

An Ecologically Motivated Analysis of Plant-Herbivore Interactions in Native Tobacco¹

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You can't always get what you want, but if you try some time, you just might find, you get what you need. . .

Mick Jagger

Mick Jagger is not a biologist, but his lyrics echo a major ecological paradigm: that tradeoffs (e.g. "costs") constrain organisms at all functional scales, from those affecting metabolism to those influencing life history traits, and that these constraints explain ecological specialization, or more aphoristically, why "a jack of all trades is a master of none." As Jagger might put it, evolutionary responses to the myriad of selective pressures faced by plants determine how they get what they need to be evolutionarily successful. This cost-benefit paradigm is broadly applicable and provides a useful construct with which to consider the consequences of variation in allocation timing and amount to different functions (Fig. 1). As such, the cost-benefit paradigm is frequently invoked by physiological ecologists and evolutionary biologists.

In contrast, the cost-benefit paradigm has not found many advocates among plant molecular biologists. The problem lies first with the scale of biological organization at which it makes predictions. An understanding of how the expression of a gene product contributes to a plant's Darwinian fitness is required. All plants provide a quantifiable estimate of fitness, but the exact parameters to be measured (seeds, pollen, tuber, etc.) differ with each mating system. Fitness measures integrate whole-plant physiological performance that can be used to quantify the fitness consequences of variation in a particular trait, but these parameters are rarely measured in molecular studies. The translation of the functional parameters (growth, reproduction, storage, and defense; Fig. 1) into the language of specific gene products is an additional challenge. Ecological success stems not only from the elaboration of particular traits that confer higher fitness in particular habitats, but just as important, from the fine-tuning of allocation patterns (the transitions 1–4 in Fig. 1). For success in a particular habitat, timing can be everything.

Unfortunately, a comprehensive understanding of internal processes is not sufficient to test the cost-benefit paradigm, because Darwinian fitness can also be influenced by processes external to the plant (Fig. 1). Consider the example of induced resistance, in which inducible expression of resistance traits is thought to be a cost-savings measure, allowing plants to time the production of a defense with the need for the defense and forgo the payment of defense costs when they are not needed. These defense costs may arise from the internal processes of allocating fitness-limiting resources to defensive traits. However, an organism's ecological context frequently alters, or worse, uncouples the relationship between physiological performance (vegetative and reproductive growth) and Darwinian fitness. These external fitness costs can occur, for example, if a defense trait has negative effects on beneficial organisms (e.g. pollinators) or, if plant defense compounds are sequestered by herbivores and co-opted for defense against their enemies (Price et al., 1980), or from positive effects of defensive compounds toward other plants enemies (Fig. 1). These ecological interactions can select for genetic associations (pleiotrophy and linkage) that are not functionally comprehensible without an intimate understanding of the plant's current and past selection pressures.

Tests of the cost-benefit paradigm require both a sophisticated reductionistic view of plant function and an equally sophisticated understanding of ecological function. None of the commonly used model plant systems meets both criteria. Many agricultural varieties have undergone intense selection for particular yield components, which must be considered with the selection regimes that prevailed during the plant's evolutionary history. Two solutions seem reasonable: develop molecular tools in a plant with a sophisticated and well-described ecology or exploit the ecological responses of near relatives to a plant with well-developed molecular tools. Both approaches are currently being explored at the Max Planck Institute for Chemical Ecology in Jena, Germany. An example of the latter strategy with *Arabidopsis* is described by Mitchell-Olds (2001). This *Update* will provide an example of the former approach with native *Nicotiana* spp.

Nicotiana attenuata, a diploid, largely selfing, native tobacco of North America, was selected as a model

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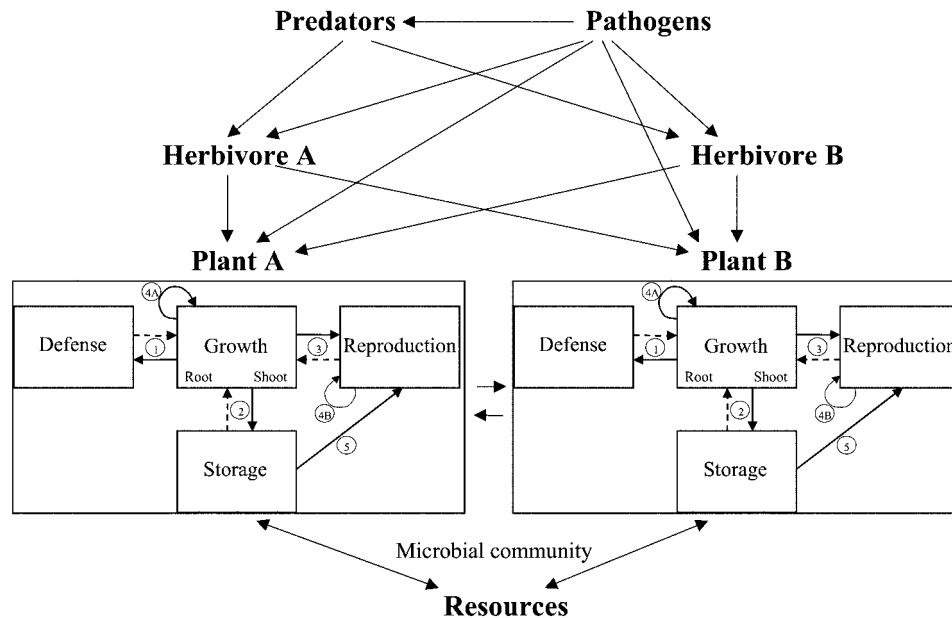


Figure 1. Fitness optimization can arise from processes both internal (inside boxes) as well as external to a plant, and hence, tests of the cost-benefit paradigm require an understanding of biochemical and physiological function as well as the plant's evolutionary history and current selective pressures. The function of genes that contribute to whole-plant growth, storage, reproduction, and defense and for the transitions (circled numbers) between these functions are rapidly being elucidated. Ecological specialization in particular environments is frequently due to processes that regulate the allocation of resources to and from growth and defense (1); growth and storage (2); growth and reproduction (3); storage and reproduction; and the reinvestment of resources from vegetative (4a) and reproductive (4b) growth into the same functions. Because all allocations other than 4a and 4b incur opportunity costs by decreasing potential growth, they are of particular importance for understanding performance in particular habitats (Chapin et al., 1990). These within-plant optimizations are played out in an ecological arena which includes interactions with a plant's competitors (plant B), herbivores, pathogens, predators, mutualists (not depicted), and environmentally available resources, all of which can uncouple the relationships among gene expression, physiological performance and Darwinian fitness.

system for two reasons: 1) it exhibits a large amount of morphological and chemical phenotypic plasticity that appears to be adaptive, and 2) it has evolved to grow in the primordial agricultural niche: the immediate post-fire environment. The habitat selection of *N. attenuata* is in large part determined by its particular germination behavior. *N. attenuata* "chases" fires in the Great Basin Desert by synchronizing its germination from long-lived seed banks with the immediate post-fire environment. The dormant seeds respond to a combination of germination stimulants found in wood smoke (Baldwin et al., 1994b) and inhibitors from the unburned litter of the dominant vegetation (Preston and Baldwin, 1999). As a consequence of this germination behavior, seeds germinate synchronously into nitrogen (N)-rich soils (Lynds and Baldwin, 1998) and hence have selected for rapid growth when water availability is high. Herbivores from more than 20 different taxa attack the plants on a variety of spatial scales, from mammalian browsers that consume entire plants to intracellular-feeding insects. The most damaging herbivores for a given population differ from year to year, as they too must recolonize these habitats after fires. How successfully a given genotype of *N. attenuata* alters its phenotype in response to these highly variable biotic selection

regimes and translates vegetative growth into seed production will determine its representation in the seed bank, and, hence, its Darwinian fitness. In short, because the habitat of *N. attenuata* is characterized by synchronized germination into N-rich soils, intense intraspecific competition, and highly variable herbivore and pathogen challenges, understanding the genetic basis of the phenotypic plasticity of *N. attenuata* might provide genetic tools to engineer greater ecological sophistication into our crop plants.

To illustrate the specificity with which *N. attenuata* tailors its defense responses to different herbivores, I contrast the response to mechanical damage (which simulates the response to some mammalian browsers) and JA elicitation with that to attack from a specialized lepidopteran herbivore, the tobacco hornworm (*Manduca sexta*). Wounding elicits a massive metabolic commitment to nicotine production, a potent direct defense, that is produced and distributed throughout the plant in a manner that optimizes plant fitness. Wounding elicits the JA-cascade, and JA-elicitation produces a) durable resistance against a suite of herbivores; b) increases in secondary metabolites that function as direct and indirect defenses; and c) substantial fitness costs when plants grow with competitors in herbivore-free environments.

Plants profoundly alter their wound responses when tobacco hornworm larvae attack, and the details of this alteration illustrate the overarching theme of this *Update*: the value of the fitness-based cost-benefit paradigm and an intimate understanding of a plant's natural history in understanding metabolism.

NICOTINE: AN EFFECTIVE JA-INDUCED DEFENSE

Nicotine is arguably one of the most broadly effective plant defense metabolites in that it poisons acetylcholine receptors and is thereby toxic to all heterotrophs with neuromuscular junctions. Given this defensive potential, one might expect that nicotine would be expressed constitutively in all cell types so as to provide maximum protection, just as many crop plants have been engineered to express the *Bacillus thuringiensis* toxin. However production and accumulation of nicotine does not meet this expectation, and the spatial and temporal details of its production are consistent with an optimization of defense allocation.

Nicotine concentrations vary 10-fold among plant parts, but are remarkably homeostatic when viewed from a whole-plant perspective. The within-plant heterogeneity results from heterogeneity in synthesis and transport. Transcripts of the rate-limiting enzyme in its synthesis, putrescine *N*-methyltransferase (*pmt*), are found only in the roots, as are protein and activity measures. *N. attenuata* has two *pmt* genes, which are tightly coregulated (Winz and Baldwin, 2001) and correlate with rates of de novo biosynthesis, which, in turn, is readily measured by mass spectrometry with ¹⁵N-pulse-chase techniques (Baldwin et al., 1994a). After its synthesis in the roots, nicotine is transported to the shoots in the xylem stream (Baldwin, 1989) and accumulated in tissues with a pattern that is consistent with predictions of optimal defense theory (McKey, 1974), which argues that defense metabolites are allocated preferentially to tissues with high fitness value and a high probability of attack. Young leaves, stems, and reproductive parts tend to have the highest concentrations; roots and old leaves, the lowest (Baldwin, 1999, and references therein; Ohnmeiss and Baldwin, 2000). Although xylem transport accounts for the initial distribution in the shoot after synthesis in the roots, it is likely redistributed from its location in the central vacuole by symplastic transport routes, particularly during elongation and flowering, when root de novo biosynthesis tends to decline (Ohnmeiss and Baldwin, 2000). Unfortunately, little is known of these symplastic transport mechanisms. Despite the high intraplant heterogeneity, whole-plant nicotine production is remarkably homeostatic. Plants produce allometrically corrected constant pools that appear to be maintained via adjustments in synthesis and biomass accumulation rather than nicotine turnover, regardless of variation in nitrogen supply rates, and

even externally supplied nicotine (Baldwin, 1999, and references therein).

Damage to leaves, such as browsing herbivores cause, dramatically increases de novo nicotine biosynthesis, the allometrically determined set points, and whole-plant nicotine accumulation 2- to 10-fold (Baldwin, 1999, and references therein). During vegetative growth, wound-induced nicotine production simply amplifies the within-shoot distribution observed in undamaged plants. However, during reproductive growth, when nicotine biosynthesis wanes, nicotine is transported preferentially to attacked tissues. In both rosette- and flowering-stage plants, the allocation of nicotine synthesized after wounding to above-ground parts is proportional to the experimentally-determined fitness value of those tissues (Ohnmeiss and Baldwin, 2000).

The sequestration of nicotine biosynthesis in the roots results in a large spatial separation between the site of synthesis and accumulation. Although little is known about the communication between roots and shoots that maintains the allometric set points, the results of many experiments demonstrate that JA is an essential component of the signaling responsible for the dramatic wound-induced increases (Baldwin, 1999, and references therein; Ziegler et al., 2001). Our current working model for the long-distance signal transduction cascade is that wounding transiently increases JA pools in shoots, which either directly through transport or indirectly through a secondary signal such as systemin (Pearce et al., 2001) increases JA pools in roots; these, in turn, stimulate nicotine synthesis in the roots and increase nicotine pools throughout the plant.

Given the defensive effectiveness of nicotine, why do plants wait for herbivore attack to up-regulate their production? The answer may well lie in the metabolic demands of production. The N investment in nicotine can be substantial. After wounding, 8% of whole-plant N is in this alkaloid alone, and this figure does not include the N used in biosynthesis, transport, and storage (Baldwin et al., 1994a, 1998). A large fraction of the increase in nicotine production is derived from N assimilated after attack, but plants are capable of mobilizing endogenous N pools when plants are grown in N-free conditions. Endogenously synthesized nicotine is not appreciably metabolized beyond demethylation to nornicotine and dehydration to anatabine (Baldwin and Ohnmeiss, 1994). Consequently, the plant is not able recoup the substantial N investments it makes in this defense and reutilize this fitness-limiting element for growth and reproduction (Fig. 1, transition 1). In addition to the large N requirements, nicotine biosynthesis costs Glc, the magnitude of which depends on the oxidation state of N used for its synthesis; nicotine synthesized from the NH₄ costs 2.86 g of Glc/g of nicotine, whereas synthesis from NO₃ costs 26% more (Gershenson, 1994). Not surprisingly, plants preferen-

tially use NH_4 for nicotine biosynthesis over NO_3 when given a choice (Lynds and Baldwin, 1998). This energy savings may represent an added benefit of the "fire-chasing" germination behavior of *N. attenuata*, which ensures germination and growth in NH_4 -rich soils for the first growing season after a fire (Lynds and Baldwin, 1998).

Although the resource costs of nicotine production may account for its inducible expression, the sequestration of nicotine biosynthesis in roots adds a 10-h delay to the transcriptional activation of this induced defense (Winz and Baldwin, 2001). Plants exhibit a "memory" in so far as they increase their rate of nicotine biosynthesis and accumulation more rapidly in response to subsequent elicitations (Baldwin and Schmelz, 1996). However, a biologically significant delay in defense activation remains and suggests that below-ground nicotine production may have benefits that offset this obvious disadvantage. Below-ground sequestration may protect the induced response: a browser may remove leaves but not the biosynthetic ability to produce the alkaloid. Protection of the biosynthetic capacity to launch a defense response is likely important during regrowth after browsing when a plant's photosynthetic capacity is severely compromised. Plants can launch a full allometrically corrected nicotine response even after a browser removes 88% of the shoot (Baldwin and Schmelz, 1994). In summary, the intraplant details of nicotine biosynthesis and accumulation are consistent with an optimization of the costs and benefits of a metabolically demanding metabolite and underscore the need to understand secondary metabolites in a whole-plant context.

JA ELICITS DIRECT AND INDIRECT DEFENSES AND DURABLE RESISTANCE

The fitness benefits of JA elicitation for plants under attack are readily seen in field and laboratory studies. *N. attenuata* plants growing in native populations induced with a root JA treatment early in the growing season had higher nicotine concentrations for the duration of the growing season, lost less leaf area to mammalian browsers, had a lower mortality rate, and produced more viable seed than size-matched controls (Fig. 2, left panel, inset). Similarly, in laboratory studies, survivorship and growth of the tobacco hornworm on JA-treated plants is dramatically lower than on untreated control plants, and when larvae have the opportunity, they move from induced plants to feed on neighboring controls (van Dam et al., 1999, 2001a). In these experiments, JA elicitation clearly increased a plant's direct defenses, which could account for the increase in resistance.

JA-elicited nicotine production is likely to account for some of the observed JA-induced resistance. Tobacco hornworm larvae, despite their nicotine resistant physiology, grow faster on low nicotine leaves

compared with leaves cultured in xylem solutions with induced nicotine concentrations (Baldwin, 1988b) and on plants with constitutive and induced nicotine production suppressed by the anti-sense expression of a *pmt* transcripts (Voelckel et al., 2001a). However, many other secondary metabolites are induced by JA elicitation of *N. attenuata* (including phenolics, flavonoids, phenolic putrescine conjugates, and diterpene sugar esters; Keinänen et al., 2001), and some of these are known to influence herbivore performance. Proteinase inhibitors (PI), for example, are up-regulated by herbivore attack and JA treatment (van Dam et al., 2001b) and are powerful anti-feedants. Moreover, a study that incorporated leaf material from plants flash-frozen at different times after JA elicitation into artificial diets to "freeze" the JA-induced chemical dynamics and examine their effects on tobacco hornworm larvae performance found that rapidly induced but uncharacterized changes in direct defenses were as important as the induced changes in PIs and nicotine (Pohlon and Baldwin, 2001). Hence, although a number of the chemical changes responsible for induced resistance have been identified, many additional ones clearly remain to be identified. A major challenge will be to understand how induced resistance emerges from all of the chemical changes brought about by elicitation.

In contrast to the situation with direct defenses, the chemical basis of indirect defense function is better understood. JA elicitation and herbivore attack from four different species of insects—but not mechanical wounding—cause plants to systemically release a bouquet of mono- and sesquiterpenes, in addition to the green leaf volatiles that are primarily released from the wounded leaves (Halitschke et al., 2000; Kessler and Baldwin, 2001). This herbivore-induced volatile release occurs principally during the day and cannot be inhibited by treating attacked tissues with lipoxygenase inhibitors (Halitschke et al., 2000). The volatile release has been verified in plants grown in native populations, where it functions as an indirect defense in two distinct ways. First, the volatile release attracts predatory bugs to tobacco hornworm eggs and feeding larvae and dramatically increases predation rates. Second, the volatile release decreases oviposition rates from adult moths (Kessler and Baldwin, 2001). These ovipositing adults are likely using the volatile release to identify host plants lacking competitors (for a single tobacco hornworm larvae requires many host plants to complete development) and to avoid plants on which predators are likely present. This indirect defense can be profoundly effective, and in a field study, the volatile release was estimated to decrease herbivore loads by 90% (Kessler and Baldwin, 2001). By synthesizing and applying single components of the herbivore-induced volatile bouquet to unattacked plants in quantities naturally emitted by the plant, it was demonstrated that individual components from all three

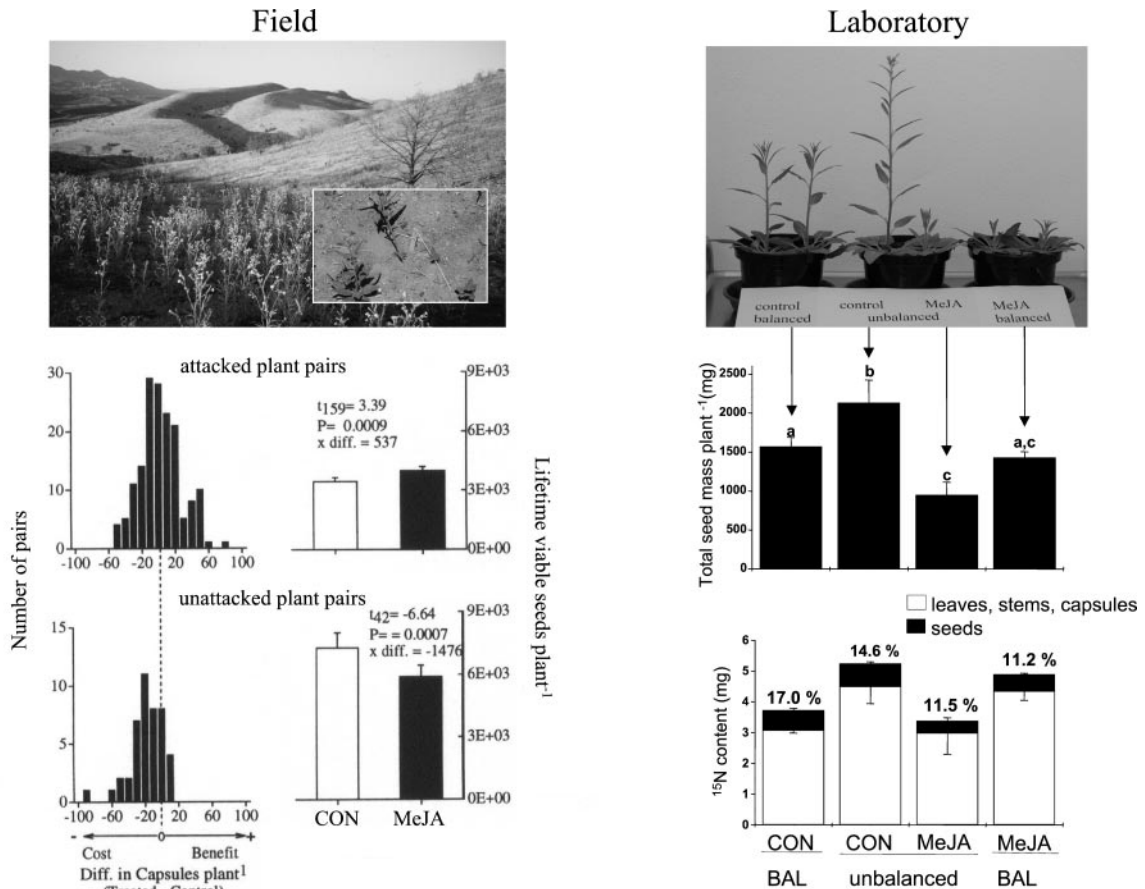


Figure 2. The fitness consequences of jasmonate (JA) elicitation in *N. attenuata* depend on selective pressure from herbivores and competitors. Treating plants with methyl ester of JA (MeJA) provides a convenient method of quantitatively simulating wound- and herbivore-induced changes in endogenous JA pools and secondary metabolites. In natural populations that germinate from long-lived seed banks after fires in the sage-juniper habitats of the Great Basin desert (left panels, Field), an early-season MeJA root treatment increases nicotine concentrations and decreases leaf loss from mammalian browsers (inset, Top right control plant, lower left MeJA treated plant) and increases lifetime seed production. When matched pairs of control and MeJA-treated plants were attacked, treated plants produced an average of 537 seeds and eight capsules more than were produced by the controls members of the pair (middle left panel). However, when plant pairs were not attacked (lower left panel), MeJA treatment decreased lifetime seed production by an average of 1,476 seeds and 13 capsules compared with untreated controls. Left panels depict frequency distribution of differences in capsules produced per plant between treated and control plants in each pair. Negative values reflect a cost of MeJA treatment (control member producing more capsules than its treated counterpart) and positive values reflect a benefit. Right panels depict mean (\pm SE) lifetime viable seed production and statistical analysis from paired t_{df} -tests (data from Baldwin, 1998). In laboratory experiments (right panels, Laboratory), elicitation of plants growing in competition with untreated controls in the same pots reduces seed production (middle right panel, Different letters indicate significant differences at $P < 0.05$) and the ability to compete for soil $^{15}\text{NO}_3$ (lower right panel) and allocate the acquired N to seed production (percent values above bars), thereby providing a large fitness benefit for neighboring controls. However, these fitness costs were not found when plants are growing without competitors (Baldwin et al., 1998) or when plants compete with similarly treated plants (data from van Dam and Baldwin, 2001).

major biosynthetic pathways contributing to the volatile bouquet, namely a monoterpene (linalool), a sesquiterpene (bergamotene), and a green leaf volatile (cis-3 hexenanol), were each active in attracting predatory bugs. The observation that enhancing the release of single components of the complex blend was sufficient to attract predators in nature makes the engineering of this type of indirect defense in crop plants, perhaps in conjunction with direct defenses, a tractable proposition.

JA-ELICITED RESISTANCE IS ASSOCIATED WITH A FITNESS COST

Although JA-elicited plants realize a higher fitness when they are under attack, this resistance comes at a substantial fitness cost if plants are not attacked. In the same field experiment in which JA-treated plants realized a fitness benefit (Fig. 2, left panel), JA elicitation reduced lifetime viable seed production by 26% (1,550 seeds) in plants protected from herbivores

by insecticide spraying and fencing (Baldwin et al., 1998) or by 20% (1,476 seeds) if plants were simply not attacked (Fig. 2, left panel). These JA-induced fitness costs mirror the costs of wound induction observed in a plantation experiment with the sibling species, *Nicotiana sylvestris* (Baldwin et al., 1990). In this experiment, plants were wounded using a standardized mechanical damage technique and had their wound-induced nicotine response suppressed with IAA applications to the wound site, a procedure that inhibits wound-induced JA production (Baldwin et al., 1997). The lifetime seed production of wounded plants that exhibited the normal wound-induced nicotine response was 32% less than that of similarly wounded plants that had had their wound-induced nicotine suppressed with IAA. The similarity between the two estimates of fitness costs supports the contention that JA-elicitation simulated the responses to wounding and that wound-induced responses (those that include nicotine production) exact a large cost from seed production.

The mechanisms responsible for these large fitness costs were explored in laboratory experiments designed to determine whether the fitness reductions could be attributed to the large investments of N into nicotine production that otherwise could not be used for growth and reproduction (Fig. 1). It is surprising that when plants were grown in individual hydroponic chambers in which the uptake and use of N could be carefully quantified, no decrements in seed production were found, even when plants were elicited so that 8% of their whole-plant N pool was tied up in nicotine (Baldwin et al., 1998). Because both of the experiments that had found fitness costs of JA elicitation were conducted under field conditions with intraspecific competitors, the fitness consequences of elicitation were then examined in the laboratory, in soil and hydroponic culture in which elicited plants were grown in competition in various combinations of elicited and control plants with varying N supply rates (van Dam and Baldwin, 1998, 2001; Baldwin and Hamilton, 2000). In these experiments, the lifetime seed production of JA-elicited plants competing with other elicited plants did not differ from that of control plants competing with control plants, although the time required for reproductive maturation was longer. The situation was dramatically different when controls competed with JA-elicited plants. In these unbalanced competition situations (Fig. 2, right panel), controls realized an opportunity benefit with a large increase in lifetime seed production at the expense of the seed production of the neighboring JA-elicited plants. The fitness cost of JA elicitation increased with N supply rate, and was associated with both a greater ability to compete for below-ground N resources, measured with ^{15}N pulse-chase techniques, as well as an increase in allocation of acquired ^{15}N to seed production (Fig. 2, right panel). Therefore, the reductions in

seed production associated with JA-induced responses could not be attributed directly to resource allocations associated with the production of resistance traits. Rather, the costly component of JA elicitation appears to be diminished competitive ability resulting from a temporary slowing of growth.

It remains open as to whether the slowing of growth, with its concomitant loss of competitive ability, is required for JA-elicited induced resistance. JA elicitation decreases transcripts of a number of photosynthetic-related genes (*lhb C1*, *chl H*, and *rbc S*; Hermsmeier et al., 2001) and this down-regulation may be required to free up resources for defense-related processes. However, these pleiotrophic effects of JA elicitation may not be strictly a result of "allocation costs." Many other metabolic processes may be responsible for these coordinated changes in metabolism, such as the autotoxicity of metabolite production (Baldwin and Callahan, 1993) or the history of JA-inducible elements recruited in the response. Direct genetic manipulations of particular resistance traits (PIs, nicotine, etc.) will allow researchers to determine precisely whether defense traits are intrinsically costly.

WHY DOES *N. ATTENUATA* ALTER ITS WOUND- AND JA-ELICITED RESPONSES AFTER TOBACCO HORNWORM ATTACK?

When attacked by the nicotine-tolerant tobacco specialist tobacco hornworm, *N. attenuata* "recognizes" the attack, as evidenced by alterations in a number of its wound- and JA-elicited responses. The induced increase in JA levels that are normally proportional to the amount of mechanical wounding erupts into a JA burst that increases concentrations two to 10 times wound-induced levels (McCloud and Baldwin, 1997; Kahl et al., 2000; Ziegler et al., 2001) and is propagated throughout the damaged leaf ahead of the rapidly foraging herbivore (Schittko et al., 1999). Wounding and JA-elicitation do not result in ethylene emissions, but tobacco hornworm attack produces a rapid ethylene burst, which is sustained during larval feeding (Kahl et al., 2000). The ethylene burst suppresses the wound- and JA- induced accumulation of nicotine biosynthetic genes, *NaPMT1* and *-2*, and the associated nicotine accumulations (Winz and Baldwin, 2001). The ethylene burst does not, however, suppress the volatile release (Kahl et al., 2000). Tobacco hornworm attack, therefore, down-regulates a major direct defense, nicotine, while up-regulating an indirect defense, the volatile release (Fig. 3, left).

All of the tobacco hornworm-induced changes in the wound responses of *N. attenuata* can be mimicked by applying larval oral secretions and regurgitants to mechanical wounds (Halitschke et al., 2001). A suite of eight fatty acid amino acid conjugates (FACs; Fig. 3) in the oral secretions are necessary and sufficient

for not only the transcriptional changes mentioned below but also the JA burst and the volatile release. If these FACs are removed from the oral secretions by ion-exchange chromatography, eliciting activity is lost and regained when synthetic FACs are added back to the ion-exchanged, inactive, oral secretions (Halitschke et al., 2001).

In addition to the changes in defense phenotype after tobacco hornworm attack, *N. attenuata* also undergoes a major transcriptional re-organization. mRNA differential display reverse transcriptase-PCR was used to gain an unbiased view of the transcriptional changes and from this study, it was estimated that more than 500 genes respond to herbivore attack (Hermsmeier et al., 2001). The herbivore-regulated genes could be crudely classified as being related to photosynthesis, electron transport, cytoskeleton, carbon and nitrogen metabolism, signaling, and a group responding to stress, wounding, or invasion of pathogens. Overall, transcripts involved in photosynthesis were strongly down-regulated, whereas those responding to stress, wounding, and pathogens and involved in shifting carbon and nitrogen to defense were strongly up-regulated. These coordinated

changes point to the existence of central herbivore-activated regulators of metabolism, which in turn are activated by minute amounts of FACs in tobacco hornworm's oral secretions (Schittko et al., 2001). Although the overall patterns of transcriptional changes agree generally with the observed phenotypic alterations, the transcriptional basis for the known phenotypic alterations remains obscure. Clearly, a functional understanding of the alterations would help generate predictions about the nature of the herbivore-elicited trans-acting factors. In other words, if we knew "why" the secondary metabolite phenotype and transcriptome of *N. attenuata* was so strongly altered after tobacco hornworm attack, we would be in a stronger position to understand how the changes come about. We are currently exploring three hypotheses.

First, these adapted larvae appear to be feeding in a "stealthy" fashion, reducing their dietary intake of nicotine by suppressing the nicotine responses below that of plants suffering comparable tissue loss (Baldwin, 1988a; McCloud and Baldwin, 1997). Tobacco hornworm larvae clearly pay a growth penalty to detoxify nicotine, as is evidenced by their higher

Down-Regulation of Nicotine During Volatile Release

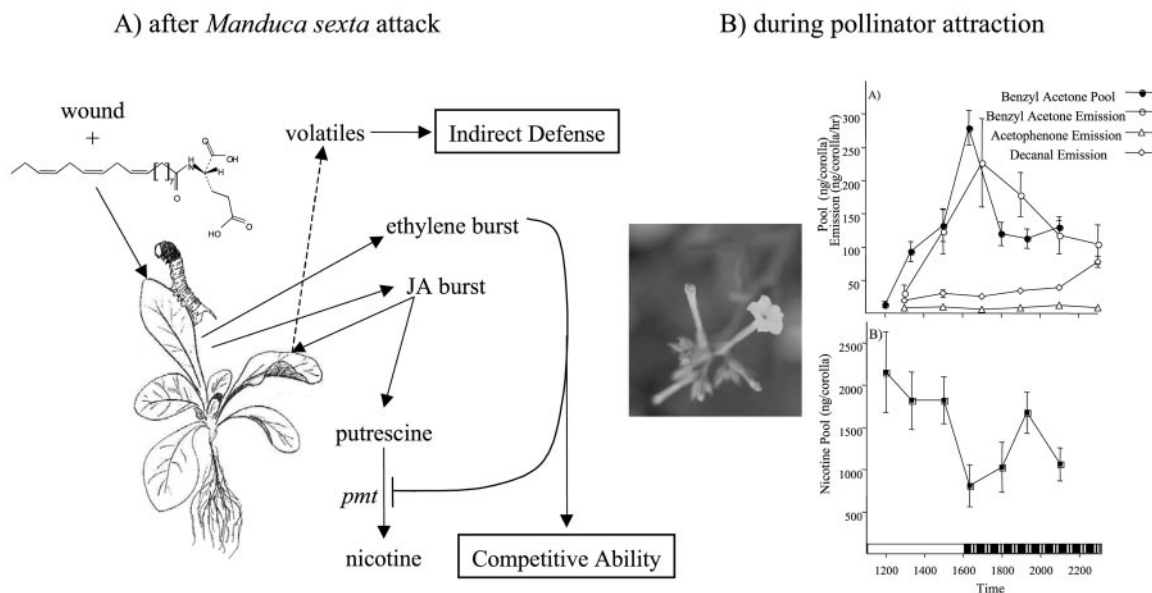


Figure 3. Two examples of the down-regulation of a direct defense (nicotine) with the release of volatiles that function to attract beneficial insects in *N. attenuata*. A, Attack by tobacco hornworm larvae is distinguished from simple wounding by the introduction of FACs at the wound site (the most abundant FAC, *n*-linolenoyl-L-Glu, is depicted) and results in JA and ethylene bursts and a systemic release of volatiles, which functions as an indirect defense by attracting predatory bugs and decreasing the oviposition rate of adult moths. The ethylene burst suppresses wound-induced nicotine production by decreasing the accumulation of putrescine *N*-methyl transferase (*pmt*) transcripts, the rate limiting step in nicotine biosynthesis and inhibits the decrease in competitive ability that results from JA-elicitation. B, Benzyl acetone (BA; 4-phenyl-2-butanone), found only in the outer lip of the corolla where pollinators come in contact with the flower, is released from the corolla in the evening. Diurnal changes in the size of the corolla BA pool closely parallel the amount emitted by flowers. Nicotine can be detected in the headspace of flowers and is localized in the basal parts of the corolla, below the attachment of the filaments and the nectar reward. The corolla pools of nicotine are stable throughout the day except during the period of peak BA production and emission when nicotine pools decrease significantly (Euler and Baldwin, 1996).

growth rates on plants with nicotine levels suppressed by the anti-sense expression of *pmt* (Voelckel et al., 2001a).

Second, *N. attenuata* may be optimizing the defensive function of its volatile release by suppressing nicotine production, which could be sequestered by the herbivore and used against predators attracted by the volatile release. Plant defense compounds are commonly sequestered by adapted herbivores for their own defense and tobacco hornworm's larvae are thought to use dietary nicotine against the larval parasitoid, *Cotesia congregata* (Barbosa et al., 1991). Thus, induced nicotine production may wreak havoc with the plant's ability to use "top-down" processes as a defense. A similar process occurs when *N. attenuata* plants attract pollinators by releasing volatile floral scents (Fig. 3, right panel). Consistent with the defense optimization hypothesis is the observation that tobacco hornworm attack does not suppress the PI elicitation (van Dam et al., 2001b). PIs are not known to be sequestered by larvae for defense and tend to slow herbivore growth, thereby prolonging the time that predators could be attracted to plants by the volatile release and attack larvae. The main predator attracted by the volatile release in nature of *N. attenuata*, *Geocoris pallens*, is relatively small and effectively kills only eggs and first and second instar larvae (Kessler and Baldwin, 2001). Direct defenses that extend the time during which larvae remain in these instars would likely increase the effectiveness of the indirect defense. Hence, the changes in the wound response may represent an optimization of defense, in which the plant assembles a suite of direct and indirect defenses particularly effective against a particular herbivore species.

Third, the plant's altered wound response may also reflect the fitness consequences of intraspecific competition and function to manipulate herbivore behavior to maximize fitness in response to selection from both herbivory and competition. *N. attenuata* plants mass germinate from long-lived seed banks after fires and hence are commonly competing with conspecifics. Because these competitive interactions may profoundly determine an individual plant's fitness (Fig. 2), they may influence the fitness consequences of anti-herbivore defense. JA-elicitation and the up-regulation of the associated resistance traits transiently slows growth and makes JA-elicited and resistant plants inferior to neighboring susceptible controls (Fig. 2). Tobacco hornworm attack results in a dramatic ethylene burst that transcriptionally down-regulates nicotine production, and this ethylene burst also inhibits the reduction in competitive ability associated with JA elicitation (Voelckel et al., 2001b). That the herbivore-induced ethylene burst inhibits both nicotine production and the reduction in competitive ability is consistent with the hypothesis that the large N demands of nicotine biosynthesis contribute to the transient slowing of growth dur-

ing JA elicitation. The ethylene burst was not found to influence the accumulation of transcripts from seven other tobacco hornworm-regulated genes (Schittko et al., 2001), but it remains to be determined whether the effect of the ethylene burst on JA-elicited growth is mediated by responses other than the suppression of *pmt* transcript accumulation and subsequent nicotine production. Although the mechanisms remain to be elucidated, the fact that *N. attenuata* suppresses a potent defense metabolite and does not down-regulate its growth as part of its herbivore "recognition" response, suggests that competitive interactions may have influenced plant-herbivore interactions and focuses attention to larval movement behavior on induced and uninduced plants.

When tobacco hornworm larvae are small (first and second instars), the costs of movement between plants are larger than the costs of remaining on JA-elicited plants (van Dam et al., 2001a). However, when they reach the third instar, they readily leave JA-elicited plants to feed on neighboring control plants. More than 98% of the lifetime *N. attenuata* leaf mass consumed by a tobacco hornworm larva is consumed during the fourth and fifth instars. Thus if herbivores are motivated by a plant's induced responses to move to neighboring plants when they are most voracious, a delayed or suppressed activation of defense might provide plants with an effective response to the combined selective pressures of herbivore attack and intraspecific competition. Specifically, plants may tolerate herbivore attack when larvae are small and move them to neighboring and competing conspecifics when they are most voracious (van Dam et al., 2001a). Plants clearly "recognize" attack from first instar larvae and have the capability of launching a lethal, however costly, defense response against first instar larvae, as is evident from larval performance studies on JA-elicited plants (van Dam et al., 1999). However, plants appear not to avail themselves of this ability. As such, the suppression of costly defense responses during herbivore "recognition" may represent a higher-level optimization of a plant's defense responses.

CONCLUSION

Native plants have clearly evolved sophisticated means of coping with the myriad of selection pressures with which they are faced. Only by measuring plant fitness attributes can one understand the plant's solutions to these selective pressures. The mechanisms responsible for this ecological sophistication are likely lurking in the details of the regulation of the "transcriptome" but without an intimate understanding of a plant's natural history, the transcriptional responses will remain functionally obscure. The cost-benefit paradigm is a useful heuristic tool to generate testable hypotheses about the function of a trait, but tools are needed to manipulate the

traits and test the fitness consequences of the manipulations in ecologically complex environments. As such, the development of gene disruption, silencing and over-expression techniques that can be used with plants growing in natural habitats may provide the fastest way forward toward a functional understanding of metabolism.

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