significantly for the 3 yr and 5 yr growth periods with live trees having 32% (in the 3 yr growth period) and 34% (in the 5 yr growth period) greater relative resin duct area. No significant differences could be found for RD density.

6.3 Prediction of tree mortality

A series of single variable logistic regression models was fitted using the combined samples from TRP and SEV (n = 80 trees with 40 living and 40 dead), the samples from TRP (n = 40 trees with 20 living and 20 dead) and the samples from SEV (n = 40 trees with 20 living and 20 dead), respectively. Since BNM contains just samples from dead trees, these data were not used for model fitting. The following internal validations were performed with random samples containing 60% of the trees and were then applied to the remaining 40% of the trees to predict the status. 200 re-samplings were used to perform this cross-validation.

6.3.1 Internal validation: TRP and SEV combined

For TRP and SEV, Table 6-4 shows the single variable models with the lowest 5 AIC values; full results are provided in the appendix. AuROC is the area under receiver operating curve, CCR_d is

the correct classification rate for dead trees, CCR_L is the correct classification rate for live trees and CCR_{all} is the combined correct classification rate.

Table 6-4: TRP- and SEV-values as a result of logistic regression, using the mean 3, 5, 7, 10, 15 and 20 year resin duct measurements of 40 pairs of living and dead trees. Only the models with the 5 lowest AIC-values are shown.

Models	Values					
	AIC	AuROC	P-value	CCR_d	CCR_L	CCR_{all}
3 yr RD mean area	70.94	0.88	< 0.001	0.77	0.76	0.76
5 yr	77.95	0.86	< 0.001	0.78	0.73	0.76
7 yr	82.91	0.83	< 0.001	0.71	0.70	0.71
10 yr	89.36	0.80	< 0.001	0.69	0.66	0.68
15 yr	91.74	0.78	< 0.001	0.67	0.65	0.66

Single variable models with the lowest 5 AIC values contain only RD mean area variables and 3, 5, 7, 10 or 15 yr growth periods, respectively. 3 yr RD mean area as the best prediction model (i.e. model with the lowest AIC-value and the highest AuROC-value) misclassified 23% of the dead trees as being alive, and 24% of the living trees are predicted to be dead. Overall, 24% of the trees are misclassified.

6.3.2 Internal validation: TRP

For TRP, Table 6-5 shows the single variable models with the lowest 5 AIC values; full results are provided in the appendix.

Table 6-5: TRP-values as a result of logistic regression, using the mean 3, 5, 7, 10, 15 and 20 year resin duct measurements of 20 pairs of living and dead trees. Only the models with the 5 lowest AIC-values are shown.

Models	Values					
	AIC	AuROC	P-value	CCR_d	CCR_L	CCR_{all}
3 yr RD mean area	33.52	0.91	0.002	0.79	0.80	0.79
5 yr RD relative area	35.36	0.90	< 0.001	0.83	0.81	0.82
5 yr RD mean area	36.09	0.90	0.001	0.79	0.74	0.77
7 yr RD relative area	36.24	0.90	< 0.001	0.79	0.75	0.77
7 yr RD mean area	36.77	0.89	0.001	0.77	0.77	0.77

Single variable models with the lowest 5 AIC values contain RD mean area or RD relative area variables and 3, 5, or 7 yr growth periods, respectively. 3 yr RD mean area as the best prediction model (i.e. model with the lowest AIC-value and the highest AuROC-value) misclassified 21% of the dead trees as being alive, and 20% of the living trees are predicted to be dead. Overall, 21% of the trees are misclassified. Despite 3 yr RD mean area is the model with the lowest AIC-value and the highest AuROC-value, the model 5 yr RD relative area shows the best classification rate for dead, live and combined trees.

6.3.3 Internal validation: SEV

Table 6-6 shows the single variable models with the lowest 5 AIC values; full results are provided in the appendix.

Table 6-6: Values as a result of logistic regression, using the mean 3, 5, 7, 10, 15 and 20 year resin duct measurements of 20 pairs of living and dead trees. Only the models with the 5 lowest AIC-values are shown.

Models	Values					
	AIC	AuROC	P-value	CCR_d	CCR_L	CCR_{all}
3 yr RD mean area	35.87	0.90	0.002	0.80	0.78	0.79
5 yr	38.78	0.89	0.003	0.81	0.77	0.79
7 yr	39.45	0.87	0.003	0.77	0.72	0.75
15 yr	39.61	0.87	0.003	0.80	0.75	0.77
10 yr	41.59	0.85	0.003	0.72	0.75	0.73

Single variable models with the lowest 5 AIC values contain only RD mean area variables and 3, 5, 7, 10 or 15 yr growth periods, respectively. 3 yr RD mean area as the best prediction model (i.e. model with the lowest AIC-value and the highest AuROC-value) misclassified 20% of the dead trees as being alive, and 22% of the living trees are predicted to be dead. Overall, 21% of the trees are misclassified.

6.3.4 External validation

The following three external validations were performed to assess the extent to which the models can be generalized to Pinyon pine populations under drought and insect pressure across New Mexico.

6.3.4.1 Combined samples from TRP and SEV against BNM

Two single variable models (3 yr RD mean area and 5 yr RD mean area, i.e. models with the two lowest AIC-values) for the combined samples from TRP and SEV are fitted, using the full data set and testing this model against the data set from BNM. BNM contains just samples from dead trees and these data have not been used for the prior model fitting.

Table 6-7: Classification table as a result of external validation with 3 yr RD mean area and 5 yr RD mean area as independent variables with data from BNM (n = 20).

Model 3 yr RD mean area, BNM	Predicted (Dead)	Predicted (Live)
Observed (Dead)	13	7
Observed (Live)	0	0
Model 5 yr RD mean area, BNM		
Observed (Dead)	9	11
Observed (Live)	0	0

While the model 3 yr RD mean area misclassified 35% of the dead trees as being alive, the model 5 yr RD mean area misclassified 55% dead trees.

6.3.4.2 TRP against SEV and BNM

Two single variable models (3 yr RD mean area and 5 yr RD relative area, i.e. models with the two lowest AIC-values) for TRP are fitted, using the full data set and testing this model against the data sets from SEV and BNM. This validation has been performed to reveal possible site differences.

Table 6-8: Classification table as a result of external validation with 3 yr RD mean area as independent variable with data from SEV (n = 38) and BNM (n = 20).

Model 3 yr RD mean area, SEV	Predicted (Dead)	Predicted (Live)
Observed (Dead)	16	2
Observed (Live)	8	12
Model 3 yr RD mean area, BNM		
Observed (Dead)	15	5
Observed (Live)	0	0

For SEV, the model 3 yr RD mean area misclassified 11% of the dead trees as being alive, and 40% of the living trees are predicted to be dead. Overall, 26% of the trees are misclassified. Two trees from the sampled twenty dead trees showed no resin ducts within the last three growth years, hence no 3 yr mean values could be computed for these trees. For BNM, the model misclassified 25% of the dead trees.

Table 6-9: Classification table as a result of external validation with 5 yr relative RD area as independent variable with data from SEV (n = 40) and BNM (n = 20).

Model 5 yr relative RD area, SEV	Predicted (Dead)	Predicted (Live)
Observed (Dead)	11	9
Observed (Live)	2	18
Model 5 yr relative RD area, BNM		
Observed (Dead)	4	16
Observed (Live)	0	0

For SEV, the model 5 yr RD mean area misclassified 45% of the dead trees as being alive, and 10% of the living trees are predicted to be dead. Overall, 28% of the trees are misclassified. For BNM, the model misclassified 80% of the dead trees.

6.3.4.3 SEV against TRP and BNM

Two single variable models (3 yr RD mean area and 5 yr RD mean area, i.e. models with the two lowest AIC-values) for SEV are fitted, using the full data set and testing this model against the data sets from TRP and BNM. This validation has been performed to reveal possible site differences.

Table 6-10: Classification table as a result of external validation with 3 yr RD mean area as independent variable with data from TRP (n = 40) and BNM (n = 20).

Model 3 yr RD mean area, TRP	Predicted (Dead)	Predicted (Live)
Observed (Dead)	10	10
Observed (Live)	0	20
Model 3 yr RD mean area, BNM		
Observed (Dead)	11	9
Observed (Live)	0	0

For TRP, the model 3 yr RD mean area misclassified 50% of the dead trees as being alive, and 0% of the living trees are predicted to be dead. Overall, 25% of the trees are misclassified. For BNM, the model misclassified 45% of the dead trees.

Table 6-11: Classification table as a result of external validation with 5 yr RD mean area as independent variable with data from TRP (n = 40) and BNM (n = 20).

Model 5 yr RD mean area, TRP	Predicted (Dead)	Predicted (Live)
Observed (Dead)	10	10
Observed (Live)	0	20
Model 5 yr RD mean area, BNM		
Observed (Dead)	9	11
Observed (Live)	0	0

For TRP, the model 5 yr RD mean area misclassified 50% of the dead trees as being alive, and 0% of the living trees are predicted to be dead. Overall, 25% of the trees are misclassified. For BNM, the model misclassified 55% of the dead trees.

7 Discussion

7.1 Relationships

Among defense

The negative correlation between RD mean area and RD density may be based on a defense-internal trade-off reflecting different strategies in allocation of carbon to defense: The amount of carbon which can be allocated to defense is limited, the tree has to choose whether to produce a denser network with smaller resin ducts or larger, but less denser resin ducts. The comparisons between live and dead Pinyon pine trees show that RD mean area differs highly significantly, but no significant differences could be found for RD density. Thus, forming larger resin ducts is a more important and perhaps more energy efficient investment into defensive structure than forming a higher density of resin ducts.

The reason for the positive correlation between RD density and RD relative area is obvious: The more resin ducts occur per square millimeter, the greater is the relative area occupied by these ducts within the increment section.

Tree size and defense

For all samples from all three sites, the scatterplot for the resin duct variable RD mean area and the tree size variable diameter at breast height DBH (Fig. 6-4) shows a strongly significant positive correlation, explaining 55% of the variation. The positive correlation could be found at all three sites; additionally, t-tests for RD mean area and DBH showed significant differences between trees at TRP and trees at SEV. These results may suggest at least a partial scaling effect: The different site qualities provide different growth limitations for the trees, meaning that potential tree height and DBH are different among sites, thus resulting in the possibility that bigger trees can host bigger resin ducts. However, genetic differences between the populations could also influence the values of RD mean area; further investigations are needed to examine the possible scaling effect and to find a comprehensive, causal explanation that clearly distinguishes between size-dependent scaling and site- and population-dependent genetic effects.

Growth and defense

Several authors have postulated a trade-off between growth and defense as a part of the secondary metabolism (Herms and Mattson, 1992; Lorio, 1986), because trees have limited resources and all requirements cannot be met simultaneously. Such a trade-off should be reflected in negative correlations between the growth measure RIA and the corresponding resin duct measures. Results in Fig. 6-5 to Fig. 6-7 show mixed relationships and in general no strong indications for large trade-offs. Inconsistent relationships between growth and defense can be found in much literature and could be partly caused by differences in measurement methods (Kane and Kolb, 2010) and the physical connection of xylem and xylem housed resin ducts. Xylem growth and the vertical resin ducts formed cambially by schizogeny in the xylem must be connected physically. This connection may contribute to the inconsistent relationships and to the absence of clearly visible trade-offs. Furthermore, the mixed relationships could reflect different climatic and genetic factors among sites.

The strongly significant positive relationship between RD production as an absolute count and growth seems to be intuitively understandable, meaning that in years of higher radial growth more xylem is available to house vertical resin ducts. However, results of other studies are equivocal: While positive relationships were found for Ponderosa pine (Kane and Kolb, 2010), Aleppo pine (Fahn and Zamski, 1970), Scots pine (Stephan, 1967) and Slash pine (Mergen and Echols, 1955), no significant relationship has been reported for Norway spruce (Wimmer and Grabner, 1997) and negative relationships for Scots pine (Wimmer and Halbwachs, 1992),

Lodgepole pine (Reid and Watson, 1966) and Canadian spruce (Thomson and Sifton, 1925). These mixed results reflect differences among tree families/genera and further genetic and environmental influences.

The weaker, but still significant positive relationship between RD mean area and growth may be partially influenced by the physical connection of xylem and xylem-housed resin ducts mentioned above, meaning that larger xylem areas could house larger resin ducts. The scatterplot also shows that the correlation was much stronger for live trees than for dead trees, suggesting different allocation strategies: Live trees allocated more resources to resin duct size even as growth increases, pointing to the importance of partitioning resources to resin defense.

The only negative correlation could be found between growth and RD density. Despite constituting weak evidence for trade-offs, the negative correlation could be caused by the existence of a threshold: The tree builds xylem and xylem housed defense up to a certain ratio, after which and despite further radial growth no more carbon would be allocated to defense. Negative correlations between ring width and RD density have also been reported for Scots pine (Wimmer, 1991; Stephan, 1967).

Temporal variations

The resin duct properties generally show moderate to high variation over time, suggesting climatic triggering or limiting of vertical resin duct formation and indicating that resin duct variables could be used as dendroclimatological variables. Several authors have investigated vertical resin duct relationships with climate: For resin duct density of Norway spruce, Wimmer and Grabner (1997) found significant positive response to above-normal summer temperatures and negative response for above-normal summer precipitation. Ruden (1987) found resin duct frequency of Scots pines to be highly correlated with summer drought, and thus he used this frequency and ring widths of Scots pines to reconstruct climate. Reid and Watson (1966) suggested evidence for a direct relation between high summer temperatures and large resin duct production of Lodgepole pine.

The chronologies of RD production, RD density and RD relative area show common patterns, suggesting relationships with climate: Decreasing values in 1996, which has been reported as a severe drought year, and diverging values for the live and dead trees within the four consecutive driest years (2000 – 2003) of the recent drought. The chronology of RD mean area shows moderate variation, and it is the only chronology that consistently shows different values between live and dead trees over the whole measured time period. This could indicate a potential genetic effect and, by clearly showing different carbon allocation to defense between live and dead trees, thus rendering *RD mean area as a key defense variable*. However, due to the relatively short time period of the chronology, one cannot say if these patterns are based on genetic differences or if past environmental effects have led to these observed different values.

More detailed studies of the temporal variation of resin duct variables and further investigations of the relationships with climate were beyond the scope of this master thesis. However, insights about triggering and limiting environmental factors and about genetic influence is important to understand tree physiological processes and related mechanisms of mortality. Longer time series of more samples, encompassing the recent severe drought and the last severe drought in the 1950s, should be subject to further investigations. An investigation of longer temporal patterns could show if resin ducts of dead trees have consistently been smaller, thus suggesting genetic effects (e.g. widespread drought-induced mortality during the drought in the 1950s acted as a genetic bottleneck) or if an inciting external factor (e.g. severe drought period in the 1950s as an inciting differentiating environmental factor) has caused a diversion between live and dead trees and has led to development on different levels observed here.

7.2 Comparisons between live and dead trees

The results show significant differences of measures of vertical resin ducts between live and dead Pinyon pine trees, suggesting that trees that have a larger defense infrastructure in xylem are more likely to survive a severe drought and drought-induced insect pressure.

Dead trees at SEV and TRP have fewer and – strongly significant for all growth periods – smaller resin ducts than live trees. Empirical studies show a positive correlation between resin duct size or abundance and resin flow (Blanche et al., 1992; Schopmeyer et al., 1954) and according to Schopmeyer et al. (1954), the yield of resin during the first 24 hours after the wounding follows the equation

$Y = \frac{KPN\overline{(ab)^2}}{\eta}$, with K = π/8, P = resin pressure at the capillary exit point, N = number of exposed RD, a and b the mean elliptical axes and η = viscosity of resin. An increase in the mean duct area and more ducts would therefore positively affect the equation-variables N, a and b and lead to an overproportional increase in resin yield. This would result in a greater capacity to produce, store and transport resin and would help the tree to repel, entrap, flush or kill the invading bark beetles. Considering the large differences between live and dead trees with 22 to 63% greater and 17 to 70% more vertical resin ducts, respectively, the superior supply of resin, acquired by allocating more carbon to resin defense, could have helped the live trees to survive. The significant differences between live and dead trees for RD relative area suggest that trees containing a greater relative area of xylem composed of resin ducts were more likely to survive.

The greatest differentiations of significant values between live and dead trees can be found within and closely around the main drought and insect attack period, with decreasing, but still strongly significant values further back in time (Table 6-2 and Table 6-3). A possible interpretation of these results could be a genetic-based adjustment of carbon partitioning towards defense, triggered by drought stress.

RD density of the sampled Pinyon pine trees is roughly a factor 2.5 higher than reported density for Ponderosa pine in Northern Arizona (Kane and Kolb, 2010), but contrary to their findings, no significant differences of RD density between live and dead Pinyon pine trees could be found at both sites. While Kane and Kolb (2010) reported that the coupled effect of greater resin duct width and denser resin duct network should result in a twofold increase in resin delivery, helping the trees to survive the drought and bark beetle attack, greater resin duct density is not a significant issue for Pinyon. Without further tree physiological studies, which clearly exceed the possibilities of this master study, interpretations are speculative: Different defense strategies and different growth-defense relationships of these two pine species may exist and affect resin duct density. Possible explanations could also be found in potentially different wood anatomy of conifers and even of pines (e.g. two-dimensional resin duct network on different radial planes versus three-dimensionally connected networks), in different forms of communication among vertical resin ducts and between vertical and radial resin ducts and/or in different forms of activating the enhanced resin flow (e.g. production of more resin within the constitutive resin ducts, resin translocation within the tree and formation of traumatic resin ducts).

This study revealed that trees having more and larger vertical resin ducts are more likely to survive a severe drought and subsequent bark beetle attack. The following four explanations are conceivable:

1) Live trees allocated relatively more carbon to resin defense than trees that died. Triggered by drought stress, this adjustment of carbon partitioning could be based on genetics.

2) Live trees had overall more carbon at disposal than dead trees, allowing them to maintain essential metabolic processes *and* to invest (more) carbon in defense. The pairwise selection of the sampled trees tried to control for similar conditions (e.g. water and nitrogen availability,

soil, aspect, slope). However, in sparse stands of Pinyon trees with scattered distribution and highly variable micro topography, a pairwise selection based on close location and similar size was not always precisely possible and the sample protocol had to be followed as good as possible. Thus, the possibility exists that live trees had more favorable microsites, which enabled them to build up a larger absolute carbon pool and to allocate more carbon to defense than dead trees. The key resources in the study regions are water/soil moisture and/or nitrogen. Whether live trees had more water/soil moisture and could therefore build up a larger carbon pool could be analyzed by isotope analysis ($\delta^{13}C$). Values of $\delta^{13}C$ are correlated with soil moisture and could thus be used as a reliable indicator of past water availability on a year-to-year basis (Saurer et al., 1995; Dupouey et al., 1993). The fact that a comparable study with similar pairwise selection of Ponderosa pines (Kane and Kolb, 2010) has found significant differences in the defense infrastructure of live and dead trees, does not support this possible limitation.

3) As a combination of the first two points, live trees had overall more carbon at disposal *and* allocated relatively more carbon two defense.

4) Structural defense elements like vertical resin ducts could not be suitable proxies for the effectiveness of the defensive system of the Pinyon pine trees. Measures of effective resin flow (constitutive and induced) are not encompassed in this study, although empirical studies show a positive correlation between resin flow and size or abundance of vertical resin ducts (Blanche et al., 1992; Schopmeyer et al., 1954). Although they are a major part of the defensive system of pines, vertical resin ducts represent only one structural part of this system. The here conducted measurements of the vertical resin ducts included the whole structure of the resin ducts, encompassing the lumen, the resin secreting epithelial cells, the sheath cells and the surrounding adjacent parenchymal cells. Relations and correlation of these "outer" parts of a resin duct to its lumen and thus its resin flow are not known to the author of this master thesis.

Thus, possible genetic differences, differences of the carbon pools between live and dead trees and investigations of the relationships between constitutive and induced resin flow and size, abundance and structural elements of vertical resin ducts should be subject to further studies.

7.3 Prediction of tree mortality

Overall, the top models performed well in predicting tree mortality and included the resin duct variables RD mean area and RD relative area. The correct classification rates of dead trees (CCR_d) of the entire models ranged from 83% to 67%, the correct classification rates of live trees (CCR_L) from 81% to 65% and the correct classification rates of live and dead trees (CCR_{all}) from 82% to 66%. These results show the importance of measures and patterns of resin defense structures in predicting tree mortality following severe drought and drought-amplified insect pressure. The results also support the findings of Kane and Kolb (2010) about the importance of resin ducts in reducing Ponderosa pine mortality during drought and bark beetle attack. Compared to their multivariable resin duct models for Ponderosa pine, internal validation of the top two single variable models showed just slightly lower correct classification rates.

Single variable models for the combined samples from *TRP and SEV* included 3yr, 5yr, 7 yr, 10 yr and 15 yr RD mean area, respectively. CCR_d ranged from 78% to 67% and CCR_L from 76% to 65%.

Single variable models for *TRP* included 3 yr, 5yr, 7yr RD mean area and 5yr and 7yr RD relative area, respectively. CCR_d ranged from 83% to 77% and CCR_L from 81% to 74%. At this site, growth rate variables were not good predictors of tree mortality (Macalady and Bugmann, in prep.). TRP showed a very high stand level mortality of 75% and an assumably high insect pressure, all sampled dead trees died between 2001 and 2005. This may suggest that drought-induced mortality processes have developed too fast to be reflected in long-term low growth rates. Under these conditions, trees that allocated more carbon to defense were more likely to survive

and under drought and drought-associated insect pressure measures of defense are more important predictors of tree mortality than measures of growth.

Single variable models for *SEV* included 3yr, 5yr, 7 yr, 10 yr and 15 yr RD mean area, respectively. CCR_d ranged from 81% to 72% and CCR_L from 78% to 72%. At this site and contrary to TRP, growth rate variables (Macalady and Bugmann, in prep.) were good predictors of tree mortality, obtaining similar correct classification rates. With 26%, SEV showed a considerably lower stand level mortality than TRP. All sampled dead trees died between 1997 and 2005, showing longer die back-processes than those at TRP with assumed lower insect pressure. Following the decline disease theory (Mannion, 1981), one might argue that longer term predisposing factors (e.g. general aridity, stand competition) and inciting factors (e.g. drought onset) may have been more present at this site, resulting in the observed different growth rates between live and dead trees. Contributing factors (e.g. persistent severe drought and drought-associated insect attacks) might then ultimately have killed stressed trees that had low carbon allocation to defense. However, the results from SEV confirm those of TRP in highlighting the role of defense variables as predictors of tree mortality under external stress factors related to defense, both in the presence and in the absence of growth-mortality relationships.

The *first external validation* with the combined samples from TRP and SEV against the data set from BNM shows an underprediction of mortality for both models. The *second external validation* with samples from TRP against the data set from SEV shows an overprediction of mortality for the model with the variable 3 yr RD mean area and an underprediction of mortality for the model with the variable 5 yr RD relative area. Against BNM, the models show an adequate prediction of mortality (3 yr RD mean area) and an underprediction of mortality (5 yr RD relative area), respectively. The *third external validation* with samples from SEV against the data sets from TRP and BNM shows an underprediction of mortality for both models. These results mean that the established single variable models can currently not be generalized to Pinyon pine populations under drought and insect pressure across New Mexico, USA. Two main explanations may be underlying these results:

1) The already mentioned tree size-dependent scaling effect (positive correlation between RD mean area and DBH) and/or genetic variation between Pinyon pine populations could lead to different value levels of resin duct variables per site and thus to different thresholds between live and dead trees in the site specific logistic regression models. Table 7-1 shows the values of the relevant resin duct variables per site:

Table 7-1: Comparison of the values of the resin duct variables that are used for the external validation.

Variables	Mean values (Standard error) per sites				
	TRP live	TRP dead	SEV live	SEV dead	BNM dead
3 yr RD mean area (mm^2)	0.031 (0.002)	0.020 (0.001)	0.026 (0.001)	0.016 (0.001)	0.020 (0.002)
5 yr RD mean area (mm^2)	0.032 (0.002)	0.022 (0.001)	0.026 (0.001)	0.017 (0.001)	0.022 (0.001)
5 yr RD relative area (%)	4.7 (0.3)	2.6 (0.2)	4.9 (0.3)	3.7 (0.4)	4.2 (0.3)

Compared to *SEV*, 3 yr RD mean area at TRP is 19% higher for live trees and 25% higher for dead trees. Additionally performed t-tests showed that these differences are statistically significant. Applying this higher TRP-threshold to the lower threshold at SEV leads to the observed overprediction of mortality. Because the 5 yr RD relative area is 4% lower for live trees and 30% lower for dead trees, the application of this lower TRP-threshold to the higher threshold at SEV leads to the observed underprediction of mortality. Compared to *TRP*, both values of the 3 yr RD mean area and the 5 yr RD mean area are considerably lower at SEV, leading to the observed underprediction of mortality. For the models with the combined samples from TRP and SEV, the combined effects lead to the observed underprediction of mortality.

2) The ultimate drivers of mortality could be the drought-amplified bark beetle pressure, which differs between the sites. A high level of beetle pressure would lead to a low model-threshold whether a tree will die or live. Application of this threshold to a site with lower beetle pressure and thus higher mortality threshold in the model could lead to an overprediction of mortality. At TRP, the stand level mortality of 75% was considerably higher than the stand level mortality of 26% at SEV. The above mentioned overprediction of mortality can be observed for the TRP-model with the variable 3 yr RD mean area, but not for the model with the variable 5 yr RD relative area. Vice versa, an underprediction of mortality can be observed for both SEV-models.

Two measures are suggested to improve the prediction power of the established models:

1) Different value levels of resin duct variables could be tackled by establishing standardized indices (e.g. obtained by dividing the values for RD mean area by DBH or by a mean value).
2) Combining single resin duct variables into multivariable defense models.

In a further step, resin duct and growth variables could be combined into multivariable growth-defense models, leading to more encompassing and thus to more accurate prediction models of tree mortality under drought.

8 Conclusions and suggestions for future research

While investigating relationships showed no strong indicators for large trade-offs between growth and defense, some evidence could be found for defense-internal trade-offs: Trees have to choose whether to produce denser networks with smaller resin ducts or larger, but less denser ducts. Relationships between resin ducts and tree size (DBH) suggest a possible tree size-dependent scaling effect: Bigger trees can host bigger resin ducts. However, genetic variation between the Pinyon pine stands may also influence the values of the resin duct variables and testing of genetic effects should be subject to further studies. Over time, mean values of resin duct variables show moderate to high variation, indicating considerable climatic influence on resin duct formation and that resin duct variables could be used as dendroclimatological variables. Chronologies of RD mean area show limited variation and dead trees with constant lower values over time, proposing the size of resin ducts with its assumed positive correlation to resin flow as a crucial factor for resin-based defense of Pinyon and indicating possible genetic variation.

The comparison of measures of vertical resin ducts between live and dead trees from two sites in New Mexico (USA) showed significant differences. Live trees have more and larger resin ducts than trees that died, thus trees with a larger defense infrastructure in xylem are more likely to survive drought-induced bark beetle attacks. This different carbon allocation to xylem resin ducts within Pinyon pine stands suggests decisive genetic variation and therefore a possible future selection of trees that invest more in defense. Defense mechanisms of conifers have a heritable component and genetic resistance can work through diverse mechanisms, e.g. modifications in chemical pathways inducing qualitative and quantitative change in defense chemicals (Franceschi et al., 2005, and references therein). Genetic variation has been reported for conifers (Rosner and Hannrup, 2004; Strom et al., 2002; Rehfeldt 1993) and findings that *Pinus* is among conifers the least sensitive genus to external effects (Evert, 2006) supports the suggestion of genetic differences.

McDowell et al. (2008) propose a very useful general framework for three main mechanisms of tree mortality under drought, where carbon starvation or failure of the carbon metabolism and translocation (Sala et al. 2010) weaken the trees, resulting in an inability to maintain structural and chemical defenses against attacks from biotic agents. Results of this master thesis cannot answer the question whether the above mentioned mortality mechanisms were relevant mechanisms of Pinyon pine mortality at the study sites. However, interpretations of the findings indicate that general expressions for mechanisms of tree mortality like “weakening of trees” or “inability to maintain defense” are too broad and that the exact ecological and tree physiological mechanisms and processes leading to tree mortality have not yet been fully revealed. The idea of water-stressed and starving trees at the investigated study sites, as a consequence of the severe and prolonged drought in the early 2000s, is intuitively understandable. But some of the drought-stressed trees died and some survived, and the results suggest that carbon allocation to xylem-based resin defense - triggered by drought stress and genetically adjusted - is an important determinant of tree mortality. Existing mechanisms of tree mortality under drought should be complemented and enhanced by the role of tree defense structures and processes, and more attention needs to be paid to the underlying ecological and tree physiological processes.

Resin duct variables are good predictors for tree mortality under drought and insect pressure, both in the presence and in the absence of growth-mortality relationships and could constitute valuable variables for modeling future vegetation dynamics. The top single variable models performed well in predicting tree mortality, obtaining correct classification rates for dead trees between 83% and 67%. However, external validations have shown that the established single variable models can currently not be generalized to Pinyon pine populations across New Mexico, USA, and thus need further improvement.

Suggestions for future research

The research conducted within this master thesis could be continued, enhanced and improved as follows:

- To improve the prediction power of the models standardized indices for the key resin duct variable RD mean area could be established. Further on, single variable models could be combined into multivariable defense models.
- The established measurements of twenty live and dead trees per site covered a time period of roughly 20 years. Based on existing samples, the sample depth could be enlarged and the measurements could be extended beyond the severe drought in the 1950s. Investigating these longer chronologies could reveal important temporal patterns and environmental and genetic influence.
- The above-mentioned longer chronologies could be combined with the outcomes of the already planned carbon isotope analysis and a possible analysis of carbon reserves in the stem. Results could reveal how climate variability triggers mortality waves and could clarify the role of physiological drivers and climatic thresholds of drought-induced tree mortality.
- For their investigation of growth-mortality relationships in space and time, Macalady and Bugmann (in prep.) sampled pairs of live and dead Pinyon pine trees both from the recent severe drought period (these samples were now partly reused to conduct this master thesis) and from the severe drought in the 1950s. With the latter samples, a similar study as presented with this master thesis could reveal if the observed differences of resin duct variables between live and dead trees also exist for the drought in the 1950s and if and what differences of the resin-based defensive system exist between these two droughts, which significantly differed in terms of temperature. Additionally, the established prediction models of tree mortality based on growth variables could be combined with the prediction models based on resin duct variables into multivariable growth-defense models.

Future research and experiments in the field of drought-induced mortality of Pinyon pine trees in the southwestern USA should, among others, address the following issues:

- Measures of effective constitutive and induced resin flow should be combined with different kind of structural measures of vertical resin ducts. This could confirm the assumed positive correlation between resin flow and size or abundance of vertical resin ducts and thus elucidate the use of vertical resin ducts as a proxy for the capacity of the defensive system.
- Which are the true causal mechanisms of Pinyon pine mortality under drought and insect pressure? Within that, examples of more specific questions could be:

  Do carbon starvation and/or carbon transport failure occur?

  Is hydraulic failure a relevant mechanism of mortality?

  Is amplified dynamics of bark beetle populations a relevant mechanism of mortality? What is the role of the beetle associated fungi?

  How are environmental and/or genetic effects influencing tree physiological processes (e.g. carbon investment in defensive structures) and how are they related to mechanisms of mortality?

  How are the mortality mechanisms interrelated?

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11 Appendix

11.1 Appendix 1: Logistic regression values for TRP and SEV combined

$`prod3	`								
	Est1	p1	AIC1	AuROC	Ss	Sp	CCR	kappa	ModSig
	0.6800	0.0012	101.4	0.7422	0.7160	0.6265	0.6711	0.3424	0.0001
	Th								
	0.4866								
$`prod5	`								
	Est1	p1	AIC1	AuROC	Ss	Sp	CCR	kappa	ModSig
	0.5400	0.0050	105.7	0.7206	0.6440	0.6639	0.6539	0.3079	0.0013
	Th								
	0.4653								
$`prod7	`								
	Est1	p1	AIC1	AuROC	Ss	Sp	CCR	kappa	ModSig
	0.6030	0.0090	106.8	0.6884	0.6524	0.6488	0.6506	0.3013	0.0025
	Th								
	0.4816								
$`prod10	`								
	Est1	p1	AIC1	AuROC	Ss	Sp	CCR	kappa	ModSig
	0.5390	0.0143	108.0	0.6859	0.6388	0.6189	0.6288	0.2576	0.0047
	Th								
	0.4791								
$`prod15	`								
	Est1	p1	AIC1	AuROC	Ss	Sp	CCR	kappa	ModSig
	0.4550	0.0296	109.7	0.6631	0.6123	0.5927	0.6025	0.2050	0.0128
	Th								
	0.4915								
$`prod20	`								
	Est1	p1	AIC1	AuROC	Ss	Sp	CCR	kappa	ModSig
	0.3870	0.0570	111.0	0.6441	0.5614	0.6008	0.5809	0.1622	0.0284
	Th								
	0.4869								
$`relarea3	`								
	Est1	p1	AIC1	AuROC	Ss	Sp	CCR	kappa	ModSig
	58.1000	0.0002	94.4	0.8009	0.7296	0.7099	0.7197	0.4395	0.0000
	Th								
	0.4865								
$`relarea5	`								
	Est1	p1	AIC1	AuROC	Ss	Sp	CCR	kappa	ModSig
	83.2000	0.0001	91.8	0.8200	0.7498	0.7454	0.7477	0.4953	0.0000
	Th								
	0.5027								
$`relarea7	`								
	Est1	p1	AIC1	AuROC	Ss	Sp	CCR	kappa	ModSig

	84.1000	0.0002	96.7	0.7763	0.6862	0.7050	0.6955	0.3911	0.0000
	Th								
	0.5007								
$`relarea10	`								
	Est1	p1	AIC1	AuROC	Ss	Sp	CCR	kappa	ModSig
	82.8000	0.0007	100.4	0.7606	0.6733	0.6679	0.6706	0.3412	0.0001
	Th								
	0.4780								
$`relarea15	`								
	Est1	p1	AIC1	AuROC	Ss	Sp	CCR	kappa	ModSig
	43.6000	0.0448	110.1	0.7119	0.5825	0.6493	0.6153	0.2315	0.0164
	Th								
	0.4820								
$`relarea20	`								
	Est1	p1	AIC1	AuROC	Ss	Sp	CCR	kappa	ModSig
	49.5000	0.0451	110.3	0.6981	0.5520	0.6277	0.5897	0.1796	0.0186
	Th								
	0.4763								
$`den3	`								
	Est1	p1	AIC1	AuROC	Ss	Sp	CCR	kappa	ModSig
	-0.0996	0.5530	114.5	0.4419	0.4496	0.4100	0.4302	-0.1404	0.5557
	Th								
	0.5088								
$`den5	`								
	Est1	p1	AIC1	AuROC	Ss	Sp	CCR	kappa	ModSig
	-0.1250	0.5910	114.6	0.4525	0.4396	0.4114	0.4256	-0.1490	0.6390
	Th								
	0.5079								
$`den7	`								
	Est1	p1	AIC1	AuROC	Ss	Sp	CCR	kappa	ModSig
	-0.3050	0.2860	113.7	0.4806	0.4777	0.4103	0.4438	-0.1120	0.2021
	Th								
	0.5130								
$`den10	`								
	Est1	p1	AIC1	AuROC	Ss	Sp	CCR	kappa	ModSig
	-0.3990	0.2120	113.3	0.5031	0.4980	0.4179	0.4578	-0.0841	0.1369
	Th								
	0.5189								
$`den15	`								
	Est1	p1	AIC1	AuROC	Ss	Sp	CCR	kappa	ModSig
	-0.5450	0.1440	112.6	0.5394	0.5381	0.4119	0.4748	-0.0500	0.0851
	Th								

```
                0.5264

$`den20         .
                Est1      p1      AIC1    AuROC   Ss      Sp      CCR     kappa   ModSig
                -0.6890   0.1250  112.4   0.5606  0.5518  0.4455  0.4994  -0.0027 0.0723
                Th
                0.5271

$`mda3          .
                Est1      p1      AIC1    AuROC   Ss      Sp      CCR     kappa   ModSig
                281.0000  0.0000  70.9    0.8839  0.7741  0.7558  0.7647  0.5294  0.0000
                Th
                0.5036

$`mda5          .
                Est1      p1      AIC1    AuROC   Ss      Sp      CCR     kappa   ModSig
                263.0000  0.0000  78.0    0.8644  0.7819  0.7309  0.7564  0.5128  0.0000
                Th
                0.4680

$`mda7          .
                Est1      p1      AIC1    AuROC   Ss      Sp      CCR     kappa   ModSig
                233.0000  0.0000  82.9    0.8319  0.7103  0.7034  0.7069  0.4137  0.0000
                Th
                0.4579

$`mda10         .
                Est1      p1      AIC1    AuROC   Ss      Sp      CCR     kappa   ModSig
                202.0000  0.0000  89.4    0.7991  0.6910  0.6648  0.6781  0.3558  0.0000
                Th
                0.4988

$`mda15         .
                Est1      p1      AIC1    AuROC   Ss      Sp      CCR     kappa   ModSig
                202.0000  0.0001  91.7    0.7844  0.6654  0.6505  0.6578  0.3157  0.0000
                Th
                0.4675

$`mda20         .
                Est1      p1      AIC1    AuROC   Ss      Sp      CCR     kappa   ModSig
                200.0000  0.0001  94.5    0.7688  0.6828  0.6189  0.6505  0.3015  0.0000
                Th
                0.5035
```

11.2 Appendix 2: Logistic regression values for TRP

```
$`prod3
  `
  Est1        p1        AIC1  AuROC  Ss     Sp     CCR    kappa
1 0.812       0.0349    53.32 0.7650 0.6611 0.6171 0.6394 0.2783
  ModSig      Th
1 0.00750032  0.4908396

$`prod5
  `
  Est1        p1        AIC1  AuROC  Ss     Sp     CCR    kappa
1 0.503       0.133     56.91 0.6938 0.5594 0.5507 0.5550 0.1100
  ModSig      Th
1 0.07037114  0.4880799

$`prod7
  `
  Est1        p1        AIC1  AuROC  Ss     Sp     CCR    kappa
1 0.535       0.159     57.32 0.6450 0.5312 0.4885 0.5097 0.0197
  ModSig      Th
1 0.09433567  0.4941275

$`prod10
  `
  Est1        p1        AIC1  AuROC  Ss     Sp     CCR    kappa
1 0.346       0.32      58.42 0.6163 0.4763 0.4830 0.4797 -0.0407
  ModSig      Th
1 0.2346211   0.4867683

$`prod15
  `
  Est1        p1        AIC1  AuROC  Ss     Sp     CCR    kappa
1 0.189       0.578     59.14 0.5750 0.4165 0.4525 0.4344 -0.1310
  ModSig      Th
1 0.6115943   0.4964796

$`prod20
  `
  Est1        p1        AIC1  AuROC  Ss     Sp     CCR    kappa
1 0.0335      0.915     59.44 0.5288 0.4236 0.4040 0.4138 -0.1724
  ModSig      Th
1 3.731593    0.4989697

$`relarea3
  `
  Est1        p1        AIC1  AuROC  Ss     Sp     CCR    kappa
1 113         0.00174   37.49 0.8975 0.7877 0.7388 0.7634 0.5267
  ModSig      Th
1 1.45E-06    0.5541974

$`relarea5
  `
  Est1        p1        AIC1  AuROC  Ss     Sp     CCR    kappa
1 153         0.000586  35.36 0.8950 0.8314 0.8065 0.8188 0.6376
  ModSig      Th
1 4.77E-07    0.4546412
```

```
$`relarea7
  `
  Est1          p1          AIC1  AuROC  Ss     Sp     CCR    kappa
1 174       0.000739  36.24  0.9025 0.7916 0.7451 0.7684 0.5368
  ModSig      Th
1 7.53E-07    0.4967324

$`relarea10
  `
  Est1          p1          AIC1  AuROC  Ss     Sp     CCR    kappa
1 196       0.0012    40.32  0.8725 0.7734 0.7430 0.7581 0.5163
  ModSig      Th
1 6.39E-06    0.4475198

$`relarea15
  `
  Est1          p1          AIC1  AuROC  Ss     Sp     CCR    kappa
1 38.9      0.209     57.4   0.7650 0.5532 0.6083 0.5803 0.1613
  ModSig      Th
1 0.10011     0.4813628

$`relarea20
  `
  Est1          p1          AIC1  AuROC  Ss     Sp     CCR    kappa
1 45.8      0.181     57.33  0.7425 0.5175 0.6197 0.5684 0.1371
  ModSig      Th
1 0.09448361  0.4725551

$`den3
  `
  Est1          p1          AIC1  AuROC  Ss     Sp     CCR    kappa
1 0.792     0.0855    56.2   0.6850 0.5551 0.5625 0.5588 0.1176
  ModSig      Th
1 0.04343776  0.4855684

$`den5
  `
  Est1          p1          AIC1  AuROC  Ss     Sp     CCR    kappa
1 1.28       0.0653    55.58  0.6425 0.4809 0.5397 0.5103 0.0206
  ModSig      Th
1 0.02920227  0.4704693

$`den7
  `
  Est1          p1          AIC1  AuROC  Ss     Sp     CCR    kappa
1 1.15       0.119     56.83  0.6300 0.4356 0.5219 0.4781 -0.0424
  ModSig      Th
1 0.0662051   0.4741764

$`den10
  `
  Est1          p1          AIC1  AuROC  Ss     Sp     CCR    kappa
1 0.474      0.563     59.11  0.5625 0.3976 0.4177 0.4078 -0.1847
  ModSig      Th
1 0.5799357   0.4902163
```

```
$`den15
  Est1      p1     AIC1  AuROC  Ss     Sp     CCR    kappa
1 -0.27     0.673  59.27 0.5150 0.3842 0.4188 0.4013 -0.1968
  ModSig    Th
1 0.856813  0.5084638

$`den20
  Est1      p1     AIC1  AuROC  Ss     Sp     CCR    kappa
1 -0.391    0.595  59.17 0.5400 0.4244 0.4148 0.4197 -0.1607
  ModSig    Th
1 0.6463777 0.5068213

$`mda3
  Est1      p1      AIC1  AuROC  Ss     Sp     CCR    kappa
1 362       0.00165 33.52 0.9075 0.7874 0.7991 0.7931 0.5862
  ModSig    Th
1 1.84E-07  0.4264547

$`mda5
  Est1      p1      AIC1  AuROC  Ss     Sp     CCR    kappa
1 350       0.00112 36.09 0.8975 0.7906 0.7404 0.7653 0.5308
  ModSig    Th
1 6.96E-07  0.5098609

$`mda7
  Est1      p1      AIC1  AuROC  Ss     Sp     CCR    kappa
1 347       0.00112 36.77 0.8925 0.7691 0.7710 0.7700 0.5399
  ModSig    Th
1 9.97E-07  0.5165822

$`mda10
  Est1      p1      AIC1  AuROC  Ss     Sp     CCR    kappa
1 299       0.00234 41.6  0.8575 0.7449 0.7653 0.7550 0.5101
  ModSig    Th
1 1.26E-05  0.4594256

$`mda15
  Est1      p1      AIC1  AuROC  Ss     Sp     CCR    kappa
1 268       0.0035  44.73 0.8275 0.6878 0.7114 0.7000 0.3993
  ModSig    Th
1 6.62E-05  0.4889414

$`mda20
  Est1      p1      AIC1  AuROC  Ss     Sp     CCR    kappa
1 243       0.0058  47.13 0.8075 0.7221 0.6701 0.6956 0.3918
  ModSig    Th
1 0.000239918 0.5158712
```

11.3 Appendix 3: Logistic regression values for SEV

```
$`prod3
  Est1     p1     AIC1    AuROC  Ss     Sp     CCR    kappa
1 0.682    0.0109 51.19   0.7388 0.6525 0.6455 0.6491 0.2980
  ModSig     Th
1 0.00223555 0.4690592

$`prod5
  Est1     p1     AIC1    AuROC  Ss     Sp     CCR    kappa
1 0.565    0.017  52.63   0.7438 0.6526 0.6695 0.6613 0.3222
  ModSig     Th
1 0.00504097 0.4492842

$`prod7
  Est1     p1     AIC1    AuROC  Ss     Sp     CCR    kappa
1 0.647    0.029  53.41   0.7213 0.7036 0.6185 0.6591 0.3204
  ModSig     Th
1 0.00792451 0.4803367

$`prod10
  Est1     p1     AIC1    AuROC  Ss     Sp     CCR    kappa
1 0.664    0.0236 53.05   0.7288 0.7064 0.6405 0.6728 0.3464
  ModSig     Th
1 0.0064183  0.4890395

$`prod15
  Est1     p1     AIC1    AuROC  Ss     Sp     CCR    kappa
1 0.652    0.0259 53.31   0.7225 0.6952 0.6581 0.6763 0.3529
  ModSig     Th
1 0.00748189 0.4754244

$`prod20
  Est1     p1     AIC1    AuROC  Ss     Sp     CCR    kappa
1 0.652    0.0274 53.41   0.7325 0.7014 0.6191 0.6603 0.3206
  ModSig     Th
1 0.00793138 0.4908735

$`relarea3
  Est1     p1     AIC1    AuROC  Ss     Sp     CCR    kappa
1 32.8     0.0597 55.39   0.7150 0.5678 0.6294 0.5988 0.1972
  ModSig     Th
1 0.02595211 0.4713419

$`relarea5
  Est1     p1     AIC1    AuROC  Ss     Sp     CCR    kappa
1 48.1     0.0416 54.32   0.7350 0.6825 0.5376 0.6091 0.2196
  ModSig     Th
1 0.01352054 0.5075808
```

```
$`relarea7   `
          Est1        p1        AIC1      AuROC  Ss     Sp     CCR    kappa
1         37.8      0.155     57.29     0.6450 0.5406 0.4972 0.5191  0.0378
          ModSig    Th
1     0.09212728    0.5028544

$`relarea10  `
          Est1        p1        AIC1      AuROC  Ss     Sp     CCR    kappa
1         44        0.104     56.57     0.6600 0.5674 0.5034 0.5350  0.0708
          ModSig    Th
1     0.05569924    0.4964359

$`relarea15  `
          Est1        p1        AIC1      AuROC  Ss     Sp     CCR    kappa
1         47.7      0.109     56.65     0.6625 0.5263 0.5860 0.5559  0.1123
          ModSig    Th
1     0.0586132     0.4861644

$`relarea20  `
          Est1        p1        AIC1      AuROC  Ss     Sp     CCR    kappa
1         53.4      0.129     56.95     0.6600 0.5241 0.5606 0.5422  0.0847
          ModSig    Th
1     0.07197762    0.485883

$`den3       `
          Est1        p1        AIC1      AuROC  Ss     Sp     CCR    kappa
1         -0.314    0.158     57.12     0.5575 0.4812 0.4292 0.4556 -0.0896
          ModSig    Th
1     0.08120815    0.5218787

$`den5       `
          Est1        p1        AIC1      AuROC  Ss     Sp     CCR    kappa
1         -0.454    0.139     57.03     0.5775 0.5275 0.4617 0.4950 -0.0109
          ModSig    Th
1     0.07643032    0.531236

$`den7       `
          Est1        p1        AIC1      AuROC  Ss     Sp     CCR    kappa
1         -0.805    0.0519    54.77     0.6250 0.5858 0.4208 0.5034  0.0066
          ModSig    Th
1     0.01770201    0.5626682

$`den10      `
          Est1        p1        AIC1      AuROC  Ss     Sp     CCR    kappa
1         -0.774    0.078     55.85     0.6125 0.6027 0.3773 0.4884 -0.0200
          ModSig    Th
1     0.03465859    0.5599249
```

```
$`den15
  `
  Est1        p1        AIC1      AuROC  Ss     Sp     CCR    kappa
1   -0.812    0.115     56.66     0.5950 0.6235 0.3484 0.4831 -0.0279
  ModSig      Th
1 0.05916682  0.5635259

$`den20
  `
  Est1        p1        AIC1      AuROC  Ss     Sp     CCR    kappa
1   -1.01     0.112     56.59     0.6100 0.6021 0.3726 0.4881 -0.0254
  ModSig      Th
1 0.05644357  0.5608709

$`mda3
  `
  Est1        p1        AIC1      AuROC  Ss     Sp     CCR    kappa
1   317       0.002     35.87     0.8986 0.8049 0.7831 0.7931 0.5855
  ModSig      Th
1 2.80E-06    0.4597167

$`mda5
  `
  Est1        p1        AIC1      AuROC  Ss     Sp     CCR    kappa
1   331       0.00257   38.78     0.8925 0.8104 0.7718 0.7913 0.5824
  ModSig      Th
1 2.85E-06    0.4823529

$`mda7
  `
  Est1        p1        AIC1      AuROC  Ss     Sp     CCR    kappa
1   361       0.00282   39.45     0.8675 0.7743 0.7156 0.7456 0.4904
  ModSig      Th
1 4.04E-06    0.5100872

$`mda10
  `
  Est1        p1        AIC1      AuROC  Ss     Sp     CCR    kappa
1   340       0.00265   41.59     0.8513 0.7220 0.7471 0.7344 0.4689
  ModSig      Th
1 1.25E-05    0.4602534

$`mda15
  `
  Est1        p1        AIC1      AuROC  Ss     Sp     CCR    kappa
1   438       0.00269   39.61     0.8650 0.7998 0.7464 0.7731 0.5462
  ModSig      Th
1 4.39E-06    0.4907444

$`mda20
  `
  Est1        p1        AIC1      AuROC  Ss     Sp     CCR    kappa
1   351       0.00498   44.52     0.8425 0.7395 0.6725 0.7059 0.4119
  ModSig      Th
1 5.92E-05    0.5012002
```