

Chapter 19

Predictability of Plant Resource Allocation: New Theory Needed?

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19.1 The Challenge: Both to the Plant, and to the Plant Scientist

The research field featured in this book pursues the challenge to clarify the potential dilemma in plants of coping with the need for growth as an expression and prerequisite of competitiveness (e.g. Chaps. 10–13), without neglecting stress defence (cf. Chaps. 2–4), however, for preventing decline (see Preface and Chap. 1). Clarification was based in this book on the disclosure of mechanisms which enable plants to regulate energy and substrate fluxes between the physiological demands, and which, as a consequence, determine the resource partitioning amongst plants in stands under the prevalent ecological scenario. Obviously, the issue is about mechanisms in resource allocation across diverse spatio-temporal scales. Such mechanisms representing cause/effect-based interrelationships differ in scale-dependent ways, regarding spatio-temporal resolution and functional specificities, i.e. within and between the levels of cells, organs, whole-plants and stands, between ontogenetic stages and, in ecological terms, between growth (or site) conditions (see Chap. 1; Sandermann and Matyssek 2004; Baldocchi 1993). The scale-dependent appearance and functionality of mechanisms in resource allocation related to growth and defence demands is one central message of the contributions to this book. Links were demonstrated between molecular and whole-plant processes and their stand-level integration, while underlining the need for considering plant, parasite and mycorrhizospheric interactions as a functional unity that drives the plant's resource allocation (Matyssek et al. 2005). Given the associated functional complexity and, thus, encountered variability in plant performance, to what extent

- Is predictability in plant responsiveness to stress manifested?
- Do trade-offs actually exist, or become apparent, in the plant's regulation of resource allocation between growth and defence?
- Do conflicting findings on plant resource allocation impede theory development?
- Does new evidence require novel theoretical concepts?

These are the questions underlying this chapter, exemplifying the explanatory capacity of the *growth–differentiation–balance theory* (GDB), as introduced in Chap. 1 (also see Table 19.1 for explanation of frequently used expressions and abbreviations). This theory claims a trade-off in parallel to increasing resource availability (primarily nitrogen, or nutrients in general) and primary productivity between growth and defence-related metabolism, in particular, emphasizing defence against parasitic stress. GDB as propagated by Loomis (1953) and Lorio (1988), and extended by Herms and Mattson (1992) and Matyssek et al. (2002, 2005), was valued in Chap. 1 to have reached evidential comprehensiveness to an extent that may foster mechanistic understanding and predictability of one crucial process underlying plant performance and persistence, namely, the regulation of resource allocation. Can it be generalized that plants under stress regulate their resource allocation in conflicting ways between the demands for growth and competitiveness *versus* those of defence?

Table 19.1 Expressions and abbreviations frequently used in this book volume and their explanation

Expression, abbreviation	Explanation (in relation to the rationale of this book volume)	Chapter with details
CBSC	Carbon-based secondary compound	1 and 17
Defence, constitutive	Defence mechanisms which are pre-formed in healthy plants;	1 and 3
induced	defence mechanisms activated by the plant upon pathogen attack	
GPP	Gross primary productivity	Preface and 1
GDB	<i>Growth–differentiation–balance hypothesis</i> , viewed in this book volume as immature theory	Preface and 1
Opportunity costs	Foregone opportunities by the plant upon resource investment in one out of several metabolic alternatives, reaching beyond the equivalent effect of resource partitioning <i>per se</i>	1
PLATHO	Acronym of numeric model, standing for “ <i>PL</i> Ants as <i>T</i> ree and <i>H</i> erb <i>O</i> bjects”	15 and 17
PR proteins	<i>Pathogenesis-related</i> proteins	3
SVM	Support vector machine: classification algorithm belonging to the class of supervised machine learning algorithms. SVM reflect the theoretical results from statistical learning theory	16
Trade-off	Two alternatives which exclude each other in terms of an inverse relationship	Preface and 1

19.2 “Opportunities” of Plants in Resource Allocation

The conflicting regulation suggested by GDB between the growth and defence-related resource allocation provided the inspiration of this book. However, do plants strictly follow such a kind of trade-off? Recent findings reported in this book emphasize the extent of such a trade-off to be circumstantial, depending both on plant-internal and external determinants. Distinctness apparently is an issue of the considered metabolites or metabolite class, by which the trade-off is presumed to be represented (cf. Koricheva et al. 1998, Chap. 1). Phenylpropanoids are an example, constituting a starting point of defence metabolism, while being linked closely to growth metabolism through their precursor phenylalanine. Hence, apple trees, which rely on phenylpropanoids in defence, conspicuously reflected the resource trade-off between growth and defence (Rühmann et al. 2002). Trade-off distinctness also is an issue of the hierarchical process level in the plant’s metabolic organization (Koricheva et al. 1998), becoming apparent in the internal carbon flux as a whole rather than at the highly resolved underlying level of functionally particular metabolites (which, in addition, may not be readily detectable and individually quantifiable due to methodological constraints). Such an impression appears to be in accordance with GDB *sensu stricto*, in postulating the trade-off to occur between growth and *constitutive* defence at the whole-plant level (Herms and Mattson 1992), at which the common currency of both demands is carbon and associated nutrient elements, in particular, nitrogen. At this level, constitutive

defence is a plant function that both competes internally with and is part of the growth-related metabolism, as this variant of defence is based both on structural resistance and preformed biochemical means (Oßwald 1995; Elstner et al. 1996). Some defence compounds may act as growth regulators and bias trade-offs (Taylor and Grotewold 2005). Remarkably, at the level of branches, defence did not vary in relation to the branch growth rate, as demonstrated in apple trees (cf. Chap. 3).

This brings us to viewing the other extreme, when presumed trade-offs do not become apparent, i.e. seemingly or actually do not exist. In fact, polyphenols as defence metabolites do not appear to incite high costs, so that trade-offs may stay minor. The latter may also be true at the whole-plant level, as the defence capacity can vary between the plant organs in relation to their risk of injury—perhaps representing “economic efficiency” in plant defence. Trade-off was not observed in beech (*Fagus sylvatica*) as infected by the root pathogen *Phytophthora citricola* (cf. Chap. 3). Here, beech trees which had survived the infection were concluded to possess a high degree of constitutive resistance. The latter appeared to have developed in parallel to (or even as an intrinsic part of) growth already *prior* to the infection, with the costs of growth and constitutive defence, in this case, being served by GPP sufficiently high to prevent mutual limitation. As mechanical robustness *via* cell wall biosynthesis (potentially including lignification) serves both mechanical requirements (i.e. “growth”) and constitutive defence (cf. Lerdaу and Gershenson 1997; Sibly and Vincent 1997), it is conducive that costs of growth and defence are not distinguishable—and hence, a trade-off neither is plausible nor presentable. Or in other terms, any resource investment in plant structure as some means of constitutive defence intrinsically fosters plant competitiveness, and hence, favours growth through enabling the plant for space occupation and related resource exploitation (cf. Chaps. 10–12).

In addition, vigour in growth can be a defence strategy *per se*, as long as increments of stress-targeted plant tissues or organs are able to (over-)compensate for the loss of respective biomass upon stress impact (cf. Maurer and Matyssek 1997). On such grounds, the postulated trade-off is not existent, as in general, the plant’s efforts in defending its above or belowground biomass may vanish, if adverse stress impacts stay minor relative to the abundance and intactness of the entire targeted biomass, e.g. the foliage or fine-root system as a whole, respectively (Zangerl and Bazzaz 1992).

Referring again to the beech trees mentioned above, which had survived pathogen impact in the absence of the claimed trade-off (cf. Chap. 3), they even displayed increase in photosynthetic performance (Fleischmann et al. 2010), which is interpreted as a means for warranting the defence costs required for survival. Such costs were spent for enhanced fine-root growth, compensating for the injury inflicted by the root pathogen. Apparently, a C sink was induced by the demand of defence that exerted stimulation on photosynthesis. The sink-driven stimulation de-escalates the claimed trade-off through raising GPP in favour of defence. Such a response reflects costliness in defence in beech, even in the absence of a trade-off with the growth-related metabolism, although the costliness was expressed in a way that differed from that in apple trees (see above). These did follow the trade-off

scheme (Rühmann et al. 2002). Hence, at high defence costs both in beech and apple trees, GDB was validated in contrary ways. A response consistent with that in beech was found in potato (Ros et al. 2004), where high N availability promoted the growth metabolism to an extent that related genes were not repressed, even though defence genes were activated upon pathogen infection.

Complementary to the response mechanisms addressed above, comprehensive resistance can be achieved by the plant through *stress-induced defence*. This latter variant tends to be specific against particular stressors and may be less costly—at a first glance, at least—being activated only on demand, as opposed to the unspecific prevalence of constitutive defence. Capability of induced defence, therefore, might be a selective advantage of plants (Walters et al. 2005). The low-cost premise, moreover, is based on the typically micro-scale restriction of induced defence to the site of stress impact, in addition, to the “baseline” of constitutive defence, although the number of induced genes can be high (Ros et al. 2004). The premise appears to be supported by the observation that the amount of resources disposable between growth and defence-related metabolism may actually be low (Häberle et al. 2009, see Chap. 11). Micro-scale trade-offs in resource allocation between growth and defence perhaps occur under induced defence at the cell or tissue level, however, they hardly become manifested in the entire plant. Again, the claimed trade-off appears to be a matter of scale, and given the scope of induced defence, to lie beyond the whole-plant perspective of GDB. Hence, a restriction of GDB is its focus on constitutive defence (Herms and Mattson 1992; Matyssek et al. 2005).

The low-cost premise of induced defence only holds, however, if all related costs actually have been recognized and are accessible to quantification. In terms of a “full-cost analysis” (Lerdau and Gershenzon 1997), hidden or indirect costs potentially exist that are related to storage and transport of defence compounds or their precursors, warranting the readiness of induced defence on instantaneous demand. If so, such costs are hard to define and assess. However, the local restriction of induced defence often encountered in plants at least signals that the immediate costs may not dominate whole-plant resource allocation. More importantly, the account shows that plants have many options for coping concurrently with growth and defence in resource allocation, by de-escalating or even circumventing conflicts as reflected by trade-offs. Such options—or “opportunities”—apparently are not only associated with “opportunity costs”, as incurring from the foregone opportunity upon following one alternative of a trade-off, but also provide means of escaping trade-offs between growth and defence. However, the multitude of options makes it very hard to predict plant behaviour under prevalent stress scenarios.

19.3 Enhancing Predictability

The predictability of resource allocation in operating between growth and defence is apparently restricted, given the range of regulatory “opportunities” plants possess. One may ask for means, therefore, that can enhance the predictability of plant resource allocation. Basically, two perspectives appear to be viable: (1) empirical

molecular research, i.e. at the ultimate highly resolved scale that controls whole-plant performance, perhaps revealing the initiation of consistent response patterns to stress, mechanistically bridging the levels of gene expression, protein synthesis and metabolic activity (cf. Sandermann and Matyssek 2004); and (2) mathematical modelling. The latter option may be conducive, if plant response is not readily accessible to validation of GDB through empirical analysis. Such latter reasons are

- difficulties in ascertaining resource availabilities plant-internally in their relevance for defence. Challenging, in particular, is the plant's operation under resource limitation, given the parabolic relationship in such a case between defence and resource availability (cf. Chap. 1, Fig. 1.1),
- restrictions in controlled experimentation on resource availability because of complex resource interdependences, and in warranting coherence in theory evaluation across diverse ecological scenarios and spatio-temporal scales; and
- uncertainties about relevant defence pools (i.e. constitutive vs. induced, whole-plant vs. organ level; see above).

Such shortcomings may result in premature or contrasting hypothesis evaluations. The question arises, therefore, if modelling can serve as a complementary approach, which may set the empirical shortcomings into perspective. Respective capacities will be elucidated after highlighting, in the following, capacities of empirical molecular research.

19.3.1 Molecular Analysis

Capacities of empirical molecular research in enhancing the predictability of plant responsiveness are exemplified by means of O₃ effects (cf. Chap. 2). Starting point in integration is microarray analysis, which reflected coordinated regulation of all shikimate pathway genes under O₃ stress, and in the case of two enzymes (3-deoxy-D-arabino-heptulosonate-7-phosphate synthase 3 and 3-dehydroquinate dehydratase/shikimate dehydrogenase), transcript and protein levels were consistently increased. In addition, upon gene expression of salicylic/gentisic acid conjugates, metabolic end products were up-regulated. Similarly, a consistent chain reaction of altered gene and metabolite expression for ethylene biosynthesis and changed physiological and structural leaf differentiation were demonstrated. Down-regulated under O₃ stress were transcript levels related to mesophyll cell structure and photosynthesis (Calvin cycle), extending in the latter case to reduced protein levels, based on proteome analysis. Although direct transcript-protein overlap was not detected, overall down-regulation of primary metabolism upon O₃ impact was apparent. Two overlaps emerged (functional category disease/defence and transcription), however, in roots of European beech infected with *Phytophthora citricola*.

Transcriptome analysis of O₃-treated beech yielded gene grouping similar to that in herbaceous plants (cf. Chaps. 2 and 16). Gene expression was more strongly affected by O₃ impact than endophyte infestation, although pathogenic effects distinctly raised transcript levels. The latter responded in leaves to ozone and to

pathogenic infection in roots, becoming assignable to similar functional categories. In addition, in beech, apple trees and potato plants, genes encoding PR proteins were identified. In conclusion, most genes of the defence category were up-regulated in the different plant species upon O₃ or pathogenic impact, corroborating the view on ozone as an “abiotic model pathogen” (cf. Matyssek et al. 2005). Transcriptional responses were more distinctly reflected in juvenile than mature trees, and gene expression typically mirrored leaf type (i.e. sun vs. shade-adapted) more distinctly than O₃ impact. Empirical molecular research does have capacities for unveiling consistent plant response patterns, fostering predictability in resource allocation between growth and defence.

19.3.2 Mechanistic Modelling

An approach complementary to empirical research is modelling, in particular, if employed as mechanistic numerical simulation models and based, in view of GDB, on the presumed parabolic relationship between resource availability and allocation to defence (cf. Fig. 1.1 in Chap. 1, Chap. 15). Given the plants’ regulatory “opportunities”, models must mirror the dynamics in resource allocation along the source–sink gradients of growth and defence-related metabolism, as determined by phenological and ontogenetic influences, and most importantly, by the internal availabilities of carbohydrates and nutrients (namely, nitrogen with respect to GDB). The internal availabilities need to respond to the resources outside, ensuring variation in interaction, as affected by fluctuating uptake capacities and variable factorial impacts. Competition with neighbouring plants needs to be considered as a crucial determinant (cf. Chap. 12), and baseline assumptions, derived from established knowledge, are to be integrated on CBSC physiology and biochemistry (cf. Chap. 1). Such requirements are comprehended in the novel PLATHO model, as introduced in Chap. 17. The advancement of PLATHO relative to the conceptual framework underlying GDB is the process-based, quantitative assessment of the plant’s operation along internal resource gradients and under the influence of ecologically relevant site conditions. Hence, PLATHO represents a quantitative and dynamic extension of GDB.

Given the functional comprehensiveness and mechanistic character of PLATHO, parameterized and validated on a broad experimental data basis (see Chap. 17), the model performance under diverse simulation scenarios is summarized in Table 19.2 as contributing to the evaluation of GDB and a related working hypothesis. The latter is conceived for reasons of comparison more “liberal” in allowing carbon, nutrients and water as driving resources but ignores the parabolic relation to defence. This kind of relation, however, is intrinsic to GDB, while focusing on nutrients (namely nitrogen, cf. Koricheva 2002; Herms and Mattson 1992; Chap. 1, Fig. 1.1) as drivers. In summary:

- Starting with plant growth that suffers from N limitation, a scenario that leads to enhanced plant-internal N availability (as mediated through fertilization or changed competition between plants) will favour growth while diminishing the

Table 19.2 The plant's resource allocation between growth and defence, as reflected by the modelling approach of PLATHO

Plant <i>limited</i> by	Scenario	Effects on growth and CBSCs	Evaluation of working hypothesis (see text) and GDB
N availability	+N	Plant growth increased CBSC concentration decreased	Both confirmed
	+C	No or small stimulating effect on plant growth (decreasing N concentration) CBSC concentration increased	Hypothesis rejected, but GDB confirmed
C availability	+N	Small stimulating effect on plant growth (enhancement of N-concentration) CBSC concentration decreased	Both confirmed
	+C	Plant growth increased Stimulatory effect on CBSC concentration	Hypothesis rejected, but GDB confirmed at low resource availability

pool of CBSCs. This outcome confirms both the working hypothesis and GDB. A scenario, however, that augments the internal C instead of N availability in the plant, will not (or just negligibly) affect growth while enhancing the pool of CBSCs. The latter result rejects the working hypothesis introduced above, but still supports GDB.

- Conversely, taking C-limited growth as a starting point (as, e.g., under shading or incipient foliage development), a scenario that raises the N availability in the plant will moderately stimulate growth, but cause the CBSC pool to decline. This outcome is in agreement with both the working hypothesis and GDB. However, if instead the C limitation is released at low N availability, then growth and the CBSC pool will increase. Although the latter result rejects the hypothesis, it does not conflict with GDB, if the initial C supply was too low for N to induce growth, and if the enhanced C availability is higher than can be “consumed” by N for growth. This latter case is mediated at low N availability through the parabolic relation to defence (cf. Chap. 1).

Using information derived from modelling, one needs to caution that models, including PLATHO, represent integrated lines of hypotheses themselves, i.e. these form the basis of model functioning, so that in principle, hypothesis evaluation by model employment is not possible. However, if mechanistic models are able to explain a broad range of experimental findings during extensive validation, as was the case of PLATHO, agreement with the empirical evidence can be taken as an indirect confirmation of the reliability of underlying presumptions. The outcome that, contrasting with GDB, the GDB-derived working hypothesis was to be rejected in some cases indicates the differential view on carbon as a driving resource and the mathematical function describing defence. Such aspects are noteworthy, as validating GDB in the past has also suffered from inadequately accounting for underlying definitions (cf. Chap. 1). Procedures followed by PLATHO

did comply, however, with fundamental requirements of science theory, namely ensuring processable evaluation of explicitly defined experimental scenarios, and as a consequence, providing new evidence about initial presumptions.

Experimental results yielded five response patterns after increasing C availability of plants, characterized by either stimulations or no response in both growth and defence, or stimulation of just one of the two plant functions—or, conforming to the trade-off presumption, decline in defence at increasing growth (cf. Chap. 17). Simulations manifested, in agreement with GDB, that low N in relation to C availability, even if the latter increased, was not reflected to promote growth, while allocation to defence could even decline (reflecting the parabolic relation between defence and nutrition). This means as a consequence, however, and still in compliance with GDB, that increasing N availability can drive both growth and defence. A general trend of favoured CBSC allocation upon increasing C availability was paralleled, in other cases, with unchanged or even declining CBSC levels. Such an increase in CBSCs was remarkably related to high N vs. C availability ratios, at a first glance conflicting with GDB. However, allocation to CBSC was reflected to become saturated at high C supply, with the saturation being reached the later the higher N availability was, and with the surplus C being allocated then to reserve storage rather than to defence. Such plant behaviour shows higher regulatory complexity than presumed by GDB. Additional cases showed stimulation in CBSC allocation, however, in the absence of growth response. Such cases are assumed to reflect growth to be determined by causes other than N availability. The five response patterns were similar in beech and spruce, although in the latter species the dependence on resource availabilities was hardly assessable, given lesser extents of C and N fluctuations relative to each other than observed in beech.

As an essential aspect of PLATHO, stand structure, and hence competition, proved to be crucial modifiers of the factorial impacts that drive the plant's resource allocation between growth and defence. A strong effect was reflected by simulations on C availability and CBSC allocation, depending on whether growth under CO₂ and O₃ regimes occurred in beech and spruce at mixed or pure stand conditions (complying with empirical observations by Kozovits et al. 2005 that "*competition dominates CO₂ and O₃ effects*"). Competition effects were mediated through changed light regimes (cf. Chaps. 8, 11–13), favouring in mixed stands the N uptake in spruce at the expense of that in beech (cf. Kozovits et al. 2005). The strong impact of competition on resource availability in beech is mirrored in the simulations (Fig. 19.1a) by a narrower range of data points both along the N and the C axis in pure than mixed stands.

Even more importantly, changes in competition turned out in the simulations to affect CBSC pools in contrasting ways, depending on species, which provides clues, why GDB evaluation may become contradictory. Modelling as exemplified by PLATHO can resolve such conflicts on mechanistic grounds, showing that hypothesis acceptance or rejection, and support of theories like GDB, can be decided by the prevalent ecological settings. The explanatory basis is provided by the variable three-dimensional relationship between plant-internal C and N availabilities and CBSC allocation. The three-dimensionality also explains

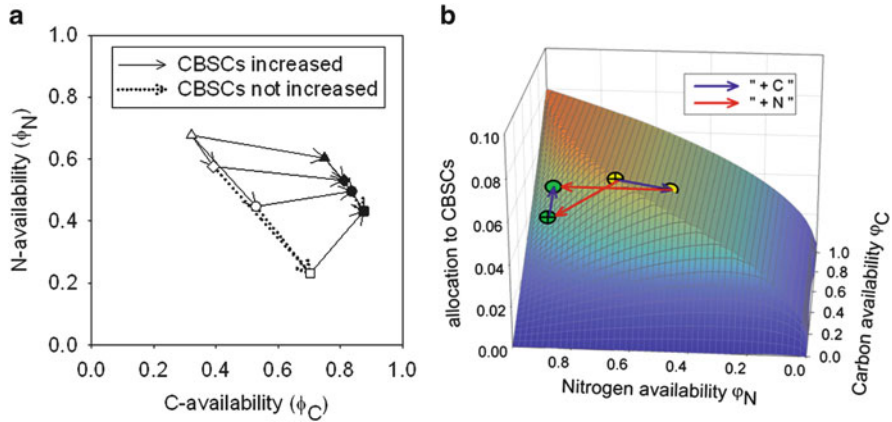


Fig. 19.1 (a) Positioning of experimental scenarios with juvenile beech (*Fagus sylvatica*) regarding plant-internally relative carbon (ϕ_C) and nitrogen (ϕ_N) availabilities. *Open symbols*: growth in mixture with spruce (*Picea abies*), *closed symbols*: pure beech stand; (*open circle, closed circle*): control, (*open triangle, closed triangle*): $+O_3$, (*open square, closed square*): $+CO_2$, (*open diamond, closed diamond*): $+O_3/+CO_2$. Vectors link simulation outcomes of two treatments, merged in the graph into one data point each. Direction of vectors point towards enhancement of CH_2O availability; *drawn vectors*: increased allocation into carbon-based secondary compound formation (CBSC), *dashed vectors*: CBSCs not increased. (b) Visualization of function in PLATHO describing the allocation of CH_2O into the CBSC pool as depending on plant-internal relative C (ϕ_C) and N (ϕ_N) availabilities. The *symbols* represent the predicted allocation to the pool of CBSCs in a fictive scenario, where relative carbon (ϕ_C) and nitrogen (ϕ_N) availabilities are averaged over the period of leaf development. The simulated scenario refers to an experiment with juvenile beech, in which nitrogen supply as well as atmospheric carbon dioxide concentration were varied: *Yellow symbols*: low N supply, *green symbols*: high N supply, *open symbols*: $700 \mu l l^{-1} CO_2$, *crossed symbols*: $350 \mu l l^{-1} CO_2$. *Red arrows* indicate the effect of enhanced nitrogen supply, *blue arrows* the effect of elevated carbon dioxide on plant-internal resource availabilities and allocation to CBSCs

inconsistent plant response to abiotic factors like, e.g., CO_2 supply (Fig. 19.1b). Ranges of different system behaviour become apparent along C and N availabilities, conforming along the N axis to the parabolic response function of defence, while the C axis communicates the saturation range of CBSC allocation, as already addressed above. The linear slope of CBSC allocation with C availability is consistent with GDB, although the latter does not claim a range of CBSC saturation. The complexity of plant resource allocation within the factorial three-dimensionality during seasonal courses was demonstrated in Chap. 17. One example is given in Fig. 19.1b, in that CBSC allocation may be increased or decreased at high CO_2 supply, depending on the level of concurrent N availability. The capacity of opposing plant behaviour in CBSC allocation becomes apparent, the more so as operation ranges of plants may change within the functional three-dimensionality during ontogenetic and phenological time courses (cf. Chap. 17).

Such kind of modelling exemplifies that judgement about hypotheses may be premature, and gain in evidence be missed, unless the mechanistic basis of plant

response is unveiled thoroughly. Modelling further ensures implications of ecologically relevant settings to be accounted for in hypothesis evaluation, and in this way, warrants biologically meaningful conclusions on plant functionality. The modelling-based analysis extends beyond conventional GDB examinations, in that resource availabilities and dynamics become quantifiable under defined ecological scenarios, accounting for variable plant-external and internal influences, i.e. seasonal as well as ontogenetic and metabolic ones. The challenge and need of such an approach was underlined by Koricheva et al. (1998) and Stamp (2003).

The other novel process-based, numeric model demonstrated in this book is BALANCE (cf. Chap. 18). This model may be viewed as an extension of the scope of PLATHO to the stand level, in particular pursuing a temporal perspective related to the prolonged lifespans of forest trees. Simulations addressed the “opportunity costs” of trees, associated with the growth-related competitiveness in mixed forests, while investing resources in pathogen defence. Here, the costs of foregone opportunities, in this case at the expense of growth and competition, were valued given the need for staying defensive *sensu* GDB trade-off—rather than opportunities of “escaping” such a trade-off (see above). Opportunity costs were expressible through interest calculation, showing that the annual compound interest of “lost opportunities” after 14 years can range in beech up to about 4 % at defence allocation of 50 % of the C pool. This kind of outcome supports GDB. Competing, non-infected spruce again was the profiteer, similar to outcomes from PLATHO, raising biomass production by up to 140 %. The trade-off between growth and defence apparently also occurring at the stand level is species-dependent, i.e. driven by the species mixture. BALANCE assesses the buffering capacities of mixed forests regarding growth/defence trade-offs, exemplifying spruce to profit most from the defence costs of competing beech, in particular, if both species grow in a randomized single-tree arrangement.

Given again the strong impact of competition on resource allocation, BALANCE reflects a non-linear allometric relationship of stem diameter *versus* above-ground biomass at the stand level, with this ratio being determined through the proportions of tree species in mixture. The structural heterogeneity of stands is implied to increase at limiting resource supply. Under stress (e.g. as by drought), the growth performance of dominant tree individuals turns out to be species-dependent. BALANCE shows the tendency, however, that at increasing resource supply the size growth of dominant trees is over-proportionally favoured. Since, correspondingly fewer resources are left for the growth of subdominant trees, competition becomes exacerbated, turning its mode from symmetric towards asymmetric interaction (cf. Chaps. 12 and 14, Pretzsch and Dieler 2012). In general, competition, resource limitation and stress limitation affect tall trees more than their smaller neighbors, which is in line with GDB at the individual tree level. Beech in addition was indicated to pursue minimization of the aboveground resource demand when maximizing belowground resource uptake. Such an outcome conforms beyond circumstantial support of GDB even to another theory on plant growth, namely that on “*optimized plant allocation*” (cf. Chap. 18).

Resuming theory examination by numeric modelling, eventually brings us to two concluding questions:

1. Would it be a drawback, if hypothesis and theory evaluation stayed controversial? The question can be negated in view of science theory, if the scenarios leading to divergent evaluation are adequately analyzed in terms of unveiling the factorial determinants. In such cases, branching in system behaviour becomes comprehensible, and hence, the controversial outcome augments system understanding. It can be stated based on the evidence presented in this book that this kind of requirement is fulfilled.
2. Would it be a drawback then, if hypotheses and theories were predominantly confirmed, as shown, e.g. in Table 19.2, given the claim of science theory that rejection promotes evidence (Popper 1969)? In its absoluteness, this question may be negated as well, as long as confirmation allows consolidation of mechanistic system understanding and strengthening of validity across ecological scenarios. Such requirements were met, regarding the findings reported in this book and in view of the subsequent considerations.

In total, process-based, numerical modelling proves to be a powerful and complementary tool in linkage with empirical research, both having the capacity in jointly enhancing predictability of plant system performance as related to prevalent ecological scenarios.

19.3.3 *Statistical Modelling*

As numerical modelling linked with empirical research proves conducive in strengthening predictability, the means of coping with the challenge addressed in this book are not exhausted yet. One further approach is statistical modelling (cf. Chap. 16), in particular, if the focus is on the degree of generality or universality of empirical findings. Typically, these originate from manifold and contrasting observational scenarios in the absence of one over-arching research concept. This is the situation, by which current knowledge becomes available in plant research on resource allocation, and such grounds have been recognized as a major impediment in fostering respective theory development (Chap. 1).

One novel means of statistical modelling is based on the theory of “*unsupervised learning*”, dating back to Vapnik (1995), as introduced in Chap. 16, which provides prediction for a yet unforeseen information input in determining the generalization error. The approach balances complexity *versus* accuracy, making use of machine learning algorithms, the so-called “*support vector machines*” (SVM), aimed at optimizing this kind of balancing in separating, i.e. classifying, different datasets by “hyperplanes”. The ultimate outcome is the identification of such variables, which most distinctly respond to same driving factors under different scenarios. The misclassification error is improved in accuracy by repeated cross-validation of test and training data randomly chosen from the database. Variables with highest prediction accuracy are combined with any other variable and its prediction accuracy of the database (according to a Greedy Variant, see Chap. 16), iteratively increasing the pool of variables under analysis until a ranking list of accuracies related to variables is established. The list yields the optimum of prediction

accuracy at a given set of combined variables. The crucial point is that the procedure does not start from a preset hypothesis, rather information is “learned” from the data, i.e., relevancy is disclosed for those data which are most indicative of the entire dataset.

Employing the SVM approach to patterns of gene expression within the data pool of diverse empirical investigations on the book subject (cf. Chaps. 2 and 16), the outcome of performed classification procedures was related to and confirmed by identified genes. As a result, hypotheses were derivable on the functional assignment of unidentified genes as based on similarities of expression patterns. The precision quality of such a kind of cluster assessment was higher as compared to conventional approaches with preceding functional classification, i.e., relating identified genes to regulatory metabolic pathways. Eventually, unknown genes become organized by their probabilities of cluster affiliation, now aiding the selection of demonstrative genes which appear to be compelling for further functional clarification. Viewing the outcome as immediately obtained from the SVM analysis across the diverse compared experimental scenarios provides the general conclusion that the number of differentially expressed genes is remarkably low, although gene response tends to be scenario-specific.

Another application of SVM analysis was demonstrated for beech in response to variable CO₂ supply under different growth scenarios (cf. Chaps. 3, 12, 16). The separating hyperplane was defined by the *C/N* ratio of leaves and the amount of cell wall-bound phenolic compounds in fine roots. This separation confirms GDB to the extent that resource limitation, as reflected by increasing *C/N*, is associated with an increase in phenolics. Maximum prediction accuracy was found to be related to six variables, namely, cell wall-bound and soluble phenolics in fine roots and leaves each, along with *C/N* and dry mass of leaves. Variables with largest effects on prediction accuracy were cell wall-bound phenolics of fine roots, and leaf *C/N* and dry mass, being those variables which represent most the resource-driven trade-off between growth and defence *sensu* GDB. The SVM analysis, therefore, conforms to the generally presumed concept that the plant’s resource allocation is mainly determined by the regulation between the growth and defence-related metabolism.

19.4 Need for New Theory?

In view of the new evidence presented in this book, namely, the manifold “opportunities” plants apparently do have in balancing the resource demands of growth and defence, do arguments emerge for a new theory on plant resource allocation, i.e. replacing GDB? It might be tempting in arguing so, as cases exist, that do not disclose growth/defence trade-offs *sensu* GDB. Conversely, it cannot be denied either that other cases support GDB. Reasons for interpreting the ambiguity, i.e. for functionally “explaining” either outcome in a case-specific way, have been detailed in this book and earlier in this chapter. According to science theory, however, the encountered situation appears to justify the rejection of GDB (Popper 1969).

Nevertheless, a respective decision would seem to be premature, acknowledging that even a scope of investigations as introduced by this book may not be adequately comprehensive for thoroughly challenging a fundamental theory in plant science such as GDB, i.e. to the full breadth of conceivable ecological scenarios. Also, the fact that plants can apparently choose from many “opportunities”, i.e. that complexity *sensu* plasticity in response is intrinsic to plant behaviour, means that resolving the growth-defence conflict through trade-offs in resource allocation is just one amongst several plant options. And it is one of these, for which GDB does hold. Attaining this kind of evidence, what GDB loses then is its claim for generality in elucidating the plant’s balance in resource allocation between growth and defence. Or, in other terms, the applicability of GDB becomes restricted, being a matter of spatio-temporal scales, of specific mechanisms in the hierarchy of internal organization and of external specificities in multi-factorial ecological settings. Hence, theory validity is to be defined, in particular, for mechanistically linking adjacent spatio-temporal scales of biological organization.

Despite this restriction, even in cases where not applying, GDB can still give orientation for designing empirical research and modelling, the latter representing one tool for hypothesis validation and theory development (see above). Such kind of orientation gives guidance to understanding plant behaviour even beyond GDB, provided the attained evidence allows the identification of functional branching points by which plants leave GDB *sensu stricto* or its defined validity ranges. Such alternative branching pathways in plant behaviour, which apparently do exist, open the wide field of functional plasticity, which is an intrinsic organismic feature of perhaps the highest evolutionary value for plant persistence and successful stress adaptation. Examples of plasticity in plant response beyond GDB are, e.g. capacities of enhancing GPP in support of defence, in using growth vigour as a means of defence strategy, or in keeping defence locally restricted at the tissue or organ level without afflicting whole-plant metabolism. Since plant plasticity beyond GDB is that flexible, hardly a new theory can currently be posed to unify the diverse observed or even further “opportunities” on mechanistic grounds. The pre-requisite is to comprehend the cause–effect based interrelationships that underlie plasticity. This book has posed, however, a guiding perspective on the grounds of advanced theory building beyond GDB.

Within the above view, GDB has not become obsolete in giving orientation as long as two requirements will be met, whatever outcome be obtained, to permit mechanistic clarification, (1) elaboration of new substantial knowledge about relevant physiological and ecological processes, and (2) assessment of the ecological settings in each individual case study. To this end, revision of GDB is mandatory, however, to warrant a more advanced rationale than currently prevailing. Quite immediately and precisely, the extended rationale must aim at the disentangling of growth and constitutive defence, “full-cost” oriented clarification of induced defence within the whole-plant metabolism, mechanistically linking the process levels of metabolic control (i.e. the molecular responses within the genome) and metabolic activity (i.e. the biochemical and physiological response level), and the overall integration into the ecological interactions at the stand and ecosystem

scales. The spectrum of biotic interactions and their mechanistic quantification is to be considered above and belowground in theory development, widening the conventional scope of host–parasite systems by the dimensions of competition and/or facilitation and the mycorrhizospheric interrelationships. As a result, the functional understanding of the varying degrees of trade-offs in plant resource allocation will be strengthened. Such a widening of the rationale appears to be conducive also towards a “holobiontic view” on the range of involved biotic interactions (i.e. beyond those between plants and micro-organisms) and approaches which expand “systems biology” to a comprehensive coverage of the relevant resource and information flows, integrating the molecular into the whole-plant system and ecosystem scale. Hence, “systems biology” must find its completion in an ecosystem biology.

Most importantly, the variability in growth/defence trade-off manifestation, ranging between distinctness to quantitative irrelevance or even absence, demands for attention on the highly dynamic and multi-functional regulatory capacity, as an expression of plasticity, in plant response. GDB-related research in the past has been fixed predominantly to steady-states in resource allocation under often mono-factorial influences. What have been overlooked were transitions, non-linearities, multi-factorial interactions and hysteretic cause–effect relationships in plant performance as well as evident branching points, at which resource allocation commences to depart from the conventional scope of GDB. The “static” way of thinking about GDB impeded a mechanistic view beyond the claimed plant-internal dilemma between growth and defence-related metabolism, creating seeming conceptual conflicts in cases of unforeseen plant response. The required extended view must comprise growth and defence, therefore, as part of the plasticity intrinsic to resource allocation, and must unravel mechanisms that control plasticity. Upon reaching such an achievement, then the presumed “dilemma” claimed by GDB would be functionally recognizable as just one facet embedded into the overall continuum of the regulation range in resource allocation to growth and defence demands. Attaining such a stage might then allow new theory formulation. The perspectives have been shown, with revised GDB appearing meanwhile as a conducive conceptual tool. Progress towards universality in attained evidence and knowledge will be fostered by theoretical approaches as represented by the diverse kinds of modelling introduced in the book and accentuated earlier in this chapter. Such latter tools are prone to forward unification across empirical observations, as enabling for sublimating response patterns that overarch the range of case study scenarios. In such respect, the linkage between modelling and empirical research has the capacity of promoting new theory building through enhancing predictability in plant system behaviour, as demonstrated in this book.

For warranting the perspective to new theory building, the mechanistically founded, even though not unifying character of GDB is to be strengthened further as discussed above. The “flaw” of not being unifying yet continues to render GDB falsifiable, and the more GDB will become mechanistically founded, the more falsification will provide mechanistic clarification and gain in evidence. By this, the field of comprehensively understanding plasticity in plant responsiveness increasingly becomes accessible. It is the challenge in research to forward the functional

variability of stress responses as an intrinsic feature of plant system biology towards process-based understanding. This book has created a basis for doing so, exemplifying conducive new evidence. Hence, mechanistic comprehension of plasticity becomes the key to plant system understanding.

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