OUT OF THE QUAGMIRE OF PLANT DEFENSE HYPOTHESES

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ABSTRACT

Several hypotheses, mainly Optimal Defense (OD), Carbon:Nutrient Balance (CNB), Growth Rate (GR), and Growth-Differentiation Balance (GDB), have individually served as frameworks for investigating the patterns of plant defense against herbivores, in particular the pattern of constitutive defense. The predictions and tests of these hypotheses have been problematic for a variety of reasons and have led to considerable confusion about the state of the "theory of plant defense." The primary contribution of the OD hypothesis is that it has served as the main framework for investigation of genotypic expression of plant defense, with the emphasis on allocation cost of defense. The primary contribution of the CNB hypothesis is that it has served as the main framework for investigation of how resources affect phenotypic expression of plant defense, often with studies concerned about allocation cost of defense. The primary contribution of the GR hypothesis is that it explains how intrinsic growth rate of plants shaped evolutionarily by resource availability affects defensive patterns. The primary contribution of the expanded GDB hypothesis is that it recognizes the constant physiological tradeoff between growth and differentiation at the cellular and tissue levels relative to the selective pressures of resource availability, including explicitly taking into account plant tolerance of damage by enemies. A clearer understanding of these hypotheses and what we have learned from investigations that use them can facilitate development of well-designed experiments that address the gaps in our knowledge of plant defense.

PLANTS PRODUCE TISSUE from raw materials and then consumers (pathogens and herbivores) harvest some of that for themselves. It appears that plants have evolved a variety of mechanical and chemical defenses primarily to ward off these consumers. In turn the consumers have evolved countermeasures, which then would act as selective pressure on plants for further defense.

It has been the quest to understand the levels of defense and the array of defenses in plants that has given rise to the body of knowledge known as the "theory of plant defense." More specifically, a central goal in plant-herbivore interactions has been to explain and predict phenotypic, genetic, and geographic variation in plant defense. The research on plant defense has been guided by a series of hypotheses that initially seemed to hold great promise for developing a general theory of plant defense, in particular one that would explain why most plants seem to be so well defended.

By the 1950s, knowledge about the

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amount, array, and biological activity of "secondary" metabolites in plants suggested that these compounds served as defense against pathogens and herbivores (Dethier 1954; Fraenkel 1959). Because these metabolites did not appear to have a direct role in primary metabolism, they were labeled "secondary" (Whittaker and Feeny 1971). Secondary metabolites are found mostly in plants, fungi, and microorganisms, specifically in organisms that lack an immune system (Williams et al. 1989). Allelochemicals, by which an organism of one species affects an organism of another species (Whittaker 1975), are a subset of secondary metabolites. Some secondary metabolites have other functions (e.g., ultraviolet protection, drought tolerance).

Further groundwork was laid with Ehrlich and Raven's (1964) correlation of related butterfly species using plants that were taxonomically and/or chemically related. To explain the diversification of terrestrial plants and insect herbivores, they proposed a "coevolution" model based on macroevolutionary patterns in plant defensive chemistry and host plant affiliations of insect herbivores. The idea is that a plant group develops a new chemical defense that reduces attack by herbivores and so eventually allows diversification as the plants proliferate and move into new niches. At some point this is followed by evolution of counteradaptations by an insect group, which also eventually allows diversification as the insects are able to exploit this diverse plant group. In theory, this process of successive adaptive radiations or repeated starbursts of speciation yields reciprocal speciation in mutant host plant and insect herbivore lineages, but it does not produce parallel cladogenesis at the level of matched species (Thompson 1999). It is not a lock-step process, with every speciation matched one on one. Importantly, because the plant lineage undergoes diversification without significant interaction with herbivores, the eventual colonization by herbivores is just as likely to start with a derived plant species as the ancestral plant species (Thompson 1999). To distinguish it as a special form of coevolution, this model has been called escape-and-radiate coevolution (Thompson 1989). The best support for this model is the consistently high diversification rates in the multiple, independent plant lineages that developed secretory canals containing latex or resin (Farrell and Mitter 1998).

Ehrlich and Raven's (1964) model can also be classified as diffuse coevolution (Futuyma and Keese 1992). The term diffuse coevolution (Janzen 1980; Fox 1981) or guild coevolution (Thompson 1989) refers to "the evolution of a particular trait in one or more species in response to a trait or suite of traits in several other species" (Futuyma and Slatkin 1983:2). Application of the term ranges from the Ehrlich and Raven (1964) scenario described above to the view offered by Fox (1981), with apparent plants (such as woody species) and their large set of herbivores involved in gradual and continuous adjustments rather than starburst (or even stepwise) changes. Although diffuse coevolution has been suggested as a potentially large influence on the diversity of terrestrial plants and insect herbivores (Futuyma and Keese 1992), there is not much concrete evidence and little in the way of rigorous tests (Farrell and Mitter 1998), and so it has been difficult to characterize. Central to this problem is that the concept can be applied too broadly—e.g., to a community of plants and herbivores—and so becomes impossible to analyze (Thompson 1989).

In contrast to diffuse coevolution, pairwise (or specific reciprocal) coevolution refers to two interacting species exerting selective pressure on each other, resulting in stepwise evolution, as first one then the other evolves a response to specific changes in the other (Janzen 1980; Fox 1981). Pairwise coevolution is probably rare (Futuyma and Keese 1992). The strongest potential cases are those in which the host plant species are primarily attacked by specialized insect herbivores (Futuyma 1983; Farrell and Mitter 1998). Such a situation may occur in unapparent plants, typically herbaceous species, which have fewer herbivores as a consequence of their small size, relative structural simplicity, and ephemeral nature temporally and spatially (Fox 1981). The phylogenic congruence of the longhorned beetle genus *Tetraopes*

and the milkweed genus *Asclepias* may be an example of stepwise or reciprocal diversification (Farrell and Mitter 1994, 1998). By itself, however, comparison of branching patterns of interacting lineages can show parallel cladeogenesis, but it does not indicate whether the lineages have coevolved (Thompson 1999). Examination of genetic variability in a plant species' resistance to a particular herbivore species and in the herbivore's ability to counter that may provide support for stepwise evolution. For example, Berenbaum and Zangerl (1998) found chemical phenotype matching between wild parsnip introduced into North America and its principal herbivore, the parsnip webworm, and for at least one furanocoumarin trait interaction seems to have contributed to escalation. The kinds of data needed to test the ideas of stepwise and diffuse coevolution have been outlined, but such data are not easily obtained (Futuyma and Keese 1992; Farrell and Mitter 1993, 1994, 1998; Thompson 1999).

Whether viewed as pairwise or diffuse coevolution, the idea has been that plants and insect herbivores are engaged in an evolutionary arms race (Feeny 1975). However, the arms race view (Dawkins and Krebs 1979) seems too simplistic now. Besides a lack of evidence that is strongly supportive of pairwise and diffuse coevolution as the dominant explanation for the patterns of plant defense (Mitter and Brooks 1983; Farrell and Mitter 1994, 1998), mathematical models suggest that in general the arms race analogy for adaptive responses by predator and prey to one another is a poor one (Abrams 1986; Seger 1992). In terms of natural history, it is also a questionable generalization. Host plant use by herbivores is shaped by predators and climatic conditions as well as host plant attributes (Fox 1981; Janzen 1985).

In sum, the concepts of coevolution and the arms race analogy have served as a useful framework for study of the evolutionary relationship between groups of host plants and their specialized herbivores, but parallel diversification due to pairwise coevolution is "most certainly the exception rather than the rule" (Farrell and Mitter 1994:67). Furthermore, the limited evidence for and the difficulty in testing ideas about diffuse coevolution favor a conservative conclusion about the degree to which it has influenced diversification. So while plant phylogeny and evolutionary history of interactions with herbivores certainly play a role in the pattern of plant defense, the coevolutionary ideas about diversification do not provide a complete or adequate explanation for the defensive pattern of most plants and, thus, the plants' relationships with their enemies. Clearly plants have evolved an array of chemical and physical defenses against a diverse set of enemies and under variable abiotic conditions. In the development of a theory of plant defense, we seek explanations that take all of that into account.

By the mid-1970s, the Optimal Defense Hypothesis, as outlined by Feeny (1975, 1976) and Rhoades and Cates (1976; Rhoades 1979), was taking shape. This framework, which will be described later, was very appealing and inspired many tests of the hypothesis. As a result, by the mid-1980s, the cumulative data, not all of which fit the predictions of the hypothesis, indicated the need for a new synthesis. Coley, Bryant, and Chapin's (1985) Growth Rate (or Resource Availability) Hypothesis provided that. At about the same time, the Carbon:Nutrient Balance Hypothesis was developed (Bryant et al. 1983; Tuomi et al. 1988). These syntheses stimulated much of the recent work on plant defense. By the 1990s, however, data gathered to test these various hypotheses suggested the need for yet another synthesis. Herms and Mattson (1992) stepped in with an ecological and evolutionary expansion of Loomis's (1932, 1953) Growth-Differentiation Balance Hypothesis. In the last decade, all of these hypotheses have served as a framework for research on plant defense. That is, *all have been cited as the theoretical basis for recently published studies*. Furthermore, often two or more are cited in a study, even though the hypotheses address different issues.

At this point in time, there is considerable confusion about the hypotheses and, consequently, about the state of the theory of plant defense. There are several reasons for this. The diversity of secondary metabolites and

their functions (which includes chemicals that negatively affect herbivores), the profusion of hypotheses that are not mutually exclusive, the difficulty in testing the hypotheses, and seemingly contradictory results have contributed to a perception that there is no tangible theory of plant defense. Furthermore, the nature of these hypotheses necessitates testing subhypotheses, which sometimes have not been appropriately constructed and/or tested adequately, and the results have added to the confusion. Not surprisingly then, it has been suggested that an all-encompassing theory may be biologically unrealistic (Berenbaum 1995). Such confusion is typical of certain stages in the development of scientific theory, however (Loehle 1987). Young and consequently immature theory lacks precise statements, which make it difficult to resolve the questions posed (Loehle 1987,1988). Progress in developing theory will be slow when time-scales for the system are long, little history of the system is left, and the system is complex (Loehle 1987).

It is unclear whether the current disarray reflects one of those immature stages or whether it signals that these hypotheses are unsuitable, and moreover that an all-encompassing theory of plant defense is impossible. Before discarding the hypotheses or accepting the conclusion that an all-encompassing theory is unobtainable, we need to consider the degree to which the hypotheses have been tested adequately and determine exactly what we do and do not know about plant defense as a result of these frameworks.

Four hypotheses of plant defense will be examined in detail here: 1) Optimal Defense (OD), 2) Carbon:Nutrient Balance (CNB), 3) Growth Rate (GR), and 4) Growth-Differentiation Balance (GDB). These plant defense hypotheses were chosen because they are the ones that are cited most often and also are routinely misinterpreted. Each of these hypotheses has assumptions and predictions, but only to some degree have these hypotheses actually been tested. In fact, the major assumptions, predictions, tests, and results have not been spelled out clearly, and so it has been difficult to evaluate what we have and have not learned from testing these hypotheses.

Hypotheses of Plant Defense Against Herbivores

the optimal defense hypotheses

The Optimal Defense (OD) Hypotheses address how defensive needs of a plant contribute to the evolution of secondary metabolites, with costs of defense paid to maximize plant fitness (McKey 1974, 1979; Rhoades 1979). The basic hypothesis (Hypothesis 1) states that, "Organisms evolve and allocate defenses in a way that maximizes individual inclusive fitness" (Rhoades 1979:12). In essence, this hypothesis states that any defensive pattern is possible if it is adaptive (McKey 1974, 1979; Rhoades 1979). However, it is difficult to test this hypothesis without more formalization of its language because neither the concepts nor predictions are obvious (Fagerstrom et al. 1987). Thus, while the conclusions have been that "any defensive pattern is possible if it is adaptive," in practice the hypothesis is not really falsifiable. Even Rhoades (1979) said that this hypothesis is probably impossible to test directly.

The second hypothesis (Hypothesis 2) states that "Defenses are costly, in terms of fitness" because they divert resources from "other needs," which mainly has been interpreted as growth and reproduction (Rhoades 1979:13). Although measuring cost of defense to fitness remains an issue, the derivative hypotheses are more testable than the basic OD hypothesis. The subhypotheses are: a) "Organisms evolve defenses in direct proportion to their risk from predators and in inverse proportion to the cost of defense" (i.e., the Plant Apparency Hypothesis); b) within an organism, defenses are allocated in proportion to risk of the plant part and value of it to plant fitness, and in inverse proportion to cost of defense; c) defense is decreased when enemies are absent and increased when they are present (i.e., allocation pattern of constitutive and inducible defenses); and d) there is a tradeoff between defense and other plant functions (growth and reproduction) such that stressed individuals are less defended (Rhoades 1979:13).

Subhypotheses 2a and 2b refer to genotypic expression of defense, whereas subhypotheses 2c and 2d refer to phenotypic expression of defense. Cost of defense is an issue in all

four subhypotheses. Examination of cost in studies of genotypic expression of defense addresses genetic constraints and microevolutionary responses to selection. In contrast, examination of cost in studies of phenotypic expression of defense focuses on allocation patterns and cost of expressing particular phenotypes.

Assumptions

The OD hypotheses assume that: 1) there is genetic variation in secondary metabolites for selection to act upon; 2) herbivory is the primary selective force for production of secondary metabolites; and 3) defenses reduce herbivory.

In general, extensive data support the assumptions. For instance, studies have shown intraspecific genetic variation in amount and type of secondary metabolites (Dirzo and Harper 1982; Zangerl and Berenbaum 1990; Vrieling et al. 1993; van Dam and Vrieling 1994; Mauricio 1998). There is considerable circumstantial evidence that herbivory is a major selective force on plants. In grasslands about 33% of the production is consumed by herbivores, and over 60% of the seaweed production is consumed by herbivores (Hay 1991). Herbivory can adversely affect plant growth and reproduction (e.g., Marquis 1984; Wisdom et al. 1989; Strauss 1991; Karban and Strauss 1993). In general, loss to herbivores exceeds the allocation to reproduction (Mooney 1972). Of course, most plants can tolerate some herbivory without a reduction in fitness, and some species or populations can tolerate a great deal of herbivory (McNaughton 1983; Paige and Whitham 1987). Nonetheless, a vast array of secondary metabolites have evolved in plants, most of which seem primarily involved in plant defense against herbivores (Berenbaum 1995). Palatability is mainly determined by plant defenses. Furthermore, ingested plant defenses can be toxic to herbivores. For example, terpenes can inhibit ATP formation, alkylate nucleophiles, disrupt molting hormonal activity, bind with protein and sterols in the gut, and disrupt the nervous system (Langenheim 1994). Not surprisingly, there is direct evidence indicating that herbivores can act as selective agents for plant traits that reduce herbivory (Simms and Rausher 1989; Mauricio and Rausher 1997).

Hypothesis 2 and its Subhypotheses

Allocation Cost of Genotypic Defense (Hypothesis 2)

The major prediction is that a cost of defense to fitness results from diversion of resources from other needs ("allocation cost"). Accordingly, when enemies are absent, less well-defended individuals should have higher fitness than better defended individuals.

As expected, there are costs to biosynthesis of secondary metabolites (Mooney et al. 1983; Baas 1989; Gershenzon 1994a,b). Metabolic cost depends on substrate and cofactor sources, and defenses have additional costs such as transport and vacuole or resin duct construction (Gershenzon 1994a,b). In addition, turnover and recycling affect cost. Large molecule nonnitrogen-containing defenses, such as lignin, are not recycled (Mooney et al. 1983). Other defenses, such as terpenes, can be degraded and the products recycled into primary metabolism (Gershenzon 1994a,b). But turnover of defenses does not necessarily mean that limited nutrients, such as nitrogen, are recovered by the plant (Baldwin and Ohnmeiss 1994).

The cost of defense can be subject to evolution, such that natural selection operating on variation in cost among individuals could eliminate individuals with high fitness cost of defense (Simms 1992; Karban 1993). Examples are the proposed cost-reducing adaptations for terpenes: sharing biosynthetic enzymes among multiple pathways, minimization of enzyme turnover, use of a single enzyme to produce a mixture of products, catabolism of products no longer needed, and use of products for more than one function (Gershenzon 1994a).

Clearly there can be tradeoffs in allocation between defense and growth or reproduction (Vrieling and van Wijk 1994; Bergelson and Purrington 1996; Strauss et al. 2002). For example, an increase across genotypes in myrosinase (which acts on glucosinolates to form isothiocyanates) was correlated with increased resistance to herbivores but at the

cost of lower seed production (Mitchell-Olds et al. 1996). Tests are difficult when the genetic backgrounds of the susceptible and resistant genotypes differ in more ways than just a resistant gene. Through genetic engineering it is possible to conduct a more controlled experiment. For example, a resistant gene transplanted into a herbaceous plant reduced lifetime seed production by 34%, illustrating that the cost of resistance to enemies can be substantial (Bergelson et al. 1996).

But total cost of resistance is likely to reflect other mechanisms than just "allocation costs" (costs via diversion of energy and nutrients), in particular ecological costs (Purrington 2000; Strauss et al. 2002). Ecological costs of resistance include deterrence of mutualists (e.g., pollinators, mycorrhizal fungi), reduction in competitive ability, traits that deter one enemy but attract another, and traits that confer resistance against one enemy but constrain resistance to another.

Furthermore, evaluating the tradeoff between growth-reproduction and defense is complicated by the "third party" tradeoff with tolerance. As opposed to resistance (traits that reduce the amount of damage), tolerance refers to traits that reduce the impact of damage on plant fitness (Stowe et al. 2000). Tolerance to tissue damage and loss is an emergent property, reflecting intrinsic growth rate, storage capacity, allocation pattern, flexible photosynthetic rate, flexible nutrient uptake, and developmental plasticity (Rosenthal and Kotanen 1994). Just as secondary metabolites are a product of various selective pressures besides herbivory, so too is tolerance. Herbivory is probably the greatest selection pressure for tolerance (or basically regrowth capacity), however. Plants exhibit genetic variation for tolerance to herbivory (Fineblum and Rausher 1995; Mauricio et al. 1997), and tolerance can counter the potential negative impact of herbivory on reproduction (Maschinski and Whitham 1989).

Plants appear to have one of three strategies: well-developed defense and poor tolerance, well-developed tolerance and poor defense, or an intermediate of both (van der Meijden et al. 1988). For instance, damage had no effect on alkaloid concentration of a plant species that typically has little herbivory but maintains a high constitutive level of defense, whereas damage resulted in lower alkaloid concentration in a second species that suffers high herbivory but exhibits substantial regrowth, and damage increased alkaloids in a third plant species that has little herbivory but also slow regrowth (van Dam et al. 1993). Some evidence indicates that defense and tolerance covary negatively (Bilbrough and Richards 1993; Fineblum and Rausher 1995), but defense and tolerance can cooccur together (Rosenthal and Kotanen 1994; Mauricio et al. 1997).

We might expect a fitness cost to tolerance in the absence of herbivory. An example of a fitness cost to tolerance in the absence of herbivory is grazing-tolerant plants being competitively inferior to grazing-intolerant plants when herbivores are absent (Painter 1987). Costs of tolerance could arise directly—e.g., allocation costs, opportunity costs (small costs early in life that are compounded as plants age)—and indirectly (i.e., ecological costs, such as altering the soil microbial relationship with the plant).

The greater an allocation to tolerance ("a third party"), the more likely that any tradeoff between growth and tolerance, or between defense and tolerance, will be undetected (Mole 1994). Paying attention to such third party tradeoffs is especially important because allelochemicals often may require relatively small allocations of resources; thus, due to third party tradeoffs, measuring costs of defense is quite difficult (Mole 1994).

It may be especially difficult to demonstrate cost of defense to fitness for plants with the strategy of "well-developed tolerance and poor defense" and plants with the strategy of "intermediate tolerance and defense" because cost of tolerance may obscure fitness costs of defense. For example, if the scale for cost of tolerance is greater than that for defense, then an increase in defense will decrease the cost of tolerance disproportionately and, thus, decrease the total cost of tolerance and defense (Simms and Triplett 1994). This could yield a positive genetic correlation between defense level and fitness in the absence of herbivory; i.e., there would be the appearance of no cost of defense.

In sum, defenses do have a cost in terms of construction and maintenance, but there is likely to have been selection to reduce that cost. Tradeoffs in allocation to different functions (e.g., growth-reproduction versus defense) can occur, but other costs, such as ecological costs, may be as important or more important. The sum of defenses in the absence of herbivory may show a cost to fitness, but detection is complicated by the third party tradeoff with tolerance. Consequently, when no cost of resistance is found, we do not know if there is no cost, or whether the investigation is incomplete (which it most certainly will be). Thus, no study has emphatically concluded that there is no cost of resistance for the focal plant(s). It seems that a more useful approach is to focus on why certain traits cost as much as they do, effect of environmental conditions on the magnitude of cost, mechanisms and implications of cost between resistance and tolerance, and the sum benefit and cost of a trait that has negative effects on some enemies but positive effects on others (Purrington 2000; Hamilton et al. 2001).

Plant Apparency (Subhypothesis 2a)

The Plant Apparency Hypothesis (Feeny 1975, 1976; Rhoades and Cates 1976) is based on the optimal defense idea (Rhoades 1979). It predicts that apparent plants (e.g., oak trees) are easily found by herbivores and, therefore, these plants have a large investment in broadly effective defenses. These dosage dependent or "quantitative" defenses (Feeny 1976) have the characteristic of interfering with nutrient acquisition by herbivores and so are called "digestibility reducing" defenses (Rhoades and Cates 1976). The investment is predicted to be large because the molecules are large, and it may take a relatively high concentration to have a negative impact. Tannins are an example of a quantitative defense. These quantitative defenses slow the growth rate of herbivores, and so are predicted to subject them to higher rates of predation and parasitism (Feeny 1976). Even herbivores that naturally feed on such plant material are subject to the negative effect of quantitative defenses on growth. In contrast, unapparent plants (e.g., crucifers) have an unpredictable distribution, and so they are expected to often escape specialist herbivores. Consequently, these plants are predicted to invest in less costly defenses that are useful against nonadapted generalists. These "qualitative" defenses are expected to be less costly because they are relatively small molecules and toxic at low dosage (Feeny 1976). Glucosinolates, cyanogenic glycosides, and alkaloids are examples of qualitative defenses.

While certainly an appropriate and useful hypothesis at the time, evidence gathered does not fully support the Plant Apparency Hypothesis (Fox 1981; Futuyma 1983; Coley et al. 1985; Waterman and Mole 1989). In support of the hypothesis, there are correlations between apparency of plants and more generalized defense (Berenbaum 1981, 1983; Coley 1983), but there are also many examples that contradict the predictions. The arsenal of defenses of plants that fit the "unapparent" lifestyle may include quantitative defenses, and "apparent" plants may have qualitative defenses (Futuyma 1976). Although described as a quantitative defense, tannins are not all-purpose digestibility reducers (Martin et al. 1987) and can act as toxins (Steinly and Berenbaum 1985). Clearly many herbivore species have counteradaptations to tannin-rich food (Berenbaum 1980; Bernays et al. 1980; Schultz and Lechowicz 1986; Martin et al. 1987). Furthermore, for many insects the deterrent effect of tannin-containing food may reflect associated qualities (e.g., low nitrogen, low water, toughness) that render the food poor for herbivores (Bernays 1981). Then there are the putative "qualitative" defenses, such as terpenes. For some plant species, terpenes seem to be qualitative defenses, but for other species they seem to be quantitative defenses (Fox 1981). In addition, even at low concentrations, qualitative defenses may be costly, due to nitrogen investment (Baldwin et al. 1990) and rapid turnover rate.

In sum, the Plant Apparency Hypothesis was a useful framework when it was proposed, and it guided a number of studies that made significant contributions to the current understanding of plant defense, but the difficulty in trying to measure apparency (Feeny 1991) and the mixed support for the hypo-

thesis have prevented further profitable research along those lines.

Optimal Defense Within Plant (Subhypothesis 2b)

Another subhypothesis of the OD hypothesis is that, within a plant, defenses are allocated in direct proportion to the risk of the particular tissue to herbivory and the value of that tissue in terms of loss of fitness (McKey 1974, 1979; Rhoades 1979).

In general, data support the idea that defenses are allocated in proportion to risk of tissue to herbivory (Bryant et al. 1983; Zangerl and Bazzaz 1992; Baldwin and Schmelz 1994; Baldwin and Karb 1995; van Dam et al. 1995; Wallace and Eigenbrode 2002). For example, the reproductive parts of wild parsnip, which had an estimated high probability of being attacked and greatest value to fitness, had the highest constitutive level of a toxic furanocoumarin, and the furanocoumarin was not inducible there (Zangerl and Rutledge 1996). Roots of wild parsnip, which were the least likely plant part to be attacked, had the lowest constitutive level of the furanocoumarin but were highly inducible. Leaves, which had a high probability of attack, had intermediate constitutive and inducible levels.

Examining this hypothesis requires measurement of three factors: 1) value of plant part, 2) benefit of defense, and 3) probability of attack (Zangerl and Bazzaz 1992; Hamilton et al. 2001). Value of plant part can and has been measured, but usually studies just infer different values of plant parts based on the generalization that reproductive parts carry higher value to plant fitness than nonreproductive parts (Zangerl and Bazzaz 1992; Hamilton et al. 2001). To determine benefit of defense would require having plants with and without defense exposed to herbivores (Hamilton et al. 2001), which is not feasible (until genetic manipulation can eliminate a plant's defense system). Instead researchers have assumed that a correlation between fitness and defense of plants exposed to herbivores is evidence for a benefit of defense proportional to defense level (Hamilton et al. 2001). But that is problematic. For example, synergerism among defenses can be high (Wittstock and Gershenzon 2002), and so benefit of defense and defense level may not correlate. In addition, to conduct a test, the same kind of defense must occur throughout the plant, so that cost of defense and effectiveness of defense only vary with level of defense (Zangerl and Rutledge 1996). Consequently, it may only be possible to conduct tests with systems in which the defensive array is relatively simple (e.g., wild parsnip). The few tests that have been done were with herbaceous temperate species (Hamilton et al. 2001). Estimating probability of attack is seldom done (but see Zangerl and Rutledge 1996) and is difficult to do with any confidence (Hamilton et al. 2001). Importantly, several populations with different levels of herbivory should be measured.

So while the logic of the Optimal Defense Within Plant Hypothesis is sound, which is based on generalities about within-plant distributional patterns of secondary metabolites and plant part function, in truth the empirical evidence is limited due to what we can realistically measure. Nonetheless, at this point in time, it seems reasonable to accept the idea that defense allocation among plant parts of herbaceous species reflects cost:benefit patterns in plant fitness.

Inducible Defense (Subhypothesis 2c)

Another subhypothesis of the OD hypothesis describes inducible defenses. Inducible defenses refer to defenses produced in response to damage and so defense levels rise above the baseline or constitutive level. Because defense is predicted to be a costly commitment, defense should be reduced when herbivores are absent and increased when plants are subject to attack (Rhoades 1979). Furthermore, because defense is predicted to be costly, the relaxation time of induced defenses is expected to be short. "Relaxation time" refers to how long it takes the level of induced defense to drop to the baseline or previous constitutive defensive level. Thus, the "defense view" of induction is that changes in phytochemistry in response to herbivory have been shaped over evolutionary time as a defense (Rhoades 1979; Haukioja and Neuvonen 1985).

Rapid induction of specific defenses in

response to herbivory (within hours, followed by relaxation over days or weeks) can be explained by the OD induction prediction. An example is the production of proteinase inhibitors in response to a wound-signaling mechanism within hours of damage in tomato leaves (Green and Ryan 1972; Broadway et al. 1986). Herbivory triggers release of systemin, which activates the jasmonic acid pathway, which in turn increases products of 15 or more genes, including proteinase inhibitor (Bergey et al. 1996). Interference in this process (via mutant genes) results in less proteinase inhibitor (Orozco-Cardenas et al. 1993) and more feeding and growth by insect herbivores (Howe et al. 1996). Other studies have shown that induced plant responses to herbivory or jasmonic acid provided higher levels of defense that resulted in greater seed production compared to controls (Agrawal 1998; Baldwin 1998).

Induced resistance can result in an allocation cost. Seed production in tobacco plants was not reduced in damaged plants in which alkaloid production was inhibited by auxin, but it was reduced in plants that increased alkaloid production in response to damage (Baldwin et al. 1990). In addition, even in the absence of enemies, inducible defenses may have allocation costs and opportunity costs, via the requirement of maintaining wound-detection pathways and defense precursors (Cipollini 1998; DeWitt et al. 1998; Purrington 2000). Induced resistance may also have ecological costs (Strauss et al. 2002).

A key issue in testing hypotheses about induction is an estimate of probability of attack (Karban and Baldwin 1997). Plants with a low probability of attack are expected to exhibit greater inducibility than those with a high probability of attack because the latter would benefit more by high levels of constitutive defense. But probability of attack is difficult to assess and can result in misinterpretation if reciprocal transplanting is not done. For instance, based on the OD induction subhypothesis, the expectation is that populations subject to high herbivory would exhibit high levels of constitutive defense and low levels of inducibility, whereas populations subject to low herbivory would exhibit low levels of constitutive defense and be highly inducible. Comparison of two populations of wild parsnip indicated that one was subject to frequent herbivory (42%) in contrast to the other (7%), but a transplant experiment showed that these differences reflected the sites (or habitats), since those levels of herbivory occurred at the sites regardless of the population source (Zangerl and Berenbaum 1990). Furthermore, the two populations did not differ in inducible levels and only in two of four constitutive defense levels (and apparently those differences were not biologically significant, as herbivory levels reflected site rather than population differences).

A prediction of the OD induction subhypothesis is that among species there should be a negative correlation between the level of constitutive defense and level of inducibility. Some data support that prediction, but in many other cases the data indicate either a positive correlation or no correlation (reviewed by Karban and Baldwin 1997). Even between populations, there may be no correlation among constitutive levels, induced levels, and level of herbivory (Zangerl and Berenbaum 1990). One explanation for a lack of negative correlation between constitutive and inducible defense levels is that perhaps plants do not take on a particular phenotype matched to a particular environment (e.g., increased defense in response to increased herbivory). In contrast, plants may respond to attack by simply changing phenotypes (levels of defenses within the defensive array change, which changes food quality) and, thus, plants present a "moving target" to enemies (Adler and Karban 1994). In the only clear test of this idea, natural populations of a postfire annual exhibited defensive changes in phenotype that matched the predictions of the OD Inducible Defense Hypothesis rather than the Moving Target Hypothesis (Baldwin 1998). Jasmonate-induced defenses reduced seed production (so had a cost to fitness), but having an inducible defense allowed the plants to avoid that cost when plants were not attacked (i.e., defenses were not induced) (Baldwin 1998). Interestingly, as the postfire environment aged and, in turn, herbivory increased, the nature of the induced defense benefits changed from that of greater seed produc-

tion in the first year after a burn, compared to noninduced plants, to greater survival to the stage of seed production in the second year.

Most likely the studies with a lack of negative correlation between constitutive and inducible levels of defense reflect the evolutionary and ecological complications of having a defense system that can handle a wide variety of enemies, but which in turn carries ecological costs (Agrawal and Karban 1999). For example, insect herbivores in the same feeding guild caused different inducible patterns: strong resistance, no resistance, or increased susceptibility (Agrawal 2000). For some plants, induced resistance against some pathogens confers resistance to other pathogens, but not to arthropod herbivores (reviewed in Agrawal and Karban 1999; Agrawal 2000). Likewise, induced resistance against herbivores may not confer resistance against pathogens. For some plants, in response to some enemies, the inducible defense pathways may be separate and for others there may be some "cross-talk" among pathways (Agrawal and Karban 1999; Felton and Korth 2000; Heil and Bostock 2002; Kunkel and Brooks 2002).

An alternative explanation for induced resistance is that it simply reflects physiological change due to tissue damage rather than an evolved defense. Strictly speaking, induction refers to a change in phytochemistry in response to stress, which can include changes in secondary metabolism (Karban and Baldwin 1997). In many cases, increases in concentration of secondary metabolites may be an unavoidable consequence of changes in primary physiology and with no functional importance to the plant (Gershenzon 1984). Furthermore, induction does not necessarily result in deterring herbivores and improving plant fitness (Fowler and Lawton 1985; Karban and Myers 1989; Karban and Baldwin 1997).

In the case of delayed induction (responses occurring in the season(s) after damage), the relaxation time is generally long (e.g., a year or more) (Rhoades 1979; Tuomi et al. 1984). In general, insect herbivore performance is less on trees with delayed induced responses compared to controls, but there are exceptions (reviewed by Karban and Baldwin 1997). Even though the phenomena of delayed induction and slow relaxation time do not fit the OD induction prediction (Rhoades 1979), they actually fit the general statement of the OD hypothesis (i.e., any observed defensive pattern is possible if it is adaptive) (Haukioja and Neuvonen 1985). This duality in predictions illustrates one reason why the basic OD hypothesis has been difficult to test and, consequently, has been a source of confusion about progress with the theory of plant defense.

Allocation Cost of Phenotypic Defense (Subhypothesis 2d)

This subhypothesis states that because allocation to defense results in less allocation of energy and nutrients to other needs (e.g., growth and reproduction), environmentally stressed plants should be less well defended than unstressed individuals (Rhoades 1979). Furthermore, under stress, plants are expected to decrease commitment to costly defenses and increase commitment to less costly but accordingly less effective defenses.

It may be that fitness costs are only significant at certain developmental stages (e.g., reproduction) or in stressful situations (Baldwin et al. 1990; Briggs and Schultz 1990; Agren and Schemske 1993; Bergelson 1994; Gershenzon 1994b). A meta-analysis that showed negative correlation of defense and plant fitness under uncontrolled environmental conditions and persistence in the presence of herbivores suggests that a cost of defense to fitness may often occur through interaction of plants and various components of their environment (Koricheva 2002). Responses to damage are strongly affected by the source-sink relationships within a plant, and tolerance decreases with nutrient stress (Chapin and McNaughton 1989; Maschinski and Whitham 1989). In general, stressed individuals have low defense levels (reviewed in Herms and Mattson 1992). But as will be explained later, the CNB and GDB hypotheses have more explicit explanations for pattern of defense across resource gradients than does the OD view. It is not clear whether commitment to defense consists of less of all defenses or less of the more costly and effective ones, as predicted.

In sum, the results are mixed for support of the subhypotheses of the OD view. Basically, the observed patterns are more complicated than originally envisioned. Of more interest now than cost of defense is *how* plants evolve and balance an array of defenses complementing an array of tolerance traits to withstand a complex of enemies within the framework of optimizing growth and reproduction. Nonetheless, all of the results are consistent with the OD statement that plants "evolve and allocate defenses in the way that maximizes individual inclusive fitness" (Rhoades 1979:12). This framework and the ensuing research led to the development of other hypotheses of plant defense.

THE CARBON:NUTRIENT BALANCE HYPOTHESIS

The Carbon:Nutrient Balance (CNB) Hypothesis (also called the Environmental Constraint Hypothesis) is a model of how the supply of carbon and nutrients in the environment influence the phenotypic expression of defense by plants (Bryant et al. 1983; Tuomi et al. 1988, 1991). Originally it was developed to explain the influence of soil nutrients and shade on plant defensive chemistry via the effects on the carbon:nutrient ratio of the plant (Bryant et al. 1983). Basically, the hypothesis is that if the carbon:nutrient ratio acquired by a plant controls allocation of resources to plant functions, then the phenotypic expression of that plant's genetic potential for defenses will be affected. The variation of phenotypic expression in defense among genotypes may range from none (complete genetic determination) to substantial (great plasticity in response to environmental conditions). For example, a species with a high constitutive level of defense that typically grows in nutrient poor soil may be well defended because: 1) herbivory in such sites selected for such genotypes, 2) chronic carbon surplus is shunted into allelochemicals, or 3) some combination of these (Tuomi et al. 1988, 1991).

Some of the confusion and controversy in the literature about the CNB hypothesis stems from conflicting statements in the original descriptions of the hypothesis. Some statements in the descriptions suggest that the original view was that *all* defensive production occurs *after* growth requirements are met; e.g., "it implies no reduction in growth to support carbon-based resistance" and "allocation to secondary metabolite production occurs only when growth demands for carbon have been met" (Tuomi et al. 1988:59). But other statements suggest that the original view was that a plant could produce some defenses to meet a fixed allocation (in conjunction with growth) and produce some defenses via flexible allocation (altered carbon:nutrient ratio yielding carbon surplus, which is shunted into defense). For instance, "Some compounds may show a fixed allocation pattern, whereas others may depend more on the constraints limiting plant primary metabolism" (Tuomi et al. 1991:92). "Thus, a model with both P_1 [proportion of carbon invested in defense when it could be allocated to growth] and P_2 [proportion of carbon surplus beyond growth requirements allocated to defense] could presumably better describe actual allocation of carbon between defense and growth. In such a model, active defense allocation could determine some background level of defense investments (i.e., P_1) that imply costs on growth. This background level of defense could also be modified by selection . . . Plant carbon/nutrient balance could thus be a factor that accounts for a part of the phenotypic variation in constitutive levels of carbon-based allelochemicals" (p 90).

Since the original descriptions of the CNB hypothesis, however, researchers have focused on the former view (i.e., allocation to defense comes *after* growth, therefore no cost to defense). Much of the criticism of the CNB hypothesis (Karban and Baldwin 1997; Hamilton et al. 2001) reflects the problems with that interpretation. But the original descriptions anticipated some of the criticisms in a way that suggests that the latter view (constitutive defense = fixed and flexible allocations) is what the authors of the CNB hypothesis intended.

Following up on the view that a plant's defense accrues from a combination of fixed allocation and flexible allocation, there are two scenarios to explain constitutive defense levels. The first, "baseline plus," is that plants may have a fixed baseline allocation to

defense (proportional to growth), the carbon:nutrient balance may be affected by environmental conditions, carbon surplus (beyond use for growth) may be shunted into defense (flexible allocation), and thus defense at any point in time is a combination of the baseline allocation and flexible allocation. The second, "variable plus," is that plants may have a fixed allocation to defense, but this allocation is proportional to both growth and carbon surplus; the carbon:nutrient balance may be affected by environmental conditions, carbon surplus (beyond use for growth) may be shunted into defense (flexible allocation), and thus defense at any point in time is a combination of the shifting-but-fixed allocation and flexible allocation. Either way, a plant's response to changes in the carbon:nutrient ratio can alter the phenotypic expression of the plant's genetic potential for defense.

Assumptions

The CNB hypothesis has several assumptions. It assumes that carbon gain and growth depend on the mineral nutrient reserves of a plant, that carbon is allocated to growth whenever the supply of mineral nutrients is adequate, and that carbon accumulated beyond the level used for growth is allocated to defense or storage; data support these assumptions (Tuomi et al. 1988). The CNB hypothesis also assumes that growth is inhibited more by nutrient limitation than is photosynthesis (Bryant et al. 1983; Tuomi et al. 1988; Tuomi 1992); data support this idea as well (reviewed by Bryant et al. 1983; Tuomi et al. 1988; Luxmoore 1991; Herms and Mattson 1992). Lastly, the CNB hypothesis assumes that herbivory is a primary selective force for constitutive secondary metabolites and that defenses reduce herbivory (Bryant et al. 1983; Tuomi et al. 1988), but it does not assume that the total amount or general type of defense (nonnitrogen-containing versus nitrogenous) is selected for by herbivory (Tuomi et al. 1988).

Although a recent review claims that the fundamental assumptions of the CNB hypothesis are now known to be "incorrect" (Hamilton et al. 2001), the so-called "assumptions" were not presented accurately. It is *not* an assumption of the CNB hypothesis that "the ability of a plant to synthesise a defence compound can be predicted based on the atoms contained in the compound relative to the availability of these atoms in the plant" (Hamilton et al. 2001:89). No such statement or implication is made in the CNB hypothesis papers (Bryant et al. 1983; Tuomi et al. 1988, 1991), nor is such an assumption required. What the reviewers are referring to is a *prediction* based on the CNB hypothesis that, for example, shade will decrease the carbon:nutrient ratio and thus cause an increase in internal nitrogen available for defense and storage. It is also *not* an assumption "that growth always takes priority over secondary metabolism in allocation of resources" (Hamilton et al. 2001:89); in other words, that growth takes priority over any level of defense including genetically determined baseline defense. What Tuomi et al. state is that "carbon is allocated to growth whenever there are sufficient mineral nutrients to construct new cells, and . . . the carbon surplus accumulated above the levels required for growth is allocated among different carbon-based allelochemicals and/or carbohydrate storage" (1988:59). This statement refers to the phenotypic expression of defense affected by the carbon:nutrient ratio and not to the level of defense in place due to intrinsic genetic determination. Statements elsewhere make this clear (Bryant et al. 1983; Tuomi et al. 1988:60–61, 1991:90–92). It is *not* an assumption "that simple mass-action drives rates of secondary metabolite production (Reichardt et al. 1991) implies that plants have little ability to control their chemical composition" (Hamilton et al. 2001:89). The CNB hypothesis acknowledges that carbon surplus is allocated to defense and/or storage (and thus regulated), and that surplus shunted into defense involves regulation of synthesis pathways; thus, precursors, substrates, and products are under control of the plant (Bryant et al. 1983; Tuomi et al. 1988, 1991). Finally, it is *not* an assumption of the CNB hypothesis that "an effect of environment is independent of the action of genes" (Hamilton et al. 2001:91). The CNB hypothesis acknowledges that plant defense is programmed genetically and that plant species exhibit a range of plas-

ticity in expression of levels and array of defenses, which is a genetically determined response to the environment (Bryant et al. 1983; Tuomi et al. 1988, 1991). Contrary to the Hamilton et al. (2001) interpretation, the CNB hypothesis has its roots in the ideas of Mooney (1972), Grime (1977, 1979), and Chapin (1980) about the effects of environmental resources on the evolution of plant functions (Bryant et al. 1983). Much of the Hamilton et al. (2001) viewpoint about assumptions and predictions of the CNB hypothesis seems to be based on the discussion of Karban and Baldwin (1997:75–77), which also provides some misleading statements (e.g., that the CNB hypothesis presents induced metabolites as being essentially waste products).

Predictions

The CNB hypothesis makes specific and testable predictions about how stressful environments affect the amount and general type of plant defense (Bryant et al. 1983). "General type of defense" refers to nonnitrogen-containing versus nitrogenous. *For genotypes with little or no phenotypic plasticity in defense,* the prediction is that any effects of resource conditions on the carbon:nutrient ratio of a plant do not translate into a change in defense levels. For example, woody plants adapted to low resource situations are expected to have a low intrinsic growth rate and therefore low capacity for compensatory growth after herbivory, which in turn would favor selection for maintenance of high defense levels (i.e., complete genetic determination of defense) and carbon surplus into storage rather than defense. *For genotypes with phenotypic plasticity in defense,* the prediction is that any effects of resource conditions on the carbon:nutrient ratio can cause a change in the total defense level. More specifically, the predictions are that fertilization or shade will decrease the carbon:nutrient ratio of a plant, reducing the excess carbon production and, consequently, decreasing nonnitrogen-containing defenses while increasing the availability of assimilated nitrogen for defense. That is, high levels of nitrogenous defenses reflect nitrogen assimilated in excess of growth requirements and, vice versa, high levels of nonnitrogen-containing defenses reflect an accumulation of excess carbon production. For high plasticity genotypes, the CNB hypothesis assumes that carbon surplus is "cheap" (Bryant et al. 1983), and so predicts that "secondary compounds are not costly" (Tuomi et al. 1988:67, 1991:89). This refers to the production of secondary metabolites from carbon surplus, however.

Importantly, the amount of defense above the baseline genetic level will reflect the carbon surplus, whereas specific types of allelochemicals presumably reflect, in part, selection over time for functions such as defense against pathogens and herbivores. Consequently, the amount and array of secondary metabolites are not expected to map precisely to function (Tuomi et al. 1988, 1991). Furthermore, the CNB hypothesis does not predict which allelochemicals will be produced, but because the limiting nutrient is usually nitrogen, the carbon:nutrient balance of a plant determines the production and accumulation of nonnitrogenous and nitrogenous defenses (Tuomi et al. 1988).

The CNB hypothesis provides specific predictions about when plants may respond to damage by increasing production of secondary metabolites (Tuomi et al. 1988). The nutrient stress view of induction, derived from the CNB hypothesis, is that herbivory can disrupt the carbon:nutrient balance, which may lead to a nonspecific accumulation of nonnitrogen-containing defenses and an associated decline in leaf protein (Bryant et al. 1983; Tuomi et al. 1984). That is, there is an optimal balance between internal reserves of carbon and mineral nutrients that promotes maximal growth (Chapin 1980; Ingestad 1982). Defoliation reduces a plant's ability to gain carbon and may affect carbon and nutrient stores. Consequently, the carbon:nutrient balance may be altered.

The alteration may or may not result in accumulation of secondary metabolites (Tuomi et al. 1991). Whether an accumulation of nonnitrogen-containing defenses occurs depends on where carbon is stored and the availability of soil nutrients (Tuomi et al. 1988). For example, evergreen trees store carbon reserves in their leaves, and thus damage to leaves (because it removes carbon

reserves) should result in a lower carbon:nutrient ratio and, even with high nutrient availability, these trees would not have carbon reserves to increase defenses. In contrast, deciduous trees store carbon reserves in stems and roots, and damage to their leaves should result in a marked and long-term increase in nonnitrogen-containing defenses when nutrients are in short supply, but less so when nutrients are more plentiful and the predamage carbon:nutrient ratio can be restored quickly. In these scenarios, the change in defense is simply passive deterioration of leaf quality due to an altered carbon:nutrient ratio for the plant. Consequently, long-term induction can occur over a few years, followed by a gradual relaxation over a few years, which may reflect the population dynamics of the herbivores (Tuomi et al. 1991). Of course, whether a plant genotype exhibits these patterns depends on the degree of phenotypic plasticity in defense.

Just as there has been confusion about the assumptions of the CNB hypothesis, there has been confusion about the predictions. An example of that is the misleading statement that "the predictions of this theory for a rapidly induced response involving the putatively nitrogen-intensive metabolite nicotine have been unambiguously falsified" (Karban and Baldwin 1997:76–77). This is misleading in three respects. First, the CNB hypothesis does *not* make predictions about wound-induced short-term rapid responses: "The carbon/nutrient balance explanations of induced responses are most unlikely in conditions where the responses are triggered by chemical cues . . . in wound-induced short-term responses" (Tuomi et al. 1991:99); "specific defensive responses are associated with the enzymatic regulation of secondary metabolism, whereas the plant carbon/nutrient balance mainly affects the amount of precursors and substrates available to the synthesis of secondary metabolites" (p 99); and "short-term as well as long-term inducible responses that spread from attacked to nonattacked parts of the plant can less likely be attributed to changes in the carbon/nutrient balance" (p 98). Second, in the study that Karban and Baldwin (1997) referred to, Ohnmeiss and Baldwin state, "[W]e interpret C/N theory to predict that as the internal C:N ratio increases there should be a concomitant decrease in the allocation to nitrogen-based secondary metabolites in order for RGR to be maximized" (1994:996). But actually what the CNB hypothesis predicts is that, relative to constitutive defense, plant species can have some combination of fixed and flexible allocation, varying between having complete fixed allocation to complete flexible allocation. Consequently, data gathered to test Ohnmeiss and Baldwin's (1994) prediction cannot be evaluated without knowing what the fixed and flexible defense allocation patterns are for the test species. These examples of misapplication are quite typical of the research on the CNB hypothesis.

Evidence

Numerous studies (200+; e.g., 147 cited by Koricheva et al. 1998) have been used to examine the CNB hypothesis. Overall, the results are equivocal. For instance, the evidence for the CNB hypothesis consists of some studies that show that fertilization can decrease concentration of nonnitrogen-containing secondary metabolites and increase concentration of nitrogenous secondary metabolites, and that shade can decrease nonnitrogen-containing defenses and increase nitrogenous defenses. Just as many studies have been interpreted as contradictory evidence.

Part of the problem with interpretation of the results of these studies is a misunderstanding of the hypothesis and, consequently, misstatement of the predictions. For example, a recent review stated, "In an early test of CNB, Bryant and coworkers determined that a slow-growing tree species adapted to a resource-limited environment did not respond as predicted (Bryant et al. 1987)" (Hamilton et al. 2001:88). That statement is a misinterpretation of the data and conclusion of Bryant et al. (1987), however, which in fact supported the CNB hypothesis. The slow-growing tree species typically growing in shaded and less fertile sites was predicted to show little plasticity in production of secondary metabolites, with fertilization and shading treatments compared to a fast-growing tree species typically growing in sunnier and more fertile sites. The pattern that Bryant at el. (1987)

found basically fit the prediction. The misinterpretation by Hamilton et al. (2001) lies in the expectation that *all plants* subject to fertilization or shading should exhibit a change in the carbon:nutrient ratio that in turn *results in less nonnitrogen-containing secondary metabolites.* That is not what the CNB hypothesis predicts. The hypothesis allows for genotypes with limited or even no phenotypic changes in defense in response to alteration in the carbon:nutrient ratio (i.e., species with complete genetic determination of defenses and therefore limited or no plasticity in "constitutive" defense levels) (Bryant et al. 1983; Tuomi et al. 1988, 1991). Importantly, the hypothesis allows for carbon surplus to be allocated to storage and/or defense.

A meta-analysis of 147 studies suggested that the concentration of pooled nonnitrogen secondary compounds and carbohydrates responded to nutrients, shade, and carbon dioxide enrichment as predicted by the CNB hypothesis, but that the concentration of particular compounds, such as hydrolyzable tannins and terpenoids, did not (Koricheva et al. 1998). However, such meta-analysis is only conclusive if the individual studies are free of methodological problems, which is not the case.

For instance, most studies only examined the changes in a few of the nonnitrogenous and nitrogenous phytochemicals. But a measured secondary metabolite may not be correlated with total secondary metabolite production (Zangerl and Berenbaum 1987). That is, it may be that the levels of some secondary metabolites are "fixed" genetically, whereas the levels of others are affected by environmental conditions and so reflect the carbon:nutrient ratio (Tuomi et al. 1991). The defensive patterns in the studies that made a strong case for having evaluated most of the major defenses concluded that there was support both for and against the CNB hypothesis; however, the predictions were in terms only of genotypes with phenotypic plasticity (Folgarait and Davidson 1994, 1995). Furthermore, none of the studies clearly estimated total defense in terms of the carbon:nutrient ratio of the plant *and* relative to the baseline genetic defense, which is what is required for a full evaluation of the CNB hypothesis. There is also the problem of erroneous conclusions due to measuring defensive concentrations (which are a function of plant biomass) when the issue is allocation patterns of molecules per plant (Koricheva 1999). Then there is the difficulty of measuring production of secondary metabolites that are dynamic or constantly turned over. It has been suggested that concentration of "static" defenses, such as condensed tannins, should fit the CNB hypothesis, whereas concentration of more "dynamic" defenses, such as terpenes, may not (Reichardt et al. 1991). Only about half of the studies with data applicable to the CNB hypothesis (e.g., as cited in Koricheva et al. 1998) support the idea that concentration of static defenses fits the CNB hypothesis. But even so, the CNB hypothesis is about a plant's carbon surplus; it does not (nor was it formulated to) predict levels of particular types of defense (Bryant et al. 1983; Tuomi et al. 1988).

It is difficult to evaluate change in the carbon to nitrogen resources within a plant. In a study that tried to examine such changes, with "carbon" being equivalent to starch plus the nonnitrogen-containing allelochemicals that were measured, enhanced CO_2 increased the "carbon":nitrogen ratio for aspen, oak, and maple (Kinney et al. 1997). High nitrate decreased the "carbon":nitrogen ratio for aspen and maple but not for oak. But even this approach leaves the question unresolved.

Some of the explanations for the equivocal results to the CNB hypothesis have been attributed to the mechanisms of secondary metabolite production. When an increase in defense requires an increase in both carbon and nitrogen, an increase in defense will not occur with a change in just carbon or nitrogen. For example, sufficient enzyme activation is also necessary to convert an accumulation of substrate to secondary metabolites (Gershenzon 1994a). No change in the concentration of a secondary metabolite in response to a change in the carbon:nutrient ratio may occur anytime there are multinutrient-requiring structures necessary to synthesize, transport, and sequester defenses (Gershenzon 1994b). Furthermore, allocation tradeoffs for carbon and nitrogen probably occur among metabolic pathways and

even within pathways (Folgarait and Davidson 1995; Jones and Hartley 1999). This would affect the amount and type of defenses produced (Muzika 1993). For example, it has been suggested that limited nitrogen affects phenolic production more negatively than terpene production (Muzika and Pregitzer 1992; Lambers 1993). Also, some chemical defenses require a delivery or canal system, which may be made from cells such as laticifers (Dussourd 1993), and production of these would require carbon and nitrogen.

Typically what is left out of the discussion is that phenotypic plasticity in defense due to the effects of the carbon:nutrient ratio is only predicted by the CNB hypothesis for genotypes programmed to put carbon surplus into defense. The hypothesis allows that some species will have primarily fixed allocation. The level of secondary metabolite production does seem to be genetically fixed for some species (Holopainen et al. 1995). Specifically, in the case of terpenes, it appears that some plant species or populations of plants exhibit much less variation in terpene production in response to environmental conditions than others (Muzika et al. 1989).

Consequently, to test the CNB hypothesis, it is first necessary to establish the baseline genetic defense. That is, what is the level and array of defenses at the optimal nutrition and maximal growth rate for the plant species? No studies used to evaluate the CNB hypothesis have established this. To achieve optimal nutrition and maximal growth rate, and then examine effects of limiting resources, requires conducting "steady-state" nutrition experiments (Ingestad 1982, 1991; Ingestad and Lund 1986).

Rapid induction of specific defenses in response (within hours followed by relaxation over days or weeks) to herbivory cannot be explained by the nutrient stress view (carbon:nutrient balance) (Tuomi et al. 1991), but does fit the defense view (OD hypothesis). For example, large amounts of monoterpenes are produced after wounding of grand fir, which are different than the constitutive monoterpenes; furthermore, the enzymatic machinery for these induced monoterpenes, which are effective against fungi and herbivores, is only apparent several days after wounding (Gershenzon and Croteau 1991; Gijzen et al. 1992). Rapid induction of specific defenses is clearly not simply chemical outcomes from a shift in the carbon:nutrient balance due to loss of acquired resources, but then the CNB hypothesis does not predict that it is.

Thus, studies suggest that neither the nutrient stress view (CNB hypothesis) nor the defense view (OD hypothesis) alone adequately account for patterns of induced resistance (Haukioja and Neuvonen 1985; Hammerschmidt and Schultz 1995). These hypotheses are difficult to test because some induced chemicals may represent passive response to damage (carbon:nutrient balance), some may reflect active response (selection for defense), some may be involved in both passive and active responses, and then of course there may be no induction (which can be explained by either the nutrient stress or defense views). Therefore, the emphasis should be on describing and understanding the passive, active, and interaction of passive-active responses in plant species chosen for contrasting patterns. Again, it is particularly important to establish the baseline defense (under optimal nutrition and maximal growth conditions) first because without this frame of reference, it will be difficult (if not impossible) to evaluate plant responses in terms of the CNB hypothesis.

In sum, there is much research purporting to test or provide insight on the CNB hypothesis, and there is also some derision of the hypothesis, much of which is flawed by misunderstanding. Although evidence relating to the CNB hypothesis suggests that the relationship between available resources and plant defense is more complicated than the original CNB hypothesis predicts, the tests are on subhypotheses and none have first established defense (fixed and flexible) allocation patterns at optimal nutrition and maximal growth. The CNB hypothesis brought attention to how resources influence both constitutive and induced plant defenses, however, and it contributed to the next hypothesis.

THE GROWTH RATE HYPOTHESIS

The Growth Rate (GR) Hypothesis addresses genotypic variation in plant defenses, via

resources shaping inherent growth rate and, subsequently, the constraints on defense against herbivores (Coley et al. 1985). Growth Rate Hypothesis is the term used by Coley (1987a,b); others have referred to it as the Resource Availability Hypothesis, but this term does not distinguish it from the GDB hypothesis or the OD Allocation Cost of Defense view (Subhypothesis 2d). The GR hypothesis is that among plant species, as maximal growth rate (at optimal resources) decreases, the constitutive level of defense increases (Figure 1). Note that there is a problem with the *x*-axis of Figure 1, in that biologically 100% of leaf mass cannot be invested in defense. Either the curves can only extend partway across the *x*-axis (from 0 up to some maximal investment that allows other leaf functions), or the *x*-axis should be renamed "Defense investment (*g actual defense/g potential possible defense per g leaf*)." Either way, it does not change the basic hypothesis. Importantly, plant growth rate is the balance between a reduction in growth due to defense costs and an increase in growth due to protection from herbivores (Coley et al. 1985).

Assumptions

The Growth Rate (GR) Hypothesis has several assumptions. It assumes that maximal relative growth rate is determined by resource availability, and so the inherent growth rate of plants matches the resource limitation in the preferred habitat. Data support this (Coley et al. 1985). The GR hypothesis assumes that herbivory is an important selective force for production of secondary metabolites and that defense has a cost. Data again support this (discussed in an earlier section). It assumes that herbivores consume plant mass as a function of herbivore mass (referred to as a "fixed amount") rather than as a fixed percentage of plant productivity, which is a reasonable assumption. It also assumes that slow turnover of plant parts is advantageous in low-nutrient environments and fast turnover of plant parts is advantageous in high-nutrient environments. Data support this (Coley et al. 1985). The idea is that fast-growing species have short-lived leaves because energy acquisition in nutrient-rich environments is maximized by rapid leaf turnover, whereas slow-growing species have long-lived leaves because slow turnover is advantageous in a nutrient-poor environment where (re)growth is constrained.

Predictions

The basic prediction is that among plant species, as maximal growth rate (i.e., intrinsic growth rate at optimal resources) increases, the level of constitutive defense should decrease. A second prediction is that fast-growing species exhibit more sharply defined peaks in the defense investment versus realized growth-rate curves, which means that "deviations from the optimal defense levels have a larger negative impact on realized growth rate than they would for slow-growing species" (Coley et al. 1985:897; Figure 1). Optimal defense level would refer to the expression of defenses at the maximal growth rate with optimal nutrition for each plant species. In practice, the predictions have been reduced to: 1) in high resource environments (e.g., agroecosystems, old-field habitats), competition favors fast-growing plant species, and so to grow fast means allocating little to defense; and 2) in low resource environments (e.g., dry or shaded habitats), resource limitation favors slow-growing plant species, which should allocate more to defense (Coley et al. 1985). This latter group cannot compensate easily for herbivory because of their slow growth, so replacement of resources lost to herbivores is more costly for plants in low resource environments.

Coley et al. (1985) make some additional predictions. One prediction is that, via more invested in defense, the actual level of herbivory experienced by slow-growing species is less than that of fast-growing species. If fast-growing species are subject to more herbivory because the constitutive defensive level is relatively low, then another prediction is that fast-growing species have greater defensive plasticity than slow-growing species. Fast-growing species are predicted to have defenses that include more secondary metabolites that are mobile, have high turnover rates, and thus have a reversible commitment to defense. In contrast, slow-growing species are predicted to have immobile defenses and, thus, a fixed

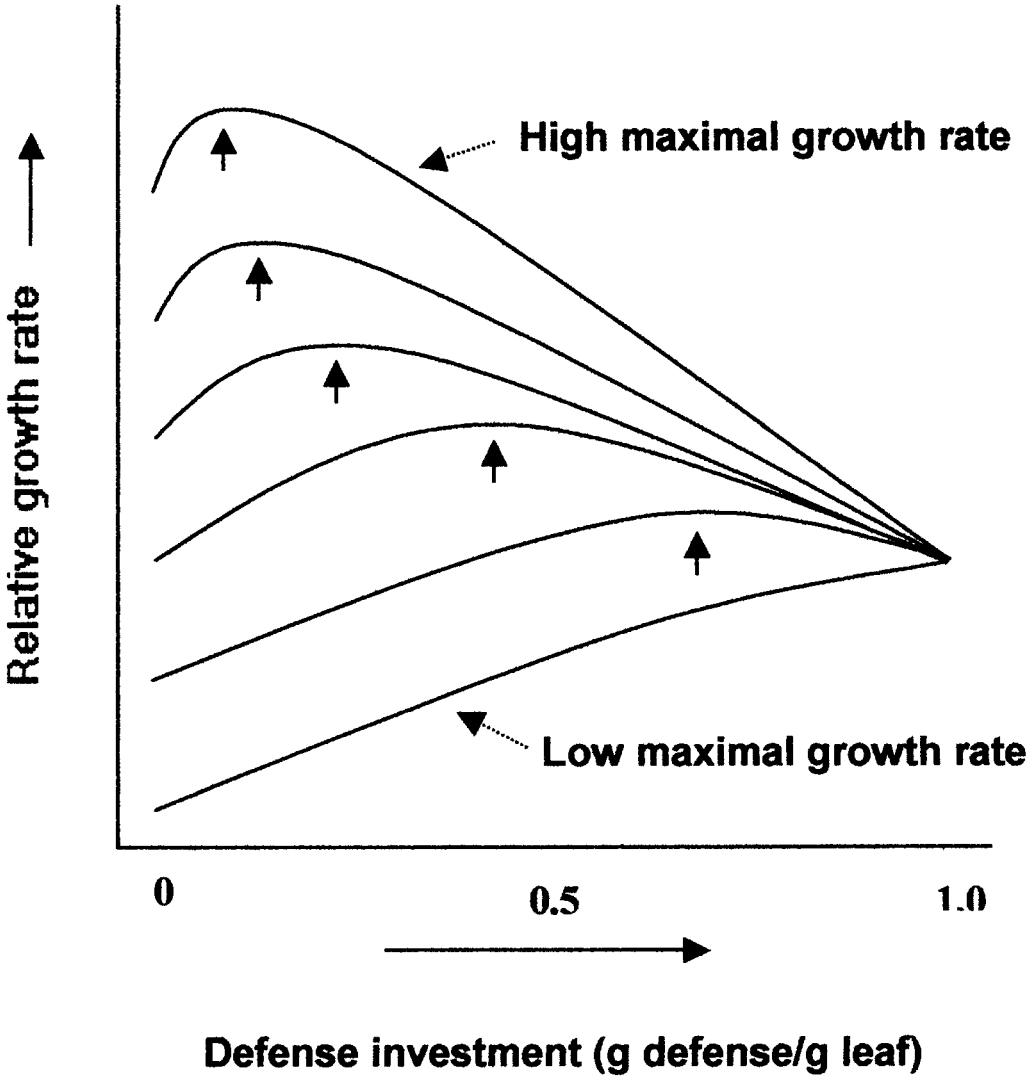

FIGURE 1. THE GROWTH RATE HYPOTHESIS: AN EVOLUTIONARY VIEW OF PATTERNS OF PLANT DEFENSE AMONG SPECIES

Effect of defense investment on realized growth. Each curve represents a plant species with a different maximal inherent growth rate. Levels of defense that maximize realized growth are indicated by arrows. Adapted from Coley et al. (1985).

investment (primarily lignins and polyphenols) for defense, in comparison to fast-growing species. Also, it is predicted that competitive, shade intolerant (fast-growing) species exhibit high induction of defenses, whereas stress tolerant (slow-growing) species exhibit low induction of defenses (Coley 1987a).

Evidence

Overall, the results are mixed in support of the GR hypothesis. A comparison of saplings in light gaps (presumably optimal conditions and maximal growth rates) of 47 tree species indicated, as predicted, a negative correlation between growth rate and tannins, and a posi-

tive correlation between growth rate and rate of herbivore damage to leaves (Coley 1987a). Growth rate was estimated in three ways: annual increase in height, annual leaf production, and highest individual tree growth rate per species (presumably an estimate of maximal growth rate). As predicted, species with long-lived leaves (i.e., slow-growing species) had higher concentrations of tannins than species with short-lived leaves (i.e., fast-growing species). But there was no negative correlation between rate of herbivore damage and tannin concentration. A comparison of pioneer versus gap tree species provided data both in support of and against the GR hypothesis (Folgarait and Davidson 1994, 1995). Tests within species that compared male and female plants, which have different growth rates, supported the GR hypothesis (Jing and Coley 1990; Herms and Mattson 1992).

Other examples clearly do not fit the GR hypothesis. For instance, fast-growing early successional conifer species (pines) have high constitutive levels of defense with relatively low induction, whereas slower-growing late successional conifer species (firs) lack the massive constitutive defenses of pine and rely on fast-acting massive increases in defense at the wound site (Cates 1996). Furthermore, it has become clear that secondary metabolites cannot simply be classified in terms of type or perceived function. Terpenes, for example, have been thought of as mobile defenses and, therefore, as appropriate defense for short-lived leaves, when actually they have traits appropriate for both short- and long-lived leaves. Although relatively expensive to produce, terpenes are cheap to maintain and mobile prior to senescence (Gershenzon 1994a), and concentrations can be high enough to produce dosage or "quantitative" effects (Langenheim 1994).

Furthermore, the predictions about the shape of the defense investment versus realized growth-rate curves, degree of defensive plasticity, and degree of induction have not been examined. In particular, while some subhypotheses have been tested, data have not been gathered to examine the hypothesis more directly. The curves of defense investment versus realized growth rate are specified mathematically (Figure 1), so in principle such a figure could be generated by comparison of plant species. But there are no studies of the GR hypothesis taking that approach. To do this would require estimating the maximal growth rate (at optimal resources for each species) and the corresponding constitutive defense level, *plus* the consequences to defense level with deviations from the maximal growth rate. Without such an assessment, it will be difficult to evaluate the GR hypothesis fully.

In sum, the research related to the GR hypothesis makes a compelling case that evolutionarily the effects of resource availability on growth are orders of magnitude more than the effects of defense cost (Coley 1987b). Furthermore, it indicates that intrinsic growth rate and correlated characteristics, such as leaf life span, are so strongly shaped by abiotic conditions that these determine basic defensive profiles.

The Growth-Differentiation Balance Hypothesis

The Growth-Differentiation Balance (GDB) Hypothesis provides a framework for predicting how plants will balance allocation between differentiation-related processes and growth-related processes over a range of environmental conditions (Loomis 1932, 1953). Growth refers to the production of roots, stems, and leaves, or any process that requires substantial cell division and elongation. Differentiation is everything else, so it refers to enhancement of the structure or function of existing cells (i.e., maturation and specialization). Some differentiation traits can limit herbivory. Secondary metabolism, trichome production, and thickening of leaf cuticle are examples of those kinds of differentiation-related processes (Herms and Mattson 1992). Allocation to differentiation includes process and product, so it includes cost of enzymes, transport, and storage structures involved in defense. Research shows that growth processes and secondary metabolism can compete for available photosynthate (Wadleigh et al. 1946; Veihmeyer and Hendrickson 1961; Mooney and Chu 1974) and, thus, full carbon allocation to all functions cannot be met simultaneously (Lorio 1986).

The GDB hypothesis states that any environmental factor that slows growth more than it slows photosynthesis can increase the resource pool available for allocation to differentiation-related products (Loomis 1932, 1953). For instance, growth is slowed substantially by shortages of nutrients and water, whereas photosynthesis is less sensitive to such limitations (reviewed by Herms and Mattson 1992). In these situations, carbohydrates accumulate in excess of growth demands and, consequently, may be converted to secondary metabolites, with low cost to plant fitness.

Although it was Loomis (1932, 1953) who originally outlined the Growth-Differentiation Balance Hypothesis for plants, it was Herms and Mattson (1992) who used the hypothesis to explain how the physiological tradeoff between growth and differentiation processes interacts with the selective forces of competition and herbivory to shape plant life-history strategies. They proposed a growth-differentiation continuum reflecting the allocation of resources to these processes. Competition in resource-rich environments selects for a growth-dominated strategy, whereas stress of resource-poor environments selects for differentiation-dominated strategy.

Assumptions

The expanded GDB hypothesis assumes that: 1) resource limitation (except for light) has a more negative effect on growth than on photosynthesis; 2) maximal relative growth rate is determined by resource availability and, consequently, the inherent growth rate of plant species reflects the resource limitation of the preferred environment; 3) there is a tradeoff between growth and differentiation due to competition for photosynthate; 4) herbivory is a major selective force for production of secondary metabolites; 5) defenses reduce herbivory; and 6) defenses can have a cost because they can divert resources from growth.

Predictions

Ecologically, the GBD hypothesis predicts: 1) Plants experiencing low levels of resources should be limited in both growth and photosynthetic capability. Limited resources should be shunted preferentially to growth processes over differentiation processes (Waring and Pitman 1985). The limitation of growth processes would yield low growth rate and moderate concentrations of secondary metabolites (Herms and Mattson 1992; see Figure 2, point A). 2) Plants experiencing intermediate resource availability should have high concentrations of secondary metabolites but an intermediate accumulation of biomass (Loomis 1932, 1953). At an intermediate resource level, growth (through cell division and enlargement) is limited by the resource level, whereas photosynthesis is less affected (Chapin 1980; Korner 1991; Luxmoore 1991). Therefore, secondary metabolites will tend to accumulate, due to the excess pool of photosynthate (or assimilates), and the defenses will be produced relatively inexpensively (Figure 2, point B). 3) Plants experiencing high resource availability should not be limited by photosynthesis or growth and, thus, should allocate a greater proportion of the photosynthate (or assimilates) to growth rather than to differentiation traits (Loomis 1932; Herms and Mattson 1992) (Figure 2, point C). Overall, the pattern of allocation to secondary metabolites should be curvilinear across a resource gradient, with a peak at intermediate resource levels. This predicted pattern contrasts with that of the OD Allocation Cost of Phenotypic Defense (Hypothesis 2d), which is that allocation to defense increases across a gradient of increasing resources. It is also similar to the predicted pattern of the CNB hypothesis, but with a different explanation. 4) The exception to the GDB prediction is the effect of light, which influences photosynthesis more than growth. In this case, with increasing light, secondary metabolites will increase proportionally with growth.

It might be useful to recast the ecological-side GDB graph to establish the real issues (Figure 3). Growth is measured as mass (or as a correlate of mass, such as height), but mass is composed of undifferentiated and differentiated products. Some of the differentiated items functionally belong in the "defense" category (e.g., glandular trichomes, secretory glands, resin ducts), others

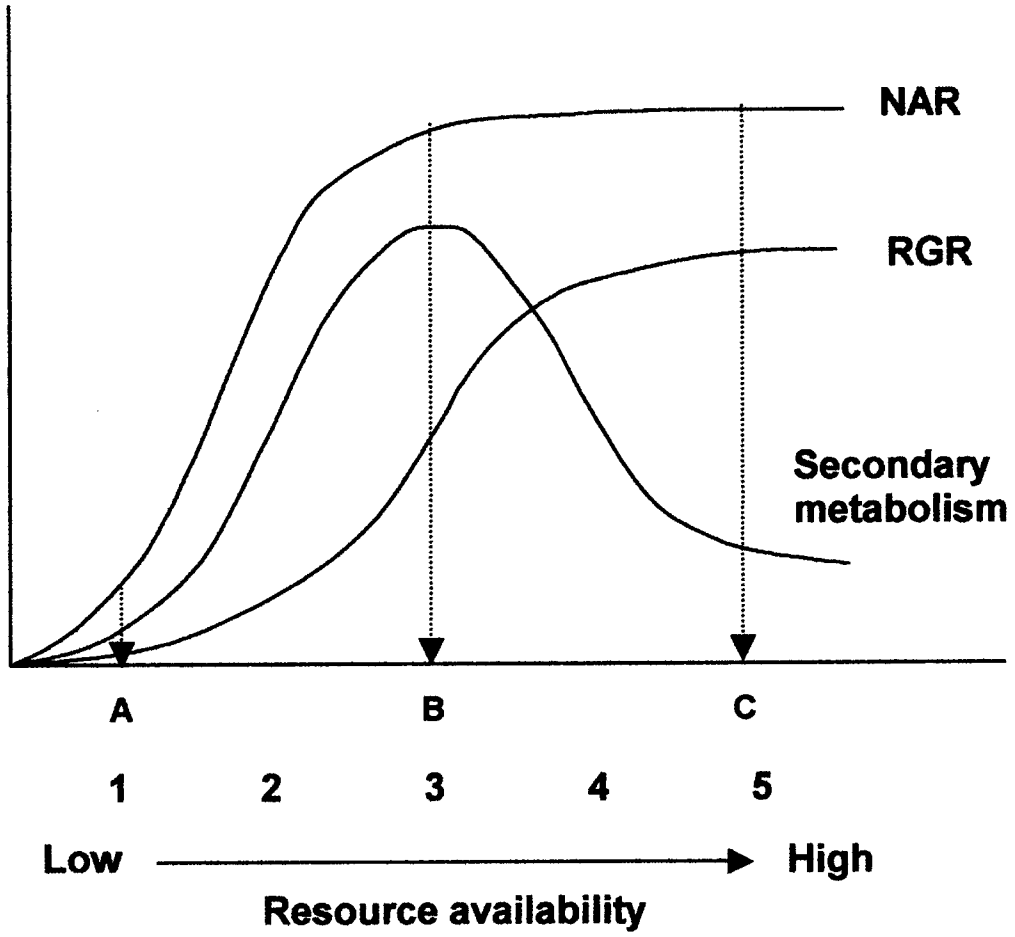

Figure 2. The Ecological Side of the Growth-Differentiation Balance Hypothesis: Intraspecific Patterns of Plant Defense Along a Resource Gradient

Relationship of net assimilation rate (NAR), relative growth rate (RGR), and differentiation (specifically secondary metabolism) across a resource gradient, for which the resource affects growth more than it does photosynthesis. Arrow A: both growth and photosynthesis are constrained by low resource availability. Arrow B: growth is more constrained than photosynthesis and thus there is more allocation to differentiation (and specifically to secondary metabolism). Arrow C: growth is less constrained and thus there is more allocation to growth. At least 5 resource levels spread along the gradient are necessary to determine the pattern. Adapted from Herms and Mattson (1992).

in the "growth" category (e.g., vascular tissue), and still others in both (e.g., secondary cell walls). In theory, the rate of production of differentiated tissues and products should lag behind that of undifferentiated tissues and products (Figure 3). Secondary metabolism is a category of differentiation processes. The rate of secondary metabolism is predicted to be greatest at an intermediate level of resources (except for light) because growth and its associated differentiation processes are more inhibited than certain other differentiation processes, in particular secondary metabolism. This does not mean that, for example, when soil nitrogen is the limiting resource, secondary metabolism can occur without nitrogen input, but rather that growth processes are so nitrogen-demanding that they are simply more limited. The recast graph makes it clearer that we need to under-

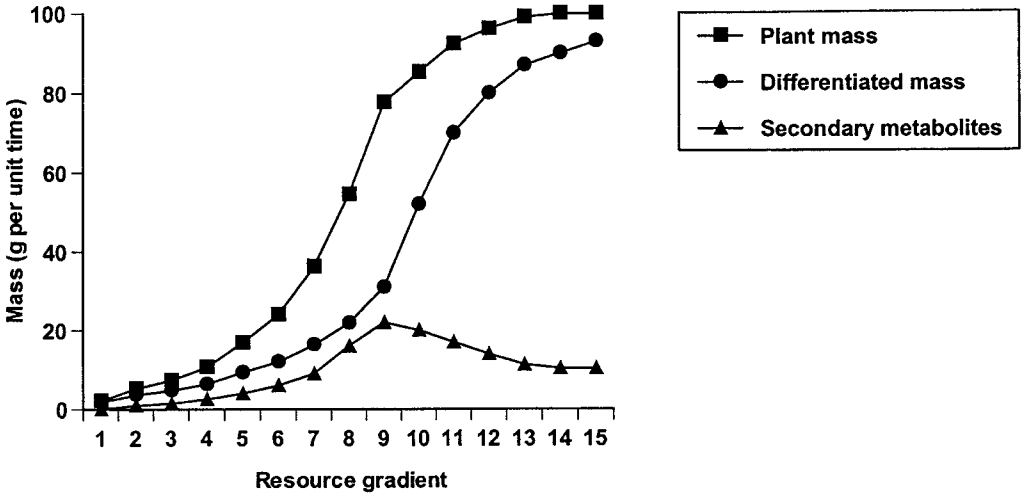

FIGURE 3. THE ECOLOGICAL SIDE OF THE GROWTH-DIFFERENTIAL BALANCE HYPOTHESIS: INTRASPECIFIC PATTERNS OF PLANT DEFENSE ALONG A RESOURCE GRADIENT
Figure 2 recast to show the relationship of rates of undifferentiation and differentiation.

stand how, across a resource gradient, allocation to different differentiation processes changes with growth requirements and limitations thereof. For instance, in resource-limited environments, it makes sense that high levels of secondary metabolism would not divert resources from growth, but that there could be a tradeoff between secondary metabolism and storage of carbohydrates and/or high maintenance respiration to tolerate poor conditions (Herms and Mattson 1992).

The expanded GDB hypothesis (Herms and Mattson 1992) predicts how over evolutionary time the relative importance of herbivory and competition have shaped plant allocation patterns (Figure 4). In the model, there is a tradeoff between photosynthate allocated to growth and that to secondary metabolism, with competition selecting for allocation to growth and herbivory selecting for allocation to secondary metabolism. Life-history strategies emerge. Growth-dominated species have adaptations that optimize the benefits of a minimal defensive investment (i.e., high plasticity)—e.g., highly bioactive secondary metabolites, inducible resistance, phenological and qualitative variation in secondary metabolites (Herms and Mattson 1992). At the other end of the continuum, differentiated-dominated species have adaptations that optimize the benefits of maximal retention and economy of acquired resources. Intraspecific genetic variation is maintained by disruptive selection, due to contrasting environments; i.e., an environment where the importance of competition outweighs that of herbivory versus one where herbivory is of greater importance.

Evidence

There have been few explicit and no rigorous tests of the GDB hypothesis. However, some data are available to evaluate the ecological side of the GDB hypothesis. Using four levels of nitrate, Mihaliak and Lincoln (1985) found a nonlinear pattern of terpene concentration in camphorweed, with the highest concentration occurring at a moderate nitrate level. Also using four levels of nitrate, Wilkens et al. (1996) found a nonlinear pattern for two phenolics in tomato plants, with the highest concentration occurring at a moderate nitrate level. These results support the ecological side of the GDB hypothesis.

Most of the evidence used for or against the GDB hypothesis comes from studies designed to answer other questions and, consequently, the evidence is problematic. For example, most of these studies only used two levels of the resource (Lincoln and Langenheim 1978; Firmage 1981; Waterman et al. 1984; Waring et al. 1985; Larsson et al. 1986; Johnson et al.

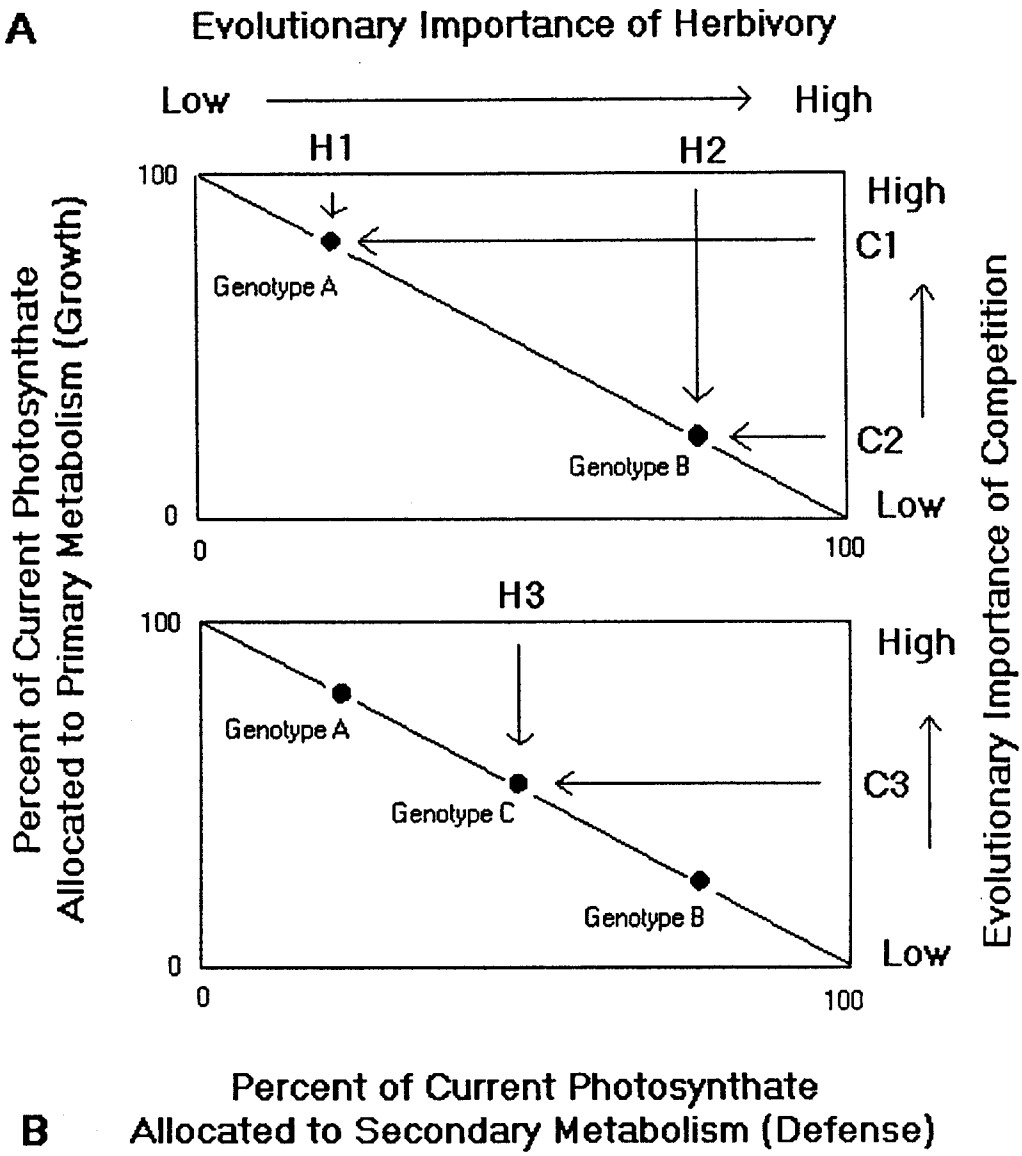

FIGURE 4. THE EVOLUTIONARY SIDE OF THE GROWTH-DIFFERENTIATION BALANCE HYPOTHESIS: PATTERNS OF PLANT DEFENSE AMONG SPECIES

There is a tradeoff in allocation of current photosynthate between growth and defense. With herbivory a strong selective pressure for defense and competition a strong selective pressure for growth, there is a tradeoff in the evolutionary importance of herbivory and competition. Stable polymorphism (Genotypes A and B) may be maintained by disruptive selection, whereas directional selection on Genotypes A and B towards the center results in evolution of Genotype C. Adapted from Herms and Mattson (1992).

1987; Bjorkman et al. 1991; Saenz et al. 1993; Wilkens et al. 1997). But the curvilinear pattern predicted for concentration of secondary metabolites relative to an increasing resource cannot be detected with just two levels of the resource, so what appears to be conflicting evidence may not be conflicting at all (Wilkens 1997). For instance, the following

results would all be supportive of the GDB hypothesis: 1) a positive correlation between concentration of secondary metabolites and low versus intermediate resource levels (Figure 2, points A and B); 2) a neutral pattern across low versus high resource levels (Figure 2, points A and C); and 3) a negative correlation across intermediate versus high resource levels (Figure 2, points B and C). Even results from studies with three levels of a resource can be difficult to interpret. For example, Muzika et al. (1989) found terpene concentration for a fir species was not affected by soil nitrogen, but it is not clear where the three nitrogen levels would fall along the ideal range for testing the GDB hypothesis (i.e., relative to the plant species and the experimental conditions). To detect a potential curvilinear pattern for plant defense, Wilkens (1997) recommends a minimum of five levels spread along an appropriate gradient of the resource.

The GDB hypothesis has been applied to within-plant patterns of defense allocation. It was suggested that terpene biosynthesis occurring primarily in young rapidly growing leaves contradicts the GDB hypothesis (Lerdau et al. 1994). But consideration of cellular growth and differentiation processes in young leaves indicates that it is not a contradiction. The epidermal cells, which mature (i.e., differentiate) relatively early in leaves, often contain high levels of secondary metabolites and most likely account for the high terpene levels (Herms and Mattson 1992).

The only analysis of the evolutionary side of the GDB hypothesis is the discussion by Herms and Mattson (1992). No tests have been reported.

Relationship of These Hypotheses

These hypotheses are not mutually exclusive (Scriber and Ayres 1988; Jing and Coley 1990; Herms and Mattson 1992; Tuomi 1992; Berenbaum 1995), and it is appropriate to integrate them (Price 1991; Herms and Mattson 1992; Tuomi 1992; Mole 1994).

Comparison and Integration of the OD and GR Hypotheses

In the GR hypothesis, herbivory is complementary to the selective pressure of resources (Coley et al. 1985) rather than being the driving force as in the OD view (Rhoades 1979). Furthermore, in the OD framework the total selective pressure from herbivores of different feeding specializations can vary for apparent and unapparent plants, and therefore determine the amount and type of defense exhibited by a plant (Feeny 1975, 1976; Rhoades and Cates 1976; Rhoades 1979). In contrast, in the GR framework, the interaction of resource availability and total herbivory, regardless of plant apparency or herbivore specialization, determines the amount and type of defense (Coley et al. 1985).

Recognizing that unapparency as an escape strategy probably only applies to ruderal species, Coley (1987a) integrated the OD and GR hypotheses by creating the Habitat Template-Plant Defense (HT-PD) Hypothesis. The HT-PD hypothesis is based on a conceptual model of plant strategies shaped by the level of disturbance and stress (Grime 1977, 1979; Southwood 1977) and relates defense to intrinsic growth rate (Coley 1987a). The HT-PD hypothesis is certainly intriguing, but until quite recently (Hodgson et al. 1999) it was difficult to know (and still is) where to assign particular species relative to the disturbance and stress axes.

The HT-PD integration did not resolve a discrepancy in predictions about types of defense. Based on the OD hypothesis, it was predicted that quantitative defenses (such as tannins) are more costly than qualitative defenses (such as alkaloids and terpenes), reflecting the differences in molecular size and concentration in a plant (Feeny 1975, 1976; Rhoades and Cates 1976). In contrast, based on the GR hypothesis, it was predicted that qualitative defenses are more costly than quantitative defenses because qualitative defenses are continually produced and metabolized, whereas quantitative defenses are produced and then kept intact (Coley et al. 1985). Effort to resolve this is probably pointless, however, because it is clear that cost of a defensive chemical is not as simple as these predictions imply.

Comparison and Integration of CNB and GR

The difference between the CNB and the GR hypotheses is that the GR hypothesis spe-

cifically incorporates the effects of herbivory and competition to select for optimal defense, whereas the CNB hypothesis addresses phenotypic expression of the genetic potential for defense. Thus, the CNB hypothesis needs the GR hypothesis to explain genotypic patterns. The GR hypothesis relies on the CNB hypothesis for predictions about defenses phenotypically (e.g., Folgarait and Davidson 1994, 1995).

Together the GR and CNB hypotheses explain what otherwise would seem like a contradiction. Among tree species, shade-tolerant species have *higher* concentrations of nonnitrogen-containing defenses than gap or pioneer species, whereas within species, shaded individuals have *lower* concentrations of nonnitrogen-containing defenses than those in full sunlight (Bryant et al. 1983: sections 2 & 6; Coley 1987a). Similarly, it takes these two hypotheses to explain the difference in inter- and intraspecific defensive patterns relative to nitrogen availability. That is, interspecific variation in defense reflects adaptations to herbivory and absolute resource levels, whereas intraspecific variation in defense reflects relative resource levels or the carbon:nutrient balance (Coley 1987a).

Comparison of CNB-GR versus GDB

At this point, it may seem that the integration of the CNB and GR hypotheses yields the GDB hypothesis. That is not the case, however.

First, the GDB hypothesis predicts that *any* environmental factor that slows growth more than it slows photosynthesis can increase the resource pool available for allocation to secondary metabolism (Loomis 1932, 1953). Thus, the GBD hypothesis goes beyond the CNB hypothesis (Herms and Mattson 1992), which focuses on the effects of shade and fertilization on allocation to secondary metabolism versus growth (Bryant et al. 1983).

Second, the GDB hypothesis explicitly acknowledges that in plant development there is a constant tradeoff between growth and differentiation requirements. Differentiation continually diverts resources from plant growth (e.g., away from production of new leaf area). Growth continually diverts resources from new differentiation (e.g., away from maturation and specialization of tissue). For instance, secondary metabolism and structural reinforcement are physiologically constrained in dividing and enlarging cells, and resin ducts and other compartmentalization of defenses depend on cell growth. Therefore, the phenotypic expression of a plant's genetic potential for defense will reflect this continual tug-of-war between growth and differentiation processes. When environmental conditions are optimal for a plant species (maximal growth rate), vegetative growth (plus differentiation to support that) is expected to receive priority for resources over secondary metabolism and storage. When environmental conditions are unfavorable for growth, the carbon pool available for allocation to secondary metabolism increases such that there is less or even no tradeoff with growth.

Third, the mathematical model of the GR hypothesis (Coley et al. 1985) assumes that all resource allocation that does not go into defense goes to growth, but that is unrealistic biologically (Moles 1994). The mathematical model of the GDB hypothesis (Herms and Mattson 1992) recognizes that phenotypic ("realized") growth rate is a function of intrinsic defense investment, competition, herbivory, and environment, and associated tradeoffs including compensatory regrowth, and thus incorporates third party tradeoffs (Mole 1994). Therefore, both the CNB and GR hypotheses need the GDB hypothesis to account for the tradeoff complexities between growth and differentiation, and in particular to include the role of tolerance.

Fourth, because plants have so many routes of entry and egress of limiting resources, evaluation of resource-based tradeoff models will require physiological and molecular genetic techniques to isolate and follow processes (Mole 1994). For instance, it appears that downregulation of growth rate, beyond that needed due to immediate resource requirements of activated defense, allows plants to reallocate resources, for example, to regrowth processes, which would increase tolerance to herbivory (Baldwin and Hamilton 2000). The GDB hypothesis provides a framework for identifying cellular and tissue growth-differentiation and third-party tradeoffs for study of plant defense issues.

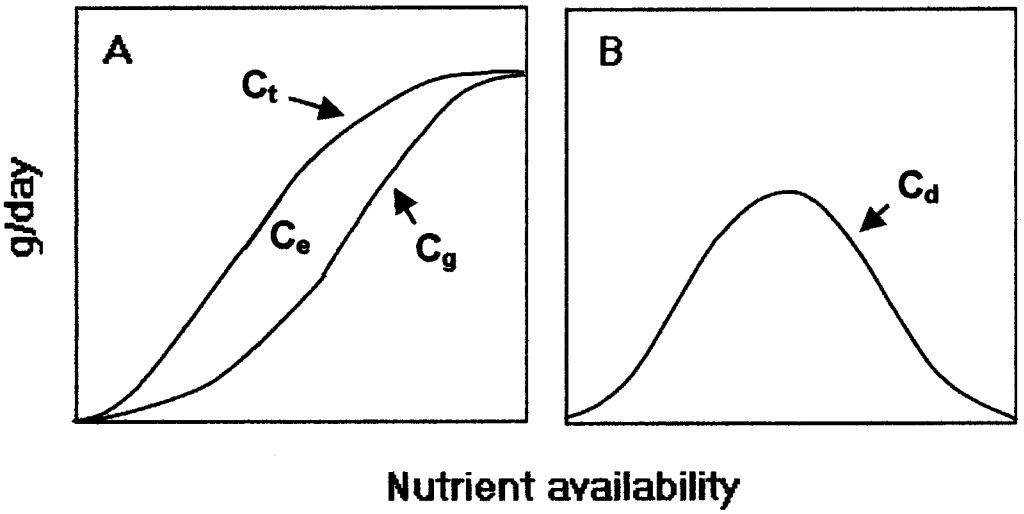

FIGURE 5. THE CARBON:NUTRIENT BALANCE HYPOTHESIS: AN ECOLOGICAL VIEW OF INTRASPECIFIC PATTERNS OF TOTAL PLANT DEFENSE ALONG A RESOURCE GRADIENT

Model of plant carbon:nutrient balance in which secondary metabolite production is supported by carbon surplus. C_t = total available carbon; C_g = carbon diverted to growth; C_e = excess carbon; and C_d = level of "carbon-based" allelochemicals. In this extreme situation, all carbon surplus is shunted to defense, and defense is determined completely by that "phenotypic plasticity." Adapted from Tuomi et al. (1988).

There is limited utility in comparing the graphical models of the CNB, GR, and GDB hypotheses, however, even though all three have growth rate on the *y*-axis. The CNB graph illustrates the *extreme* situation, wherein all carbon surplus is shunted to defense, and defense is determined *completely* by that "phenotypic plasticity" (Figure 5). The ecological-side GDB graph is too simplistic (Figure 2). The exact form of the tradeoff between growth and secondary metabolism has not been established for any species (Herms and Mattson 1992). Furthermore, the graph fails to capture the intimacy of growth and differentiation processes, and the implications of that for withstanding herbivory, which is the key contribution of the GDB hypothesis. The GR graph illustrates "defense investment" on the *x*-axis, but it does not suggest the degree to which that investment comes from fixed and/or flexible allocations (Figure 1). It is also difficult to incorporate tolerance into "defense investment" because tolerance refers to more than storage reserves; it also refers to processes, such as regulation of photosynthetic rate and nutrient uptake.

COMPARSION OF THE OD AND EXPANDED GDB HYPOTHESES

As discussed by Herms and Mattson (1992), the expanded GDB hypothesis also addresses the evolutionary aspects of the OD and GR hypotheses. The expanded GDB hypothesis includes a model of the evolution of plant allocation trajectories that takes into account the selective pressures of herbivory and competition (Figure 4). In contrast to the OD view, the GDB explanation resides in recognition of the physiological tradeoffs between growth and differentiation (and which includes third party tradeoffs) at the cellular and tissue levels relative to the selective pressures of resource availability. Predictions can be made and tested. Therefore, by subsuming the other hypotheses and providing explicit and logically compatible predictions, the expanded GDB is the most theoretically mature of the hypotheses of plant defense.

Conclusions About the Theory of Plant Defense

None of the plant defense hypotheses have ever been firmly rejected (Herms and Mattson 1992; Berenbaum 1995). Considering how theory develops, this is not surprising. The theory of plant defense has the characteristics of immature theory. To expect that we would have a mature theory at this point is unreasonable.

So where do we go from here? The current theory of plant defense addresses the diverse and ubiquitous ways that plants cope with enemies, in particular why some plant species are so well defended and others not so, but it is in danger of stagnation (and so remaining in a state of confusion) if hypotheses are not rigorously tested. Each of the hypotheses has contributed to our current understanding and could contribute more, but we need to recognize that each has its limitations and each has different kinds of contributions. However, while the basic OD hypotheses served a useful purpose at the outset, they are no longer appropriate as the focal framework for investigation because the tendency is that, whatever the results of a test, they can be (and have been) interpreted as supporting the OD view. In contrast, the subhypotheses of the OD view are more testable, but they should be referred to with specific names that distinguish them from the two basic OD hypotheses. In particular, focus on cost per se will be less useful than that on mechanisms of cost, such as for ecological costs. Any use of the CNB and GR hypotheses should clearly and fairly address the crux of those hypotheses (as outlined herein). Further research on the subhypotheses of the CNB and GR hypotheses is unlikely to be productive, however. The expanded GDB hypothesis, which subsumes the other plant defense hypotheses (Herms and Mattson 1992) and, furthermore, is the most mature of the hypotheses, should be tested carefully so that we can determine the degree to which it is a useful hypothesis.

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